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Full Length Research Paper

Hexavalent chromium reduction by metal resistant and halotolerant *Planococcus maritimus* VITP21

Sangeetha Subramanian, Silviya Sam and Gurunathan Jayaraman*

School of Biosciences and Technology, VIT University, Vellore, 632014, Tamil Nadu, India.

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Halotolerant Cr (VI) reducing bacteria (VITP21) was isolated from kumta coastal region, India and was identified by biochemical methods and 16S rRNA analysis as *Planococcus maritimus*. The halotolerant bacteria exhibited complete reduction of 100 and 200 mg/L of Cr (VI) within 24 and 28 h, respectively and greater than 90% reduction was observed for higher concentration of Cr (VI) in the range of 300 to 500 mg/L (pH 7, 35°C, and 4% NaCl). The optimum parameters for chromium reduction was found to be pH 7.0, 35°C and 4% NaCl with an agitation rate of 140 rpm under aerobic condition. The isolate was capable to grow and reduce chromate even in the presence of different divalent metal ions (50 mg/L of Pb^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Mn^{2+}) under saline conditions. Experiments with resting cells, permeabilized cells, sonicated cells and cytosolic fractions demonstrated that the chromium reduction is mainly associated with the soluble cytosolic fraction of the cell. To our knowledge, this is the first detailed report on effective reduction of Cr (VI) by halotolerant bacteria *P. maritimus* VITP2l. The current investigation indicates that the isolated organism can promisingly contribute for the treatment of toxic hexavalent chromium present in saline industrial waste water.

Key words: Bioremediation, chromium reduction, chromium removal, halotolerant bacteria, *Planococcus*, saline wastewater.

INTRODUCTION

Chromium (VI), a heavy metal ion is released into the environment mainly due to chrome tanning processes, electroplating, paint and pigment manufacturing industries (Sharma et al., 2011). Among the different oxidation states, trivalent and hexavalent chromium exist as stable species. Compared to trivalent chromium, hexavalent chromium is highly toxic, mutagenic and carcinogenic (Sultan and Hasnain, 2007). The permissible limit recommended by environmental protection agency (EPA) for Cr (VI), in drinking water, is less than 50 µg/L (Baral and Engelken, 2002). Therefore treatment of water polluted with hexavalent chromium becomes indispensable. Conventional methods like precipitation, electrochemical treatment, ion exchange have several disadvantages (Sannasi et al., 2006). Use of microorganisms has been shown as an alternative, as they have an ability to tolerate bio-accumulate, precipitate, adsorb or bio-convert toxic hexavalent chromium. Extensive work has been carried out on the removal of chromium by a variety of organisms (Thacker et al., 2006; Srinath et al., 2002; Jeyasingh et al., 2005; Sultan et al., 2007). However, very few microbes have been studied for their potential in removing hexavalent chromium under saline conditions. Metal removal study in the presence of salt becomes important as waste water generated by most of the industries, especially tannery waste stream contains higher concentration (1- 10 % by wt) of salt as NaCl (Sivaprakasam et al., 2008).

Saline environment which includes sea water, salterns, soda lakes, evaporation pools inhabits several groups of organisms. The microbes which are flourishing in this habitat can withstand extreme or at least moderate saline conditions, depending on the habitat and adaptability.

*Corresponding author. E-mail: gjayaraman@vit.ac.in. Tel: + 91 416 2202573. Fax: + 91 416 224 3092.

Halophilic microbes grow well in higher salt concentration whereas halotolerant microbes are capable of growing even in the absence of salt. Organisms from saline habitat have been shown to be resistant to many toxic metals as they survive in highly stress conditions (Gnanamani et al., 2010). Biological detoxification of Cr (VI) has been reported by a few halophilic/halotolerant organisms (Kiran et al., 2008; Amoozegar et al., 2007; Shapovalova et al., 2009; Vanengelen et al., 2008; Donmez and Kocberber, 2005). Despite the fact that these studies show promising contribution from halophilic/halotolerant species, extensive investigation on chromium reduction capability under various conditions and localization of chromium reduction is not reported for halotolerant bacteria. In order to give such insights, this report details the potential capability of a halotolerant bacterium, *Planococcus maritimus* VITP2l, in reducing toxic hexavalent chromium to non-toxic trivalent chromium.

MATERIALS AND METHODS

Materials

Chemicals and culture media were purchased from Himedia, Mumbai. All reagents were of analytical reagent (AR) grade. All the glasswares were washed with nitric acid solution (1:1) and rinsed thrice with distilled water before use.

Halotolerant bacteria and culture condition

Halotolerant bacterial strains were isolated from Kumta costal region of Karnataka, India and were maintained in the laboratory. Morphologically different colonies were transferred on Luria agar plate amended with 100 to 1000 mg/L of Cr(VI) as potassium dichromate and investigated for chromium resistance. Selected Cr (VI) resistance bacterial cultures were studied for their Cr (VI) removal potential under aerobic condition with an initial Cr (VI) concentration of 200 mg/L. Luria Bertani medium, supplemented with 4 % (w/v) NaCl was used for all the experiments. The pH of the media was adjusted to 7.0 with either 1 M NaOH or HCl. All experiments were performed in duplicates under identical conditions.

Identification of isolated strain

Morphological and physiological characteristics of isolated strain were studied as per Bergey's manual of determinative bacteriology (Holt et al., 1994). The strain was further identified by 16S rRNA amplification and nucleotide sequencing. Megablast was used to identify homologous sequences in the nucleotide database and phylogenetic tree was constructed using the neighborhood joining method.

Batch removal study

The potential halotolerant bacterial cells, maintained in agar plates, were sub cultured in 25 ml of culture media in 100 ml flask and used for inoculating the experimental flask. The medium (100 ml) in 250 ml flask containing various initial chromium concentrations ranging from 100 to 500 mg/L was inoculated with 1% (v/v) of overnight grown cultures with optical density of 1.0 (at 600 nm). Samples were collected at regular intervals and the cell growth was monitored by measuring the optical density at 600 nm. The samples were then centrifuged at 8000 rpm for 5 min and the supernatant was used for determining the concentration of chromium. Each experiment was carried out for a period until the residual concentration of hexavalent chromium was found to be same with time.

Chromium removal study under varying salt (NaCl) and chromium (VI) concentration

The effect of salt concentration on Cr (VI) removal efficiency of the isolated strain was determined in the presence of varying concentration of NaCl (2% (w/v) to 10% (w/v)). Different initial chromium concentration (100 mg/l to 500 mg/L) was also used in the study. Experiments were carried out in 100 ml LB media amended with varying initial salt concentration and varying chromium concentration. The initial pH was maintained at 7.0. The removal efficiency was studied after 24 h of cell growth at 35°C and 140 rpm.

Effect of pH and temperature on chromium removal

Chromium removal by the isolated bacterial strain was investigated by varying initial pH and temperature. For pH dependence study, the strain was grown in LB media with 4% NaCl at different pH (6.0 to 10.0). The experiments were carried out at 35°C with an agitation rate of 140 rpm. After 24 h of growth, amount of hexavalent chromium in the cell free supernatant was determined. The influence of temperature on growth and chromium removal was studied by incubating the culture at different temperature (30, 35, 40 and 45°C). These experiments were performed at pH 7.0 at constant NaCl concentration (4%w/v), initial chromium concentration (200 mg/L) and agitation rate (140 rpm).

Effect of metal ions on chromium removal

The isolated bacterial strain was grown in the presence of cobalt chloride, nickel chloride, cadmium chloride, lead nitrate, zinc chloride, manganese chloride and copper chloride. The chromium removal was investigated in the presence of 50 mg /L of these different metal ions in the presence of 200 mg/L of chromium. Chromium removal was monitored after 24 h of growth at pH 7.0, 35°C, 4% (w/v) and an agitation rate of 140 rpm.

Chromium reduction by resting cells

Overnight grown bacterial cells (200 ml Luria broth) were harvested by centrifugation (8000 rpm for 30 min at 5°C), washed twice with 20 ml of 10 mM Tris HCl (pH 7.2) and were resuspended in 20 ml of the same buffer. An aliquot of the resting cells was amended with 25 and 50 mg/L of hexavalent chromium (final concentration). Heat killed cells served as control (Thacker et al., 2006). All experiments were performed at 35°C. After incubation, the suspension was centrifuged and the cell free supernatant was investigated for chromium removal.

Chromium reduction by permeabilized cells

Overnight grown culture (200 ml of Luria broth) was centrifuged (8000 rpm for 30 min), washed with 10 mM Tris HCl buffer (pH 7.2)

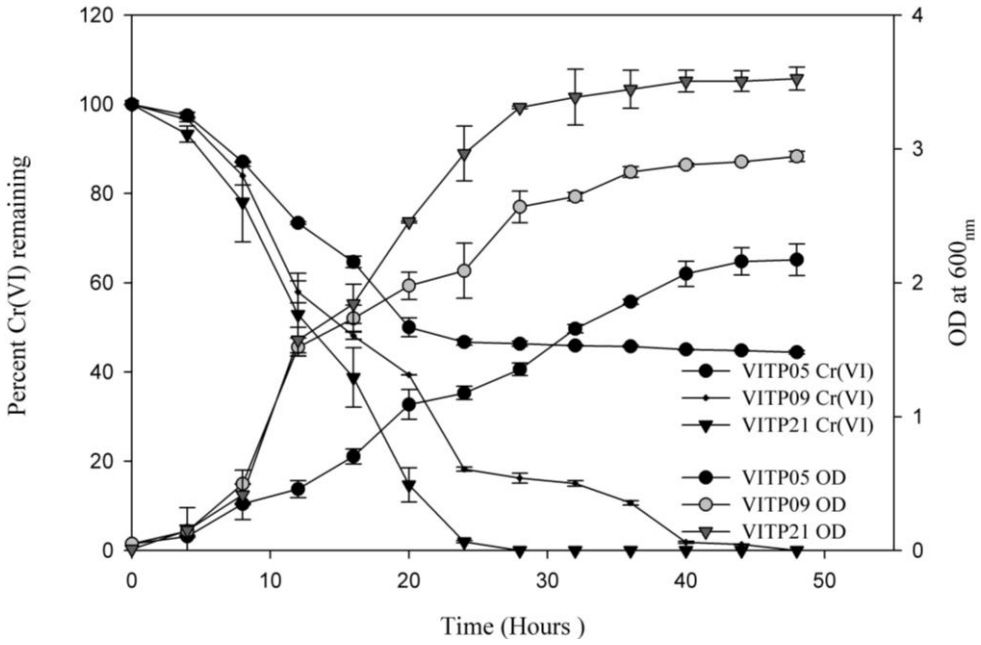

Figure 1. Microbial growth and Cr (VI) removal by different halotolerant isolates. Initial Cr (VI) concentration was 200 mg/L (4% NaCl; pH 7; temperature, 35°C; agitation rate, 140 rpm).

and resuspended in 20 ml of the same buffer. Toluene (0.01% v/v) and Triton X -100 (0.2% v/v) was added to the cell suspension. The prepared cell suspension was vortexed to permeabilize the cells (Thacker et al., 2006). Experiments were carried out for two different initial concentration of hexavalent chromium (25 and 50 mg/L). Heat killed permeabilized cells served as control. All the experiments were performed at 35°C.

Chromium reduction by sonicated fraction

Experiments for chromium reduction were carried out by three different types of sonicated fractions. Bacterial cells grown overnight (200 of Luria broth) were harvested by centrifugation (8000 rpm for 30 min), washed with 10 mM Tris HCl buffer (pH 7.2) and were resuspended in 10 ml of the same buffer. Cells were disrupted by sonication for 15 min (with amplitude of 40 and 60 for 10 and 5 min, respectively) in ice cold condition with 2 s of pulses (Sonics INC model VC 130). The resultant suspension was centrifuged at 8000 rpm for 60 min at 5°C. Both the cytosolic and membrane suspensions were used for the experiments. In these experiments, 50 mg/L hexavalent chromium concentration was used. For all these experiment, heat killed cells were kept as control. In addition to the Diphenyl Carbazide (DPC) assay for chromium, the chromium reduction was also monitored from the change in absorbance in the wavelength range of 325 to 400 nm, as a function of time (Shimadzu UV 2401PC).

Determination of hexavalent chromium concentration and biomass

In all experiments, concentration of hexavalent chromium was determined by diphenylcarbazide method (Thacker et al., 2006). Accordingly, the hexavalent chromium is determined spectrophotometrically by reaction with diphenylcabazide in acid solution (6 M H_2SO_4). Cell free supernatant (200 or 400 µl) was made up to 1 ml using distilled water followed by addition of 330 µl of 6 M H_2SO_4 and 400 µl of diphenylcarbazide (0.25 % w/v in acetone) and final volume was made to 10 ml using distilled water. The absorbance of the samples was read at 540 nm to determine the concentration of hexavalent chromium. Samples for total chromium concentration was first acid digested and were oxidized with potassium permanganate before analyzing by DPC method at 540 nm (Philip et al., 1998). The biomass concentration was inferred from the optical density value at 600 nm (Shimadzu UV 2401PC).

RESULTS AND DISCUSSION

Preliminary screening for potential halotolerant isolates

The halotolerant strains isolated from the Kumta coast were screened for their resistance against the toxic hexavalent chromium. Only three strains (VITP05, VITP09 and VITP21) were found to have significant growth in agar plates amended with higher concentration of potassium dichromate (1000 mg/L). Further confirmation of their chromium resistance was obtained by growing these bacterial strains in LB media amended with Cr (VI). All the three strains (Figure 1) showed capability of chromium removal as well as growth in the presence of initial Cr (VI) concentration of 200 mg/L at 35°C (7.0 pH, 4% NaCl and 140 rpm). After 24 h of growth, VITP21 showed 98% of chromium removal compared to VITP09 and VITP05 which showed only 82 and 53%, respectively. However, after extended hours of incubation, complete chromium removal was achieved for

strain VITP21 (28 h) and VITP09 (48 h), respectively, whereas strain VITP05 showed incomplete (44%) chromium removal even after 48 h of incubation. It was also inferred, from the optical density at 600 nm, that greater biomass was obtained with VITP21 than either VITP09 or VITP05. The chromium (VI) removal rate of VITP21, VITP09, VITP05 was found to be 7.14, 4.35 and 2.42 mg/L (per hour), respectively, thus revealing significant differences in the ability of the three halotolerant strains to remove chromium. Based on the highest removal rate and growth pattern, halotolerant bacterial strain VITP21 was used for further characterization and chromium removal studies.

Characteristics of potential bacterial strain

Morphological, physiological and biochemical characterization of the potential strain VITP21 was carried out as per the guidelines given in Bergey's manual of determinative bacteriology. The selected strain VITP21 was found to be Gram positive, circular, optically transparent filiform bacterium which formed orange-yellow colonies on the agar surface. Cells were non-sporulating, non-motile with surface texture to smooth and shiny. The strain was found to be positive only for catalase and negative to various tests like oxidase, urease, methyl red, indole production, citrate utilization, triple sugar-iron agar, mannitol motility and Voges-Proskauer tests. It was found that none of the carbon sources (maltose, lactose, xylose, sucrose, fructose and dextrose) were fermented by the organism. According to the Bergey's manual of determinative bacteriology, with consideration of the physiological and biochemical tests performed, the strain was tentatively named as *Planococcus* sp. VITP21.

The phylogenetic tree construction using the 16S rRNA sequence in MEGA 3.1 software by neighbour joining method confirmed that the organism belonged to the *Planococcus* cluster, with immediate neighborhoods as *Planococcus maritimus* strain KP8 (Gene Bank Accession number:EU594443) and *Planococcaceae bacterium* NR115 (Gene Bank Accession number:DQ520812). The sequence identity between the isolated organism and *P. maritimus* strain KP8 was found to be 95%. Hence the isolated organism was designated as *P. maritimus* VITP2l. The ribosomal RNA gene sequence of the isolated organism has been submitted to GenBank (HQ829427).

Effect of initial metal ion concentration on microbial growth and Cr (VI) removal

Growth of the selected *P. maritimus* VITP2l strain in the presence of varying Cr (VI) (100 to 500 mg/l) concentration was also monitored at regular intervals of time. There seems to be an increase in the lag phase with increase in Cr (VI) concentration (Figure 2A). It has to be noted that the maximum biomass obtained is almost the same in all the Cr (VI) concentrations studied and this is similar to the reports obtained by Sarangi and Krishnan (2008). At 600 mg/L of Cr (VI) concentration, bacterial growth decreased remarkably, with prolonged lag period (40 h), then reached to a maximum biomass concentration (3.307 OD_{600}) in 80 h of incubation (data not shown). This implies that though there was a longer lag period at higher Cr (VI) concentration, the organism could adapt suitable mechanism so as to be resistant even in the presence of higher Cr (VI) concentration. *Bacillus megaterium* strain TKW3 isolated from multiple-metal-contaminated marine sediments of Tokwawan, Hong Kong SAR was reported to be resistant to 0.34 mM $Cr_2O_7^{2-}$ (Cheng and Gu, 2005). *P. maritimus* SS-06 was reported to be highly resistant to metals like arsenic, mercury, cobalt, cadmium, lead and selenium (300 mmol L^{-1}) along with other bacterial isolates from Palk Bay sediment (Nithya et al., 2011). Though metal resistance/chromium resistance by moderately halophilic/halotolerant was reported for wide range of bacterial species, there is no extensive report on chromium reduction by *P. maritimus* strain.

The effect of initial Cr(VI) concentration on hexavalent chromium removal by *P. maritimus* VITP2l, was studied over a Cr (VI) concentration range of 100 to 500 mg/L in aerobic conditions (pH 7.0, 4% NaCl and 35°C). Substantial chromium removal occurred over the entire Cr (VI) concentration used in the study (Figure 2B). It could be seen that complete removal of chromium was achieved within 24 and 28 h of incubation for the initial Cr (VI) concentration of 100 and 200 mg/L, respectively, but when the initial Cr (VI) was increased further, there was an incomplete and delayed response with respect to Cr (VI) removal. It was observed that 97% (32 h), 94% (36 h) and 91% (40 h) was maximally removed for 300, 400 and 500 mg/L, respectively. However it should be noted that more than 90% is removed even with an initial Cr (VI) concentration of 500 mg/L. Decrease in Cr (VI) as function of time could be either due to bioaccumulation or reduction of the metal ion. Reduction of Cr (VI) to Cr (III) could be confirmed by reoxidising the culture supernatant using potassium permanganate. Comparative analysis of Cr (VI) concentration, before and after oxidation is given in Table 1. It is clear that there is an increase in Cr (VI) upon oxidation, which implies that the decrease in Cr (VI) concentration is due to changes in the oxidation state of Cr (VI) to Cr (III). Partial removal of Cr (VI) (of initial concentration of 200 mg/L or more) by organisms such as *Ochrobactrum intermedium* SDCr-5 (Sultan and Hasnain, 2007), *Providencia* sp. (Thacker et al., 2006), *Lysinibacillus fusiformis* ZC1 (He et al., 2011) has been reported. Even bacterial sp. isolated from chromium contaminated sites had shown to exhibit less than 90% removal, even after incubation for 48 h or above in the

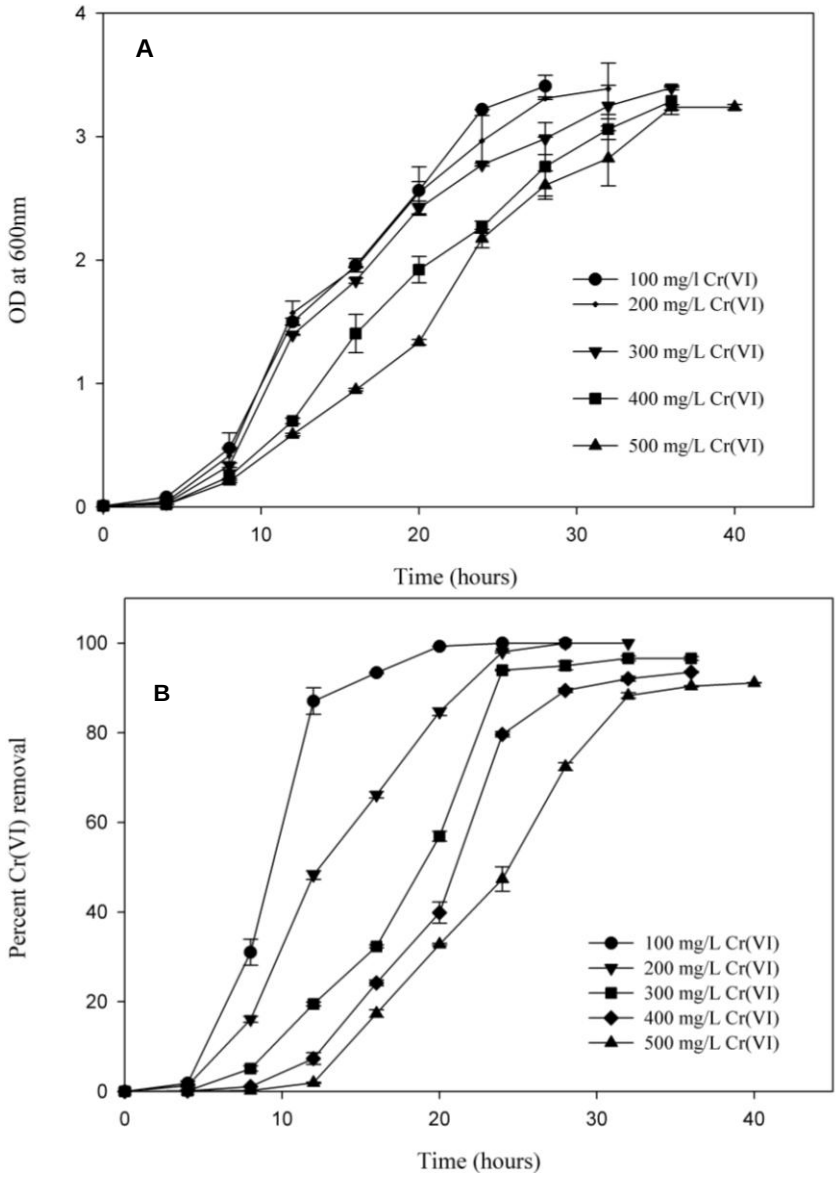

Figure 2. Effect of initial Cr (VI) concentration (100 to 500 mg/L) on (A) growth of *Planococcus maritimus* VITP21, (B) Cr(VI) removal (4% NaCl; pH 7; temperature, 35°C; agitation rate, 140 rpm).

presence of lower Cr (VI) concentration (Zhang and Li, 2011; Amoozegar et al., 2007; Zakaria et al., 2007; Desai et al., 2008b; Kathiravan et al., 2010). Complete removal/ reduction of initial chromium concentration of 200 mg/L or more, was reported using *Pannonibacter phragmitetus* LSSE-09 (Xu et al., 2011) and *Bacillus* sp., MTCC 5514 (Gnanamani et al., 2010).

Effect of salt concentration on chromium removal

As the organism is halotolerant, the ability to remove Cr (VI) in the presence of increasing concentration of NaCl (2 to 10% (w/v)) was studied. Figures 3A and B depicts the chromium removal and biomass growth of the isolate after 24 h of incubation. It could be generally observed that the effect of NaCl is dependent on the initial Cr (VI) concentration. Present investigation reveals that for 100 and 200 mg/L of initial Cr (VI) concentration, greater than 90% of removal was observed even in the presence of 2 to 8% NaCl. However a substantial decrease in removal efficiency is observed in the presence of 10% NaCl. Such dependence of Cr (VI) removal on NaCl concentration was more prominent at higher initial concentration of Cr (VI). When the initial Cr (VI) concentration was 500 mg/L, substantial removal was observed only in the presence of

Table 1. Decrease in Cr (VI) concentration as a function of time.

Time (h)	Concentration of Cr (VI) before oxidation	Concentration of Cr (VI) after oxidation
0	200±0	200±0
8	160.5±7.6	189.9 ±5.2
12	105.7±18.8	198.8 ±0.8
16	86.7±27.4	191.6 ±8.3
20	31.4±9	192.0±11.4
24	4.7±0.6	197.0±4.2
28	0	197.0±4.2

Increase in Cr (VI) concentration after oxidizing the culture supernatant grown in the presence of 200 mg/L of Cr (VI) with potassium permanganate, indicate that Cr (VI) is reduced to its lower oxidation state.

4% NaCl (50%) and 6% NaCl (48%) and decreased to less than 20% in the presence of 10 % NaCl (in 24 h of incubation). Chromium reduction in presence of NaCl by halophilic as well by non halophilic organisms has been reported (Ibrahim et al., 2011, Amoozegar et al., 2007, Cheung and Gu, 2005, Shapovalova et al., 2009 and Wani et al., 2007). Some of the reports suggest that the Cr (VI) removal efficiency of the organism decreases with increase in NaCl concentration (Okeke, 2008; Cetin et al., 2008). Thus the present investigation corroborate with these reports on the interdependency of Cr (VI) removal and NaCl concentration.

Effect of temperature and pH on chromium removal

In order to investigate the effect of temperature on organism's growth and chromium removal, the organism was grown at different temperatures (30, 35, 40 and 45°C) in the presence of 200 mg/L Cr (VI) (Table 2) and in the presence of 4% NaCl. *P. maritimus* VITP21 showed growth as well as Cr (VI) reduction in the temperature range investigated, with the maximum chromium removal of 98% and growth of 3.18 OD at 35°C. Beyond this temperature, there is decrease in organism growth as well as chromium removal, indicating the inter-relationship between these two parameters. Similar one-to-one correlation between growth and Cr(VI) reduction was reported by the strains *Nesterenkonia* sp. stain MF2 (Amoozegar et al., 2007) and *Exiguobacterium* sp.GS1 (Okeke, 2008).

The effect of initial pH on organism growth and chromium removal was investigated at an initial Cr (VI) concentration of 200 mg/l (Table 2) in the presence of 4% NaCl. The percentage removal of chromium was greater than 80% in the pH range of 6.5 to 9.0, with a maximum at pH 7.0 (97%). Substantial growth and removal was not observed at pH ≤ 6.0. However, the growth and Cr(VI) removal was significant at alkaline conditions. Even at pH 10.0, greater than 45% of 200 mg/L of Cr (VI) was removed. Similar results indicating pH dependent bacterial growth and Cr (VI) reduction have been reported (Thacker et al., 2006; Philip et al., 1998). Recently highest reduction rate in alkaline condition (8.0 to 10.0 pH) was reported using *Pannonibacter phragmitetus* LSSE-09, but in the absence of NaCl (Xu et al., 2011).

Effect of other metal ions on chromium removal

The efficiency of Cr (VI) reduction in the presence of different heavy metal ions (50 mg/L) was also investigated, as the effluents from the industrial sites could be contaminated with various heavy metal ions. Figure 4 depicts a differential effect on Cr (VI) removal in the presence of heavy metal ions in the presence of 4% NaCl. Greater than 85% reduction of Cr(VI) and microbial growth was observed in the presence of Ni^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , $Pb^{2+}$and Mn^{2+} whereas comparatively lesser chromium removal as well as microbial growth was observed in the presence of Cd^{2+} (57%). Sultan and Hasnain (2007) have reported that the efficiency of Cr (VI) reduction by *O. intermedium* SDCr-5 is decreased in the presence of Pb^{2+} (100 µg/ml), Zn^{2+} (100 µg/ml) and relatively lower concentration of Cd^{2+} (20 µg/ml). *Bacillus sphaericus* (Pal and Paul, 2004) isolated from serpentine soil have shown inhibition of chromium reduction by metal ions like Ni^{2+}, Co^{2+} , Cd^{2+} and Pb^{2+}. These and other reports (Zakaria et al., 2007; Masood and Malik, 2011) indicated that Cd^{2+} interferes in Cr(VI) removal. Though in a few cases Cu^{2+} has been shown to have an inhibitory effect on Cr (VI), Camargo et al., (2003) have reported that Cu^{2+} stimulates the reduction of Cr (VI) by *Bacillus* sp.ES 29. Thus, the present study reveals that *P. maritimus* VITP2l can be efficiently used for Cr (VI) removal even in the presence of these heavy metal ions and the order of efficiency is $Ni^{2+} \approx Cu^{2+} \approx Zn^{2+} \approx Co^{2+} \approx$

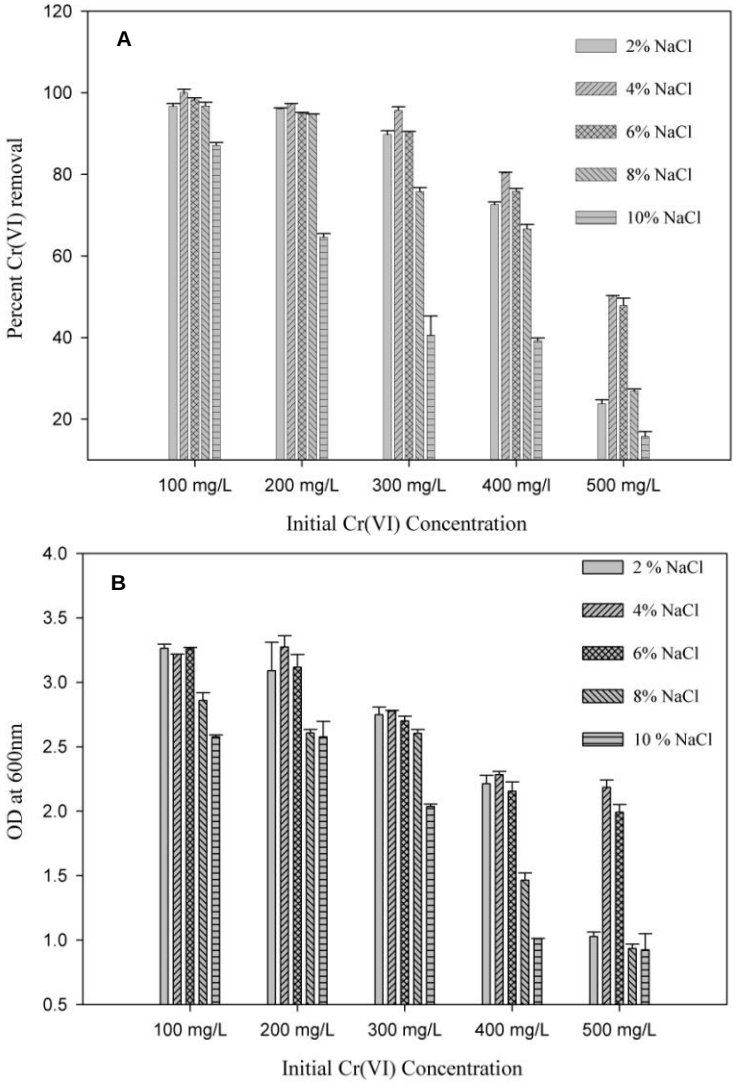

Figure 3. Effect of NaCl concentration (2 to 10% w/v)) on (A) Cr (VI) removal and (B) microbial growth in the presence of different initial Cr (VI) concentration(100 to 500 mg/L) by *Planococcus maritimus* VITP21 (pH 7, temperature, 35°C; agitation rate, 140 rpm; incubation time, 24 h).

$Mn^{2+} > Pb^{2+} > Cd^{2+}$.

Chromium reduction by resting cells and permeabilized cells

In order to get further insight into the mechanism of Cr (VI) reduction, both resting cells and permeabilized cells were used for the study for two different initial Cr (VI) concentrations (25 and 50 mg/l). Table 3 indicates that the resting cells was able to reduce only 58% of Cr (VI) in 30 min and increased to 90% after incubation for 2 h. However, when the cells were treated with either TritonX100 or toluene the percentage of Cr (VI) reduction increased significantly, indicating that 100% Cr (VI) reduction could be achieved in the presence of these permeabilizing agents. Permeabilization induced enhancement in Cr (VI) reduction has been reported using *Ochrabactrum intermedium* SDCr-5 sp. (Sultan and Hasnain, 2007) and *Providencia* sp. (Thacker et al., 2006).

Chromium reduction by sonicated fractions

Different cell fractions were investigated to ensure the localization of chromium reduction in the isolated halotolerant bacteria. The cells were sonicated (after removing the extracellular medium) and centrifuged. It has to be pointed out that the extracellular medium did

Table 2. Effect of temperature and initial pH on Cr(VI) removal and growth.

Parameter	Percent Cr (VI) removal	OD at 600 nm
Temperature		
30°C	82.1±4.7	2.1±0.01
35°C	98.2±0.38	3.2±0.04
40°C	57.6±1.5	1.4±0.14
45°C	15.9±1.06	0.6±0.001
pH		
6	4.3±0.8	0.2 ±0.0
6.5	96.5±0.7	3.1±0.01
7	98.01±0.6	3.2±0.06
8	86.7±0.9	3.1±0.09
9	81.9±1.4	2.7±0.24
10	45.5±4.1	1.1±0.16

The experiments were performed with an initial Cr (VI) concentration of 200 mg/Land in the presence of 4% NaCl.

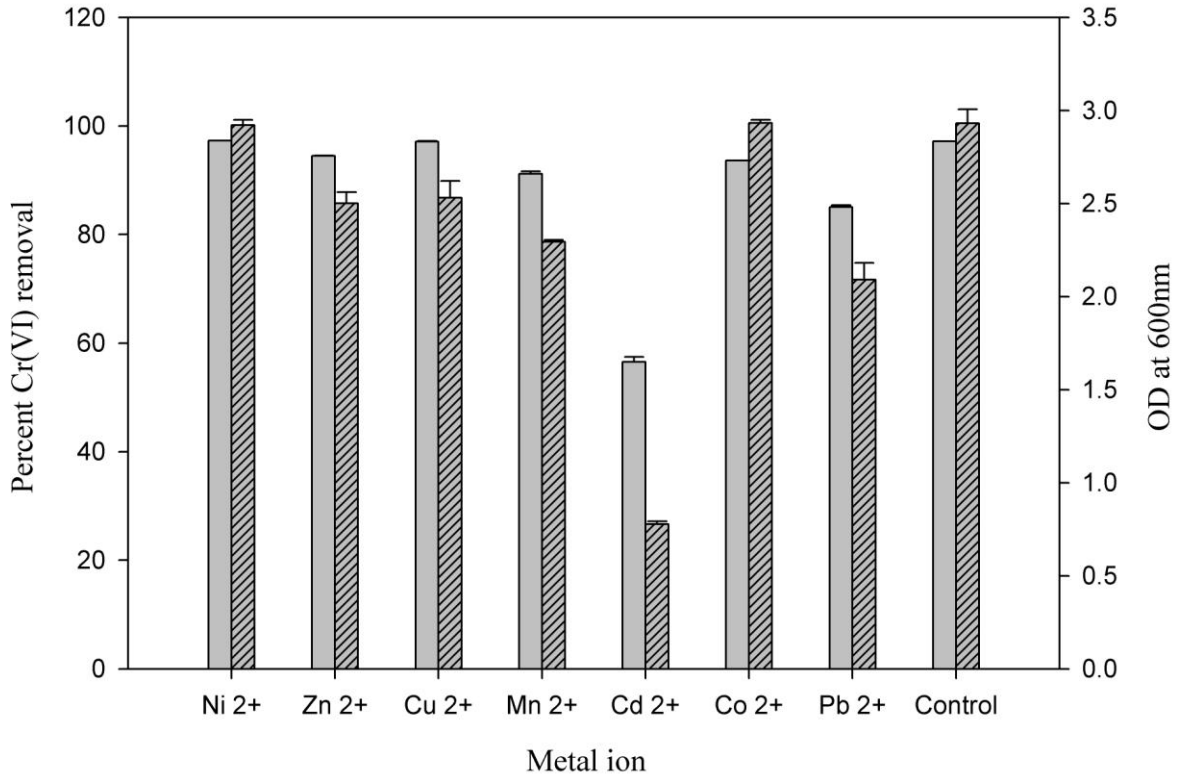

Figure 4. Effect of different metal ions (50 mg/L) on Cr (VI) removal by *Planococcus maritimus* VITP21. Initial Cr(VI) concentration was 200 mg/L (4% NaCl, pH 7; temperature, 35°C; agitation rate, 140 rpm; incubation time, 24 h).

not contribute to Cr (VI) reduction (data not shown). The sonicated fractions were then incubated with 50 mg/L Cr (VI). Table 4 indicates that among the different fractions used, the cytosolic fraction was capable of reducing Cr (VI) to the extent of 85% in 30 min and 97% in 2 h. However, no significant reduction of Cr (VI) was observed with the membrane suspension. To confirm this, the cytosolic fraction was mixed with the membrane

Table 3. Percent Cr (VI) reduced by resting and permeabilized cells.

Cell type (mg/l)	30 min	2 h
Resting cells		
25	58.2	90.6
50	25.6	80.8
Permeabilized cells (Triton X100 treated)		
25	68.4	100
50	40.5	91.6
Permeabilized cells (Toluene treated)		
25	64.4	100
50	34.5	94..0

Table 4. Percent Cr(VI) reduced by sonicated fractions (Cr(VI) 50 mg/L)

Sonicated fraction	30 min	2 h
Soluble cytosolic fractions	85.3	97.2
Soluble cytosolic fractions and insoluble membrane fractions	91.6	97.5
Insoluble membrane fractions	1.5	36.6

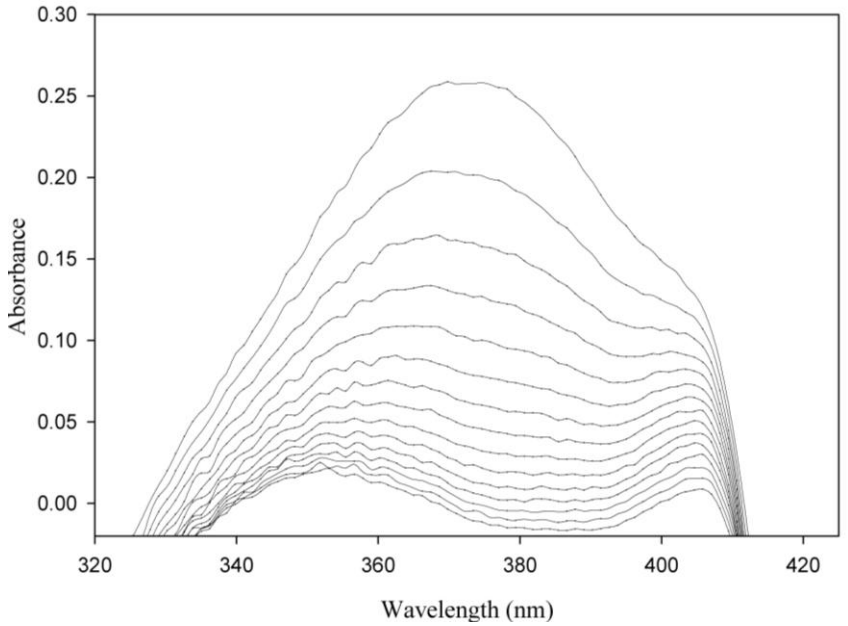

Figure 5. UV spectrum of the cytosolic fraction as a function of time. The decrease in absorbance and shift in λ_{max} is indicative of change in oxidation state of Cr (VI).

suspension. This formulation revealed 97% reduction in 2 h, which is equivalent to the contribution of the cytosolic fraction indicating that only the cytosolic fraction contributes to Cr (VI) reduction. Further confirmation in this regard, was achieved by monitoring the changes in the absorbance of the Cr (VI) reduction spectrophotometrically. Figure 5 indicates that the absorbance of the Cr (VI) treated with the cytosolic fraction decreases as a function of time, further confirming that the reduction of Cr (VI) is effected by the

cytosolic fraction. Such reports on the localization of Cr (VI) reductase activity in the soluble component of the cell had been demonstrated in *Brucella* sp. (Thacker et al., 2007), *B. sphaericus* AND 303 (Pal et al., 2005), *Bacillus* sp. (Desai et al., 2008a), *Providencia* sp. (Thacker et al., 2006), *Arthrobacter* sp. and *Bacillus* sp. (Megharaj et al., 2003). Membrane associated Cr (VI) reduction was also reported in *Pseudomonas fluorescens* strain LB300 (Bopp and Ehrlich, 1988), *Enterobacter cloacae* HO1 (Wang et al., 1990) *Thermus scotoductus* SA-01 (Opperman et al., 2008). However in the present study, cytosolic fractions of halotolerant bacteria, *P. maritimus* VITP2l grown in LB medium (amended with 4% NaCl) and in the absence of hexavalent chromium reduced Cr (VI), was capable of reducing Cr (VI). This vividly indicates the enzymes involved in Cr (VI) is not induced by the presence of Cr (VI) but is naturally expressed in the system.

In conclusion, the bacterial isolate *P. maritimus* VITP2l, reported in this study shows remarkable capacity to reduce Cr (VI) under saline environment over a broad pH range and in the presence of different heavy metal ions. The reduction is effected by the cytosolic components. The study also reveals that the isolated strain is capable of tolerating higher concentration of Cr (VI). Thus the results of this study provide the first detailed report of the potential application of *P. maritimus* VITP2l in chromium bioremediation, even under saline conditions and in the presence of other heavy metal ions.

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