

## Plant growth promotion by a hexavalent chromium reducing bacterial strain, *Cellulosimicrobium cellulans* KUCr3

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**Abstract** This article reports on the isolation and characterization of a Cr(VI) resistant bacterial strain, having plant growth promoting properties to improve general growth of plant in chromium-contaminated soil through rhizosphere colonization. The strain was isolated from the sludge of waste canal carrying industrial effluents. The minimum inhibitory concentration of chromium to this strain was found to be 450 and 400 mM in complex and minimal media, respectively. The strain also showed varied degree of resistance to Cd, Co, As, Ni and Zn. It exhibited potential Cr(VI) reducing ability under aerobic culture conditions, and the factors affecting Cr(VI) reduction by this strain were evaluated. The optimum pH and temperature required to achieve maximum Cr(VI) reduction values were 7 and 35°C, respectively. Higher concentration of Cr(VI) slowed down the reduction, but with longer incubation time it reduced nearly all detectable amount of Cr(VI). The strain showed positive response to IAA production and phosphate solubilization. It promoted the growth of chilli plants in waste-fed soil with or without additional Cr through its establishment in rhizosphere. The successful establishment of KUCr3 in the rhizosphere of chilli plants helped to reduce Cr uptake by the test plant. This strain shows a promise that the multifarious role of this strain would be useful in the Cr-contaminated rhizosphere soil as a good bioremediation and plant growth promoting agent as well. Through biochemical characterization and 16S rDNA sequence analysis, the strain KUCr3, as the name given to it, was identified as a strain of *Cellulosimicrobium cellulans*.

**Keywords** Cr(VI) bioremediation · PGPR · *Cellulosimicrobium cellulans*

### Introduction

Contamination of soil and ground water due to the use of chromium (Cr) in various anthropomorphic activities has become a serious global threat (Shanker et al. 2005; Abdel-Sabour 2007). The toxic effect of Cr primarily to the biota depends on its electrochemistry (Losi et al. 1994; Kotas and Stasicka 2000; Marsh and McInerney 2001). The hexavalent species i.e. Cr(VI) is most toxic because of its high mobility (Losi et al. 1994; Becquer et al. 2003). Accumulation of such toxic form by the plants from the environment contaminated with Cr is a matter of growing agro-environmental concern. The toxicity of Cr in plants is observed at multiple levels from reduced yield including overall growth to inhibition on enzyme function and mutagenicity (Mei et al. 2002; Shanker et al. 2005). In this context, microbial reclamation has been a promising aspect using Cr-resistant bacteria to detoxify Cr(VI) in the rhizosphere environment. Detoxification of Cr(VI) can occur directly by enzymatic reduction to less mobile form Cr(III) by Cr(VI) reductase or indirectly through making complexes with metabolites (Losi et al. 1994; Silver and Phung 1996; Wang et al. 1997; Camargo et al. 2003; Cheung and Gu 2005; Sau et al. 2008; Pei et al. 2009). Cr(VI) resistant strain having such detoxifying ability with plant growth promoting features has raised high hope for cost effective and eco-friendly measures for sustainable agriculture in soil tract contaminated with Cr (Rajkumar et al. 2005, 2006; Faisal and Hasnain 2006). The rhizosphere offers a complex and dynamic microenvironment where microbes develop unique communities interacting with root systems

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that have potential application to detoxify hazardous compounds including toxic metals (Burd et al. 2000; Denton 2007; Rajkumar and Freitas 2008). Therefore, improvement of the interaction between plants and beneficial rhizosphere microbes would be an important component of bioremediation technology in agriculture (Glick 2003; Jing et al. 2007; Zhuang et al. 2007).

The objective of this work was to isolate a Cr-resistant bacterial strain, which could detoxify Cr(VI) and promote plant growth as well by successful colonization in rhizosphere soil of green chilli grown in waste-fed soil. The study was also aimed to evaluate the efficacy of the strain to reduce chromium accumulation in that test plant.

## Materials and methods

### Isolation and purification of a chromate resistant bacterial strain

Chromate-resistant bacterial isolate was obtained from the sludge of waste canal carrying industrial effluents nearby Kolkata, India. The sample was diluted appropriately and inoculated on PYG (10 g peptone, 5 g yeast extract, 3 g glucose in 1 l, pH 7.2) agar (2% w/v) plates having different concentration of Cr (0.5, 1, 1.5, and 2 mM) as  $K_2CrO_4$ . The colonies that could tolerate 2 mM Cr were selected randomly and assessed for its Cr(VI) removal ability. The isolate that showed significant Cr(VI) removal was selected for further experiment in this study and the isolate was designated as KUCr3. The isolate was then purified to have a pure culture by cycles of single colony isolation and liquid culture transfers on minimal medium (3 g  $K_2HPO_4$ , 6 g  $Na_2HPO_4$ , 5 g NaCl, 2 g  $NH_4Cl$ , 0.1 g  $MgSO_4$ , 8 g glucose in 1 l, pH 7.2) supplemented with 2 mM Cr(VI). A single culture was eventually chosen for further experiment on the basis of its Cr(VI) removal ability and was being maintained. The minimum inhibitory concentrations (MIC) of Cr(VI) to this strain were measured both in PYG and minimal liquid media supplemented with higher concentrations of Cr.

### Characterization of the Cr-resistant bacterial isolate

The isolate was identified based on the morphological and standard biochemical tests. The strain was also tested for its resistance to different toxic metals viz. Cd ( $CdCl_2 \cdot H_2O$ ), Co ( $CoCl_2 \cdot 6H_2O$ ), As ( $As_2O_3$ ), Ni ( $NiCl_2 \cdot 6H_2O$ ) and Zn ( $ZnCl_2$ ). The MIC values of those metals were determined both in PYG and minimal liquid media. Furthermore, the genomic DNA was isolated and purified using bacterial genomic DNA SPIN kit (RKT 09, Chromous Biotech Pvt.

Ltd., Bangalore, India) following manual instructions. The 1.45 Kb 16S rDNA fragment was amplified using 16S rRNA bacterial specific forward (5'-AGAGTRTGATCM TYGCTWAC-3') and reverse (5'-CGYTAMCTTWT ACGRCT-3') primer sets. The PCR amplification reaction was performed using high-fidelity PCR Master Kit (Roche Applied Science, Germany). The base sequencing of the PCR product was done at Chromous Biotech Pvt. Ltd., Bangalore, India. The sequence data were aligned and analyzed to identify the bacterium and its closest neighbors using BLAST function (Altschul et al. 1990) at NCBI database and the Ribosomal Database Project (Maidack et al. 1997).

### Chromium(VI) analysis

Chromate removal was measured as the decrease of chromate with the time using Cr(VI) specific colorimetric reagent S-diphenylcarbazide (DPCZ). About 1 ml of 0.05% DPCZ (w/v in acetone) was added to 1 ml of culture supernatant and additionally 3 ml of 0.16 M sulfuric acid was poured for minimizing the deterioration (Urone 1955). A purple complex was formed due to the reaction of DPCZ with chromate. The absorbance was taken immediately at 540 nm in a spectrophotometer (Cecil CE7200, England). Quantity of Cr(VI) was measured obtaining the calibration curve using  $K_2CrO_4$  solution as standard.

### Growth and chromate removal in PYG medium

The effect of Cr(VI) on cell growth under aerobic condition was determined by inoculating young cell suspension of KUCr3 (finally to have  $\sim 6.0$  log CFU  $ml^{-1}$ ) to PYG broth supplemented with 2 mM Cr(VI) as  $K_2CrO_4$  and incubated on a rotary shaker at 37°C, and compared with control set without Cr(VI). The growth responses were determined by counting the colony forming units (CFU) on PYG agar plate by dilution plate technique. The chromate removal was measured at different time intervals by measuring the Cr(VI) left in the cell-free supernatant following centrifugation.

### Effects of initial inoculum concentration on chromate removal

To assess the effect of initial inoculum load on chromate removal young cell suspension of KUCr3 (finally to have  $\sim 4.2$ , 5.1 and 6.0 log CFU  $ml^{-1}$  separately) was inoculated to PYG broth supplemented with 2 mM Cr(VI) as  $K_2CrO_4$  and incubated on a rotary shaker at 37°C. The chromate removal was measured at an interval of 24 h by measuring the residual Cr(VI) in the cell-free supernatant following centrifugation.

### Effect of pH and temperature on chromate removal

The influence of pH on chromate removal under aerobic culture conditions was measured in PYG broth having different pH values separately and was inoculated with young cell suspension (finally to have  $\sim 6.0$  log CFU ml<sup>-1</sup>) and incubated at 37°C on a rotary shaker. The effect of incubation temperature on chromate reduction was assessed in PYG broth (pH 7.2), inoculated with same volume of young cell suspension and incubated on a rotary shaker at different temperature. Chromate removal was measured after 120 h.

### Chromate adsorption by cell mass

In order to assess the Cr adsorption ability of the KUCr3, same amount of washed live and dead cell mass (wet) were put into K<sub>2</sub>CrO<sub>4</sub> solution in sterilized saline buffer or PYG broth finally having 2 mM Cr(VI). Bacterial cells of 24 h old were harvested by centrifugation at 7,000×g for 10 min to have the live cell mass or thereafter autoclaved to get the dead cell mass. The Cr removal by the dead and live cells was measured at different time intervals by measuring the residual Cr(VI) in the supernatant following centrifugation at 7,000×g for 10 min. Cell-free sets were maintained to determine the artifacts might arise due to the metal sorption on the glass surface of the container.

### Characterization of plant growth-promoting features of KUCr3

For qualitative assay of indole acetic acid (IAA) production by KUCr3, cells were grown at 37°C for 48 h on PYG with or without tryptophan (500 µg ml<sup>-1</sup>) and then the bacterial cells were removed from the culture medium by centrifugation at 7,000×g for 10 min. About 1 ml of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (150 ml concentrated H<sub>2</sub>SO<sub>4</sub>, 250 ml H<sub>2</sub>O, 7.5 ml 0.5 M FeCl<sub>3</sub>·6H<sub>2</sub>O) and development of a pink color indicated IAA production. The phosphate solubilizing activity of the isolate was qualitatively analyzed on Pikovskaya's agar medium (HiMedia, India). Development of a clear zone around the growing colony indicated phosphate solubilization.

### Bioassay for plant growth promotion

Study of plant growth promotion and rhizosphere colonization were done with a stable kanamycin resistant mutant (150 µg ml<sup>-1</sup>) of the bacterial strain KUCr3. Surface sterilized green chilli seeds were allowed to imbibe sterilized water and then were sown in solarized clay pots containing sterilized waste-fed soil (Cr content,  $\sim 450$  µg gm<sup>-1</sup>; pH 7.4) and kept in the growth chamber to avoid microbial contamination. Five

sprouting seeds were allowed to grow in each pot. To see the effects of Cr toxicity, seedlings were irrigated every 4 days either with water (control) or with 200 ml of 500 µM Cr as K<sub>2</sub>CrO<sub>4</sub> solution. The choice of such lowered Cr concentration was chosen in order to avoid severe toxic effect of high concentration of Cr on test plants. The plants were harvested after 45 days, and growth parameters were measured. The rhizosphere colonization ability of the strain was studied and the possible microbial contamination was also monitored (Sinha and Mukherjee 2008). To study the rhizosphere colonization, soil sample adhering to the roots was removed and the bacterial population (CFU gm<sup>-1</sup> soil) was determined by dilution plate technique on PYG agar plates with kanamycin (150 µg ml<sup>-1</sup>) and 2 mM Cr(VI), and without kanamycin and Cr(VI) for the assessment of contaminants, if any.

### Estimation of Cr in plants

Root and shoot samples were vigorously shaken with 0.01 M EDTA solution and water to exclude contaminant Cr on the surface. The washed root or shoot samples were then dried at 105°C and digested in a mixture of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (4:1, v/v) following Sinha and Mukherjee (2008). Total Cr content in the digest was determined by atomic absorption spectroscopy (AAnalyst 400, Perkin Elmer, Singapore).

### Chlorophyll assay

The Chlorophyll content was measured from 1 g of mature leaf slices following the method of Arnon (1949).

## Result and discussion

### Characterization of the Cr-resistant bacterial isolate

#### *Phenotypic and biochemical characterization of the isolate*

The selected KUCr3 strain was found to be Gram-positive, motile and pleomorphic one, which developed yellowish-white circular and convex colonies on PYG agar medium. The strain showed a positive result to catalase, amylase, methylene red test, nitrate reduction, cellulose degradation, IAA production and phosphate solubilization. The strain could utilize glucose, sucrose, maltose and lactose as carbon source. The strain showed negative response to Voges–Proskauer test, citrate utilization, H<sub>2</sub>S production, urease production, gelatin hydrolysis, arginine hydrolysis, mannitol utilization and siderophore production. In addition to Cr resistance the strain showed varied degree of resistance to Cd, Co, As, Ni and Zn both in PYG and minimal media. The MIC values of the test metals have been presented in

**Table 1** Minimum inhibitory concentration of different heavy metals

Medium used	MIC values of the test metals (mM)					
	Cd <sup>a</sup>	Co	As	Ni	Zn	Cr
PYG	3.0	5.0	5.0	6.0	20.0	450.0
Minimal	1.5	4.0	4.5	5.0	18.0	400.0

<sup>a</sup> The chemical form of metals used are CdCl<sub>2</sub>·H<sub>2</sub>O, CoCl<sub>2</sub>·6H<sub>2</sub>O, As<sub>2</sub>O<sub>3</sub>, NiCl<sub>2</sub>·6H<sub>2</sub>O, ZnCl<sub>2</sub> and K<sub>2</sub>CrO<sub>4</sub>, respectively

Table 1, for Cr it was 450 and 400 mM in PYG and minimal media, respectively. The differences in MIC values in PYG and minimal media might be explained by the fact that bioavailable metal content was reduced due to complexation with undefined components in the PYG medium. Nevertheless, this high tolerance to different toxic metals could be attributed to the fact that the strain was isolated from an industrial waste-fed canal where a selection pressure was posed.

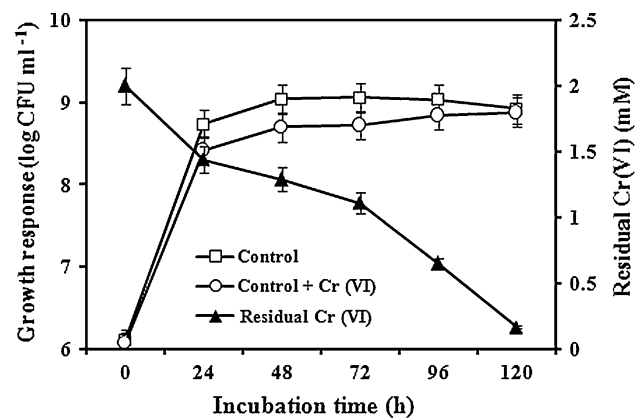
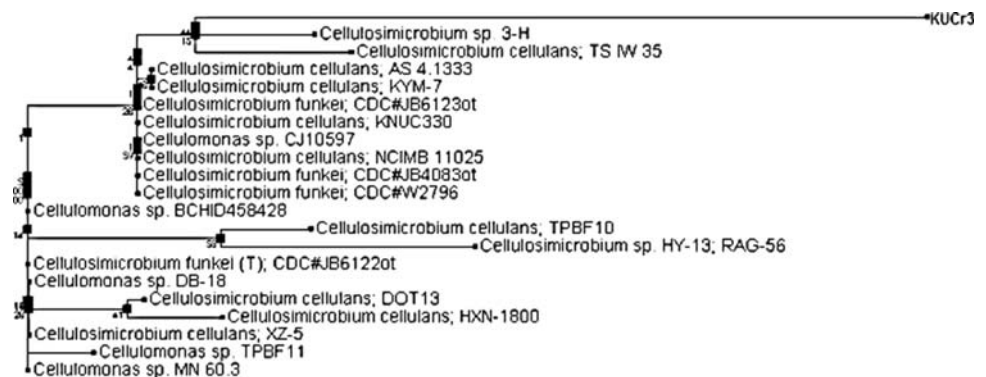
#### Molecular characterization of the isolate

Based on the biochemical tests and analysis of the 16S rDNA sequence (1.45 Kbp) using BLAST function at NCBI database and Ribosomal Database Project, the isolate KUCr3 (NCBI GenBank Accession No. FJ793202) was identified as a strain of *Cellulosimicrobium cellulans* (Schumann et al. 2001). The phylogenetic lineage of KUCr3 drawn from 16S rDNA sequence databases of 20 closely related members is presented in Fig. 1.

#### Growth and chromate removal

Growth and chromate removal by KUCr3 in the PYG medium supplemented initially with 2 mM Cr(VI) were measured at time intervals (Fig. 2). Due to less cell mass yield by the strain at the maximum tolerance level of Cr(VI) as per MIC data, in this experiment 2 mM Cr(VI), which is much higher than that found in industrial effluent-fed waste canal water, was used to have a substantial bacterial cell mass. The strain grew in presence of 2 mM

**Fig. 1** Phylogenetic position of *Cellulosimicrobium cellulans* KUCr3 showing relatedness with 20 closely related members based on 16S rDNA sequence comparison



**Fig. 2** Comparative growth responses of KUCr3 grown in PYG broth with or without (*Control*) chromate (2 mM) and its Cr(VI) removal ability in Cr(VI) supplemented medium with the time course. Data are the mean of three replications with SE

Cr(VI) comparably with the control set and removed Cr(VI) steadily. During early stationary phase of growth, the rate of Cr(VI) removal slowed down after initial steady rate, however, it increased after 72 h and removed almost 95% from the medium within 120 h. The Cr(VI) removal was found to be cell mass dependent in this strain, active microbial biomass dependency of Cr(VI) removal was also documented earlier (McLean et al. 2000; Rahman et al. 2007).

The strain showed two steady phases of Cr(VI) removal in the course of its growth, one during active growth and another during mid-stationary phase, it raised a question whether dead cell mass had any role to immobilize chromate or not; because bioaccumulation of chromium could not be ignored as such phenomena were documented earlier (Nurbap Nourbakhsh et al. 2002; Faisal and Hasnain 2004). Answering to this question both dead and live cell mass were put into sterilized saline buffer and PYG broth having 2 mM Cr(VI). The dead cell mass insignificantly contributed to remove chromate (<3%), even the live cells could not show any significant role on removal in saline buffer (Table 2), suggesting major contribution of catalytic

**Table 2** Chromium (VI) removal (%)<sup>a</sup> by the live or dead (wet) cell mass

Incubation time (h)	PYG + 2 mM Cr(VI)		Saline buffer + 2 mM Cr(VI)	
	Dead mass	Live mass	Dead mass	Live mass
24	2.28	44.46	2.20	2.25
48	2.68	55.06	2.52	2.85
72	2.88	61.72	2.80	2.85

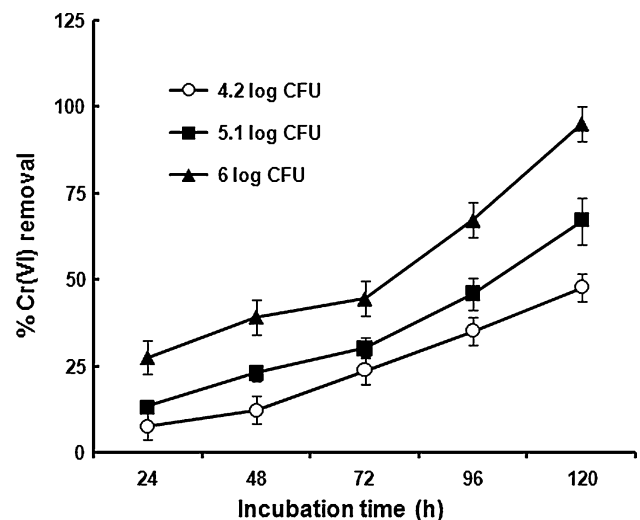
24 h grown cell mass (dead or live) were suspended in sterilized saline buffer or PYG broth having 2 mM Cr(VI)

<sup>a</sup> Data (%) obtained from the mean of three replications

reduction of Cr(VI) by the active cells or its cytosolic enzymes, perhaps a soluble reductase (Pal et al. 2005). This findings contradict the report on the reduction of Cr(VI) under non-growth conditions by cell mass of a related isolate, *Cellulomonas* sp. (Sani et al. 2002; Viamajala et al. 2007). The insignificant removal of chromate by live cells in saline buffer might be due to the absence of external electron supply which is essential during the reaction process (Cervantes and Campos-Garcia 2007), considering it is as a redox reaction. It seems an additional amount of reductase from the still growing cells during stationary period, in addition to remnant enzymes produced during active growth phase, sustained Cr(VI) reduction steadily. However, it is difficult to preclude the possible role of other metabolites produced during nutrient scarce conditions i.e. during stationary phase, on Cr(VI) immobilization. Viamajala et al. (2007) earlier reported that *Cellulomonas* sp. ES6 could reduce Cr(VI) without external electron donors under non-growth conditions for a long period and suggested the role of endogenous reductants. Insignificant Cr(VI) reduction by the cell mass under non-growth conditions in saline buffer discarded such possibility in this strain. Thus it warrants further investigation to resolve the exact mechanism which could contribute to immobilize Cr(VI) other than enzymatic reduction in this particular strain during stationary phase. Study of reduction kinetics with cell-free extracts is being conducted to elucidate the catalytic reduction of chromate by *C. cellulans* KUCr3.

#### Effects of initial cell density on chromate reduction

The rate of chromate removal showed a positive relation to the initial cell density in the inoculum under aerobic growth conditions (Fig. 3). The Cr(VI) removal increased with the increase of initial inoculum load. Irrespective to inoculum load reduction rate increased steadily after 72 h except in the medium where initial load was 4.2 log CFU ml<sup>-1</sup>, in that case Cr(VI) was reduced steadily after 96 h. It seems a substantial biomass was required to attain



**Fig. 3** Contribution of initial inoculum load on the Cr(VI) removal by KUCr3 grown in PYG liquid medium having 2 mM Cr(VI). Data are the mean of three replications with SE

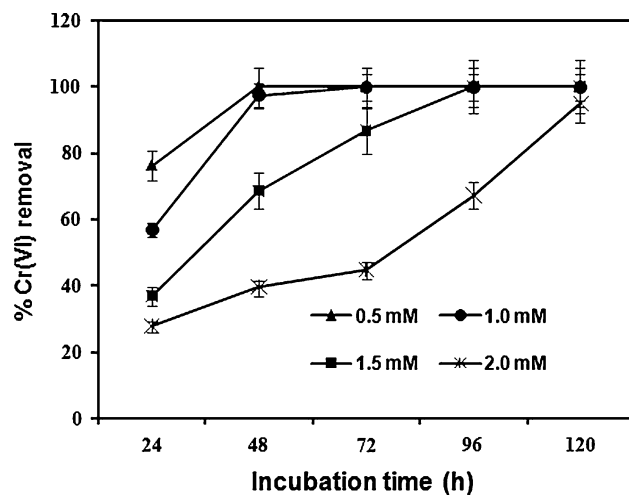
the optimal reduction rates (Wang et al. 1989; McLean et al. 2000).

#### Effect of initial Cr(VI) concentration on chromate reduction

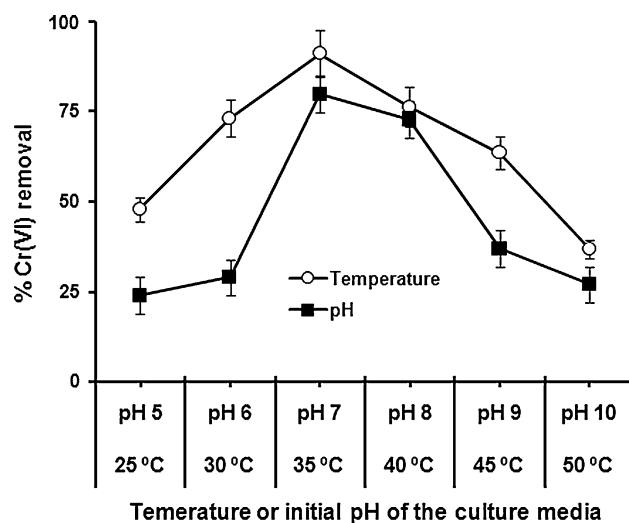
The effect of Cr(VI) concentration in the medium on the chromate reduction by KUCr3 was studied at different concentrations (0.5–2 mM). After 120 h the strain could reduce chromate more than 90% irrespective to the initial concentration of Cr(VI) supplemented in the media (Fig. 4). At lower concentration (0.5 and 1.0 mM) chromate reduction rate increased sharply within 24 h and metal was completely reduced from the medium after 48 h. In the first 24 h the reduction rate was slowed at 1.5 and 2 mM of Cr(VI), then at 1.5 mM it took 96 h to reduce completely and 120 h for 95% reduction at 2 mM concentration. It seems higher concentration poses a selection pressure due to Cr toxicity and thus lengthened the growth phase, and for having substantial cell mass it took longer period for chromate reduction (McLean et al. 2000; Sau et al. 2008). Cr(VI) concentration dependent reduction kinetics in *Bacillus* was also reported earlier (Wang and Xiao 1995; Camargo et al. 2003).

#### Effect of pH and temperature on chromate reduction

Bacterial detoxification by reducing Cr(VI) from the medium was found to be dependent on the pH and incubation temperature. The KUCr3 showed varied growth responses at different pH (data not shown), but optimal pH for the growth and maximum chromate removal ability were within a range of 7–8 and Cr(VI) removal achieved its



**Fig. 4** Effect of initial Cr(VI) concentration in the culture media (PYG) on Cr(VI) removal by KUCr3. Data are the mean of three replications with SE



**Fig. 5** Effect of pH of the medium (PYG) or incubation temperature separately on the Cr(VI) removal by KUCr3. For pH study the incubation temperature was 37 °C and for temperature study pH of the medium was 7.2. The results were obtained after 120 h of incubation. Data are the mean of three replications with SE

maximum value at pH 7 (Fig. 5). The correlation between pH and chromate reduction might be due to the occurrence of dominant Cr(VI) species in the aqueous environment at pH 6.5–9 (McLean and Beveridge 2001). Similarly, KUCr3 grew better at a temperature ranging between 30 and 40 °C, and maximum Cr(VI) removal was achieved at 35 °C (Figure 5). Earlier Losi et al. (1994) reported that optimum temperature of 30–37 °C required for Cr(VI) detoxification by reduction in culture condition. Requirement of incubation temperature for Cr(VI) removal by reduction in *Bacillus* sp. was also documented (Wang and Xiao 1995; Camargo et al. 2003; Sau et al. 2008). The most

of the isolates so far reported, the optimal pH and temperature for growth correlated with highest rate of Cr(VI) removal by reduction (Wang et al. 1990; Shakoori et al. 2000; Camargo et al. 2003; Sau et al. 2008).

#### Plant growth-promotion and Cr(VI) immobilization by KUCr3

The Cr(VI) reducing strain *C. cellulans* KUCr3 was qualitatively examined for some selected number of features thought to be the contributors for plant growth promotion. The strain showed the ability to produce IAA and to solubilize phosphate; additionally it has the ability to detoxify Cr(VI) which would be an effective feature in rhizosphere to protect the root system of the crops from Cr toxicity.

The strain colonized successfully in the rhizosphere soil of chilli plant grown under either control or chromium added conditions. The rhizosphere colonization was determined by dilution plating on PYG agar plates with kanamycin (150  $\mu\text{g ml}^{-1}$ ) and Cr (2 mM), and without kanamycin and Cr for the assessment of contaminants, if any. The bacteria persisted in the rhizosphere comparatively better (7.1 log CFU  $\text{gm}^{-1}$  soil after 45 days) under conditions without added Cr than when Cr exogenously added (6.2 log CFU  $\text{gm}^{-1}$  soil). It might be accounted for the toxic effect posed to the bacterial population by added Cr.

Nevertheless, the findings suggest successful colonization of this strain in the rhizosphere of chilli plants when inoculated and subsequently promoted growth of the plants (Table 3 and Fig. 6). Plants showed better visible growth when inoculated with KUCr3 under control conditions, additional Cr supplementation reduced the growth, but the growth retardation was relieved by KUCr3 inoculation (Fig. 6), suggesting the potentiality of this strain as plant growth promoter and as an effective bioremediator. Remediation of toxic metal at contaminated sites might be related to the presence of higher proportion of metal resistant microbial population in the soil conferring protection to the plants (Doelman 1985, Rajkumar and Freitas 2008). Under control conditions i.e. soil without extra chromium, KUCr3 increased the shoot length (26.53%) and root length (16.67%) over the control one suggesting its bioefficacy as plant growth promoter. It might be due to the IAA production and phosphate solubilization by this strain (Husen 2003; Rajkumar et al. 2005, 2006; Rajkumar and Freitas 2008). Additional Cr significantly decreased the value of each growth parameter when uninoculated and the effects were relieved when soils were with KUCr3. The study showed that the shoot length, root length and the production of chlorophyll were severely hampered by Cr, reducing 25.79, 25 and 29.23% over the respective values of the control plants (without additional Cr). The overall reduction in plant growth seems to be a consequence of toxic effect of

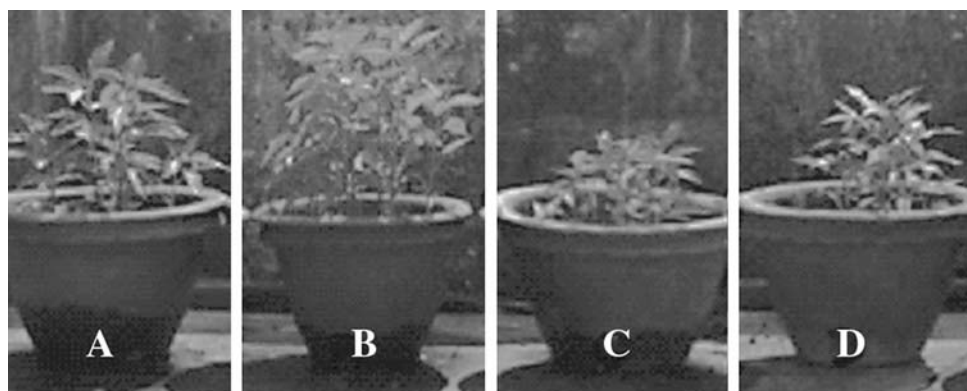
**Table 3** Contribution of KUCr3 on plant (chilli) growth and Cr accumulation

Parameters <sup>a</sup>	Treatment <sup>b</sup>				CD (at 5%)
	Control (A)	A + KUCr3	A + Cr	A + Cr + KUCr3	
Shoot length (cm)	19.00 (±0.676)	24.04 (±0.108)	14.10 (±0.071)	17.00 (±0.071)	0.987
Root length (cm)	6.00 (±0.346)	7.00 (±0.418)	4.50 (±0.248)	8.00 (±0.282)	1.011
Chlorophyll content (mg g <sup>-1</sup> )	0.65 (±0.001)	0.66 (±0.011)	0.46 (±0.002)	0.61 (±0.003)	0.017
Cr in shoot (µg g <sup>-1</sup> )	32.42 (±0.118)	20.11 (±0.193)	156.34 (±0.226)	98.13 (±0.231)	0.605
Cr in root (µg g <sup>-1</sup> )	185.93 (±0.283)	134.25 (±0.256)	934.03 (±0.386)	395.10 (±0.302)	0.960

<sup>a</sup> Data for each treatment are the mean of five observations with SE; CD ( $P < 0.05$ ) extracted from analysis of variances

<sup>b</sup> Plants grown in sterilized waste-fed soil (A) without extra Cr, with inoculants only (A + KUCr3), with extra Cr only (A + Cr) or with extra Cr and inoculants (A + Cr + KUCr3)

**Fig. 6** Effect of KUCr3 inoculation on plant (chilli) growth grown in sterilized waste-fed soil (B), and in sterilized waste-fed soil with additional Cr (D); sets A and C were uninoculated ones of B and D, respectively



Cr after its accumulation on different plant parts. When KUCr3 was used as seed inoculant, it protected the plant significantly from the toxic effects of Cr by minimizing the reduction in agronomic parameters (Rajkumar et al. 2005, 2006).

Irrespective to the treatment regimes the root system accumulated higher amount of Cr than the shoot parts (Table 3). The reason of the high accumulation of Cr in root systems might be due to the immobilization of Cr(VI) in root cells (Shanker et al. 2005), though rate of Cr accumulation in plant is also dependent on other several soil factors (Kabata-Pendias and Pendias 2001). The successful establishment of KUCr3 in the rhizosphere of chilli plants helped to reduce Cr uptake (Table 3). It reduced 37.97% of Cr uptake in shoots and 27.80% in the roots when grown in soil without extra Cr. Even KUCr3 reduced Cr accumulation in both shoots and roots by 37.23 and 56.70%, respectively when the plants were grown in soils with extra chromium. Interestingly, the rate of Cr mobilization from root to shoot whatever the plant accumulated remained almost same (~15%) irrespective to the treatment regimes, suggesting insignificant role of KUCr3 in internal Cr mobilization in the plant system. The decrease in Cr uptake by the plants might be due to the bacterial

Cr(VI) immobilization in the rhizosphere soil by reducing its valence state or making metabolic complexes leading ultimately to lower availability of Cr.

## Conclusion

This study demonstrated that *C. cellulans* KUCr3, when applied to the rhizosphere soil of plants, served two major purposes. First, bioinoculation improved the growth parameters of plants perhaps due to its IAA production and phosphate mineralization, at least in the pot experiment, secondly and more importantly, it decreased the Cr uptake in plants most likely due to Cr(VI) reduction by the strain in the rhizosphere. Earlier Yu et al. (2006) also reported plant growth promoting activity of *C. cellulans* Ha8 on cucumber plant by mitigating the repress of cinnamic acid to the growth. Our study, therefore, shows that the multifarious role of the strain KUCr3 could be applied in the Cr-contaminated rhizosphere soil as a good bioremediation agent, although the extent of stimulation of plant growth and reduction in Cr uptake under actual metal-polluted field conditions and its formulation need further study.

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