

Research Paper

Hexavalent chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden

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Abstract

Microbiological analysis of overburden samples collected from chromite mining areas of Orissa, India revealed that they are rich in microbial density as well as diversity and dominated by Gram-negative (58%) bacteria. The phenotypically distinguishable bacterial isolates (130) showed wide degree of tolerance to chromium (2-8 mM) when tested in peptone yeast extract glucose agar medium. Isolates (92) tolerating 2 mM chromium exhibited different degrees of Cr⁺⁶ reducing activity in chemically defined Vogel Bonner (VB) broth and complex KSC medium. Three potent isolates, two belonging to *Arthrobacter* spp. and one to *Pseudomonas* sp. were able to reduce more than 50 and 80% of 2 mM chromium in defined and complex media respectively. Along with Cr⁺⁶ (MIC 8.6-17.8 mM), the isolates showed tolerance to Ni⁺², Fe⁺³, Cu⁺² and Co⁺² but were extremely sensitive to Hg⁺² followed by Cd⁺², Mn⁺² and Zn⁺². In addition, they were resistant to antibiotics like penicillin, methicillin, ampicillin, neomycin and polymyxin B. During growth under shake-flask conditions, *Arthrobacter* SUK 1201 and SUK 1205 showed 100% reduction of 2 mM Cr⁺⁶ in KSC medium with simultaneous formation of insoluble precipitates of chromium salts. Both the isolates were also equally capable of completely reducing the Cr⁺⁶ present in mine seepage when grown in mine seepage supplemented with VB concentrate.

Key words: chromite overburden, hexavalent chromium, chromate reduction, *Arthrobacter*, *Pseudomonas*.

Introduction

Chromium, the transition metal with its most stable hexavalent [Cr⁺⁶] and trivalent [Cr⁺³] forms are widely distributed in the environment as a result of diverse anthropogenic activities (Megharaj *et al.*, 2003; Mishra *et al.*, 2010) as well as geogenous occurrence (Pal *et al.*, 2005). Mining operations, both open cast and underground, in chromite ore deposits often generate huge overburdens in and around the mining areas. These overburden materials are solid waste piles containing rejected minerals, waste rocks, very poor or low grade chromite ores and soils. Natural leaching and weathering of these waste overburden materials often lead to the generation and percolation of hexavalent chromium, which is of serious concern for environmental contamination and pollution (Dhal *et al.*, 2010).

In India, the state of Orissa represents about 97% of the countries chromite deposits, which are localized mainly in Sukinda Valley and Baula-Nuasahi belt of Jajpur and Keonjhar districts respectively. Over the years, mining activities both by the government and private agencies have led to the deposition of about 7.6 tonnes of overburden (Tiwary *et al.*, 2005). This has not only disrupted the ecological balance of the area but has also become the main source of hexavalent chromium contamination (Godgul and Sahu, 1995; Samantaroy *et al.*, 1999). Health hazards of mine workers as well as inhabitants of the surrounding areas have attracted the attention of researchers and environment protection agencies and emphasized detoxification of Cr⁺⁶. Hexavalent chromium mediated toxicity in most living forms is primarily due to its easy uptake across the membrane, development of oxidative stress, DNA dam-

age, carcinogenicity, mutagenicity and altered gene expression (Bagchi *et al.*, 2002).

Since 1970s, microbial detoxification of Cr^{+6} has been identified as the eco-friendly and superior alternative over those of conventional physico-chemical strategies (Komori *et al.*, 1990; Camargo *et al.*, 2003), which often generate huge amount of secondary sludge (Thaker and Madamwar, 2005). A large number of organisms capable of reducing Cr^{+6} under aerobic (Ganguli and Tripathi, 2002; Pal and Paul, 2004; Thaker *et al.*, 2006; Wani *et al.*, 2007) as well as anaerobic (Opperman and Heerden, 2007) conditions have been reported from both anthropogenic (Basu *et al.*, 1997; Viti *et al.*, 2003; Thaker and Madamwar, 2005) as well as geogenous (Pal and Paul, 2004) sources and have the potentials for the development of *in situ* or *ex situ* Cr^{+6} bioremediation strategies.

The present study attempts to isolate chromate resistant and reducing bacteria from chromite mine overburden and evaluate their chromate reducing potentials. Heavy metal and antibiotic resistance along with reduction of Cr^{+6} in mine seepage by selected bacterial isolates have also been reported.

Materials and Methods

Collection of samples

Twelve mine overburden samples collected from open cast as well as under ground chromite mines in and around Sukinda valley and Baula-Nuasahi areas of Orissa, India were used during the present study. The samples were collected from subsurface zones in sterile containers and stored at 4 °C until used for microbiological analysis.

Microbial analysis

Population density of aerobic, heterotrophic bacteria in overburden samples was estimated by dilution and plating on peptone yeast extract glucose (PYEG) agar medium (Wang and Xiao, 1995). The medium contained (grams per liter): peptone, 10.0; yeast extract, 5.0 and glucose, 3.0 (pH 7.0). The plates were incubated at 28-32 °C for 2-4 days and the population density was determined by the number of colony forming units/g of the overburden sample. Phenotypically distinguishable bacterial colonies were further purified and maintained on slopes of PYEG agar at 4 °C.

Hydrolysis of fluorescein diacetate (FDA) (Schnurer and Rosswall, 1982) was used to determine the microbial activity of overburden samples. One gram of freshly collected overburden sample, 24 mL phosphate buffer (0.2 N, pH 7.0) and 1 mL of FDA (final concentration 10 µg/mL) was agitated on a rotary shaker (120 rpm) for 3 h at 35 °C. The reaction was stopped by adding equal amount of analytical grade acetone, filtered through WHATMAN No. 1 filter paper and optical density measured at 490 nm. The amount of fluorescein released was determined from the calibration curve prepared in the same way. FDA hydrolyzing activity was expressed as µg fluorescein/h/g of oven dried sample.

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Screening for chromate tolerance and reduction

Bacterial tolerance to chromate was tested by agar dilution method (Cervantes and Ohtake, 1988). PYEG agar supplemented with different concentration of (2-8 mM) hexavalent chromium (as chromate) was inoculated with overnight grown cultures, incubated at 35 °C for 3-4 days and tolerance to Cr^{+6} was determined by visual growth.

Reduction of hexavalent chromium by bacterial isolates was determined during growth in Vogel Bonner (VB) broth and modified KSC medium. Vogel Bonner broth was made up of 2.0% sterile stock solution of VB concentrate. The VB concentrate contained (grams per liter): K_2HPO_4 , 500.0; $\text{Na}(\text{NH}_4)\text{HPO}_4 \cdot 4\text{H}_2\text{O}$, 175.0; citric acid, 100.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10.0 and 2.0% of 25% D-glucose 20.0 mL (pH 7.0) (38). Modified KSC medium contained (grams per liter): NH_4Cl , 0.03; K_2HPO_4 , 0.03; KH_2PO_4 , 0.05; NaCl , 0.01; sodium acetate, 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; CaCO_3 , 0.005; $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$, 0.005; and glucose 10.0, (pH 7.2) (37). Media (20 mL/100 mL flask) supplemented with 2 mM Cr^{+6} was inoculated with overnight grown culture and incubated at 35 °C under continuous shaking (120 rpm). Reduction of chromium was estimated by measuring the decrease in hexavalent chromium content of culture filtrate following 1, 5-diphenyl carbazide method (Park *et al.*, 2000).

Bacterial tolerance to heavy metals

Bacterial tolerance to heavy metals was evaluated following broth dilution method of Calomiris *et al.* (1984). The VB broth (5 mL/tube) supplemented with increasing concentration of heavy metals was inoculated with 0.2 mL overnight grown cultures and incubated at 35 °C for 24-48 h under continuous shaking (120 rpm). Medium without metal served as control. The lowest concentration of metal ions that inhibited the growth of organism was taken as the minimum inhibitory concentration (MIC) of that metal.

Antibiotic susceptibility

Susceptibility of selected isolates to different antibiotics was evaluated by disc-diffusion method. Antibiotic impregnated discs (6 mm, dia. HIMEDIA) were placed on freshly prepared lawns of each bacterial isolates on PYEG agar medium and incubated at 35 °C for 24 h. The diameter of inhibition zone was measured to nearest mm and isolates were identified as sensitive, resistant and intermediate following standard antibiotic sensitivity testing method (Difco Laboratories, 1984). Discs containing the following antibiotics were used: streptomycin (25 µg/disc), tetracycline (30 µg/disc), neomycin (30 µg/disc), kanamycin (30 µg/disc), chloramphenicol (30 µg/disc), doxycycline (30 µg/disc), ampicillin (10 µg/disc), polymixin B

(50 units/disc), penicillin G (10 units/disc), erythromycin (15 µg/disc), methicillin (5 µg/disc), nalidixic acid (30 µg/disc), gentamycin (10 µg/disc), rifampicin (30 µg/disc), netilin (30 µg/disc), novobiocin (30 µg/disc) and norfloxacin (30 µg/disc). The antibiotic resistance index (ARI) of an organism was expressed as the ratio of the number of resistant antibiotic to the total number of antibiotics tested.

Measurement of growth

Growth of chromate reducing bacteria in liquid media was measured following the viable count method. The cultures were serially diluted and plated on PYEG agar medium and the number of colony forming units/ml was calculated after incubation at 35 °C for 2-4 days. Growth of the organisms was also determined by measuring the optical density at 540 nm using a Systronics Photoelectric Colorimeter (Model 112).

Characterization and identification of bacterial isolates

The selected bacterial isolates were characterized morphologically and physio-biochemically following standard microbiological methods as described in Gerhardt *et al.* (1994). The characteristic features of the isolates were compared with those described in Bergey's Manual of Systematic Bacteriology Volume 1 (Holt and Kreig, 1989), Volume 2 (Holt *et al.*, 1989) and Bergey's Manual of Determinative Bacteriology 9th edition (Holt *et al.*, 1994) for determining their taxonomic identity.

Results

Microbiological characteristics

Microbial density of the chromite mine overburden samples as revealed by plate count technique was in general low but showed a wide degree of variability, ranging from 0.2-145 x 10⁴ cfu/g (Table 1). Fluorescein diacetate hydrolyzing activity of these samples as a measure of the microbial activity also showed a significant variation and maintained a strong positive correlation with total microbial count. The coefficient of correlation (r) between microbial count and FDA hydrolyzing activity of samples from Sukinda Valley and Baula-Nuasahi area were 0.98 and 0.982 respectively.

A total of 130 morphologically distinguishable aerobic heterotrophic bacteria were obtained in pure form. Majority (58%) of them were Gram-negative, motile rods forming mostly white to creamish colonies but without any diffusible pigment.

Chromium tolerance of the isolates

Relative tolerance of these heterotrophic bacterial isolates to hexavalent chromium was evaluated in PYEG agar medium supplemented with 2-8 mM Cr⁺⁶. The num-

Table 1 - Microbiological analysis of overburden samples collected from chromite mines of Orissa.

Locality	Site	Bacterial count ^a , cfu/g x 10 ⁴	Microbial activity ^b (µg fluorescein/g/h)	Number of bacterial isolates
Sukinda valley				
Sukinda	SUK1	0.28 ± 0.02	1.18 ± 0.21	11
	SUK2	145.0 ± 0.05	2.36 ± 0.26	14
	SUK3	1.79 ± 0.06	2.23 ± 0.26	11
	SUK4	130.0 ± 0.08	2.16 ± 0.21	12
South Kaliapani	SKP1	0.37 ± 0.04	1.03 ± 0.20	10
	SKP2	6.0 ± 0.06	1.71 ± 0.50	13
	SKP3	0.20 ± 0.04	1.07 ± 0.20	11
Saruabil	SAR1	8.60 ± 0.08	1.79 ± 0.20	10
	SAR2	15.50 ± 0.07	1.90 ± 0.26	12
Baula - Nuasahi area				
Baula	BAU1	0.27 ± 0.80	1.14 ± 0.20	9
Nuasahi	NUA1	1.81 ± 0.40	1.54 ± 0.30	10
Bangur	BAN1	0.24 ± 0.29	1.03 ± 0.21	7

^aBacterial count was determined following dilution and plating on peptone yeast extract glucose agar medium. Plates were incubated at 28-32 °C for 2-4 days.

^bMicrobial activity was estimated by fluorescein diacetate (FDA) hydrolytic activity following the method of Schnurer and Rosswall (1982).

Results represent mean ± standard error of triplicate sets.

ber of tolerating isolates decreased gradually with increasing concentration of Cr⁺⁶ in the medium. A total of 51 isolates representing about 40% of the total population tolerated 8 mM of Cr⁺⁶. It also revealed that the frequency of occurrence of Cr⁺⁶ tolerance among isolates of Baula - Nuasahi area was higher (54%) than those from Sukinda valley (38%) (Figure1), even though, the number of bacterial isolates obtained and screened from Baula - Nuasahi was much lower compared to those from Sukinda Valley.

Screening for chromate reduction

A total of 92 isolates (nearly 70% of the total population) tolerating 2 mM of Cr⁺⁶ was further screened for their chromate reducing potentials in synthetic VB broth and complex KSC medium. Reduction potential of these isolates was more pronounced in KSC medium than in synthetic VB broth. In all, only three bacterial isolates, SUK 1201, SUK 1205 and SUK 1207 were able to reduce more than 50 and 80% of initial 2 mM Cr⁺⁶ in VB broth and KSC medium respectively, and were selected for in depth studies (Table 2).

Heavy metal tolerance

The selected isolates showed a wide degree of tolerance to Cr⁺⁶ with MIC values ranging from 8.6 to 17.8 mM.

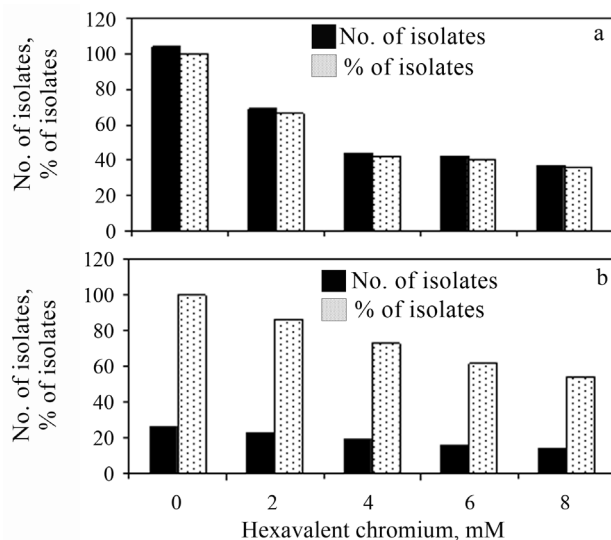


Figure 1 - Tolerance of hexavalent chromium by bacteria isolated from chromite mine overburden of Sukinda valley [a] and Baula - Nuasahi areas [b].

In addition, the isolates were also tolerant to a number of metals like Ni^{+2} (MIC, 4.9-7.8 mM), Fe^{+3} (MIC, 4.0-6.0 mM), Cu^{+2} (MIC, 2.8 - 5.1 mM) and Co^{+2} (MIC, 2.2-4.6 mM), but were extremely sensitive to Hg^{+2} followed by Cd^{+2} , Mn^{+2} and Zn^{+2} (Table 3).

Antibiotic sensitivity

Antibiotic sensitivity profile of the 3 selected potent Cr^{+6} reducing bacterial isolates (SUK 1201, SUK 1205 and SUK 1207) as evaluated by disc-diffusion assay against 17 different antibiotics revealed that they are also resistant to a number of antibiotics like penicillin G, ampicillin, novobiocin, norfloxacin, neomycin, methicillin and polymyxin B but sensitive to streptomycin and chloramphenicol (Figure 2). The antibiotic resistance index (ARI) was highest (0.71) for isolate SUK 1207, while it was 0.65 and 0.53 for isolate SUK 1201 and SUK 1205 respectively.

Table 3 - Minimum inhibitory concentration of heavy metals against selected bacterial isolates from chromite mine overburden.

Metals	Minimum inhibitory concentration, mM ^a		
	Bacterial isolate		
	SUK 1201	SUK 1205	SUK 1207
Cr^{+6}	17.8	11.8	8.6
Ni^{+2}	6.4	7.8	4.9
Fe^{+3}	6.0	4.0	4.0
Cu^{+2}	5.1	4.8	2.8
Co^{+2}	4.6	4.2	2.2
Mn^{+2}	3.2	3.1	1.2
Zn^{+2}	4.2	2.8	2.1
Cd^{+2}	2.1	2.1	3.1
Hg^{+2}	0.001	0.001	0.0015

^aMIC of metals was determined by broth dilution assay (Calomiris *et al.*, 1984) using VB broth.

All metals were used as chloride salts, while Cr^{+6} was used as chromate.

Characterization of isolates

All together some 56 characters including morphological, physiological and biochemical properties of the 3 selected potent bacterial isolates were evaluated. Isolate SUK 1207 was Gram-negative motile rod, but both SUK 1201 and SUK 1205 were Gram-positive and showed a unique rod to cocci cycle during growth. The morphological and physio-biochemical characteristics of the isolates were compared with those described in Bergey's Manual of Systematic Bacteriology and Bergey's Manual of Determinative Bacteriology. The isolates SUK 1201 and SUK 1205 were tentatively identified as members of *Arthrobacter* sp. but differed in terms of colony morphology, motility, production of cellulase, lipase and arginine dihydrolase along with utilization of some carbon sources. On the other hand,

Table 2 - Chromate reduction capacity of chromium resistant bacteria isolated from chromite mine overburden.

Locality	No. of resistant isolates ^a	Percent Cr^{+6} reduced							
		0-25		26-50		51-75		76-100	
		A	B	A	B	A	B	A	B
Sukinda valley	69	31	10	35	19	3	37	0	3
Baula-Nuasahi area	23	9	1	14	7	0	15	0	0
Total	92	40	11	49	26	3	52	0	3
% of organism	100	43.47	11.95	53.26	28.26	3.26	56.52	0	3.26

^aBacterial isolates able to tolerate 2 mM Cr^{+6} in peptone yeast extract glucose agar medium.

A - Growth and Cr^{+6} reduction in VB broth, incubation: 10 days at 35 °C under continuous shaking (120 rpm).

B - Growth and Cr^{+6} reduction in KSC medium, incubation: 10 days at 35 °C under continuous shaking (120 rpm).

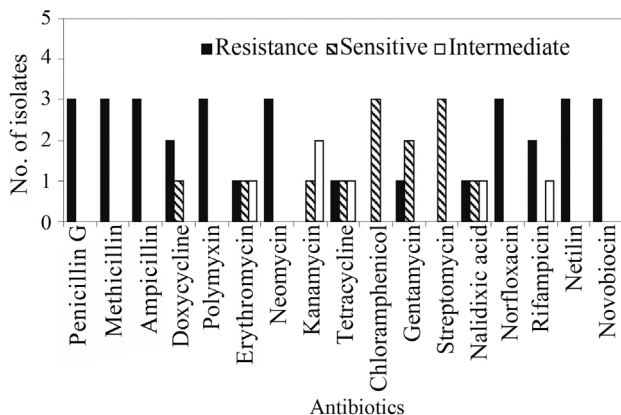


Figure 2 - Antibiotic sensitivity profile of selected bacterial isolates of chromite mine overburden.

the isolate SUK 1207 was designated as *Pseudomonas* sp. (Table 4 and 5).

Time course and chromate reduction

In chemically defined VB broth, the isolates SUK 1201, SUK 1205 and SUK 1207 reduced nearly 67, 64 and 52% of initial 2 mM Cr⁺⁶ respectively. During the course of the reduction of hexavalent chromium in culture, there was a gradual increase in cell count up to 7th day of incubation, which was 8.42 ± 0.10, 9.06 ± 0.12 and 8.8 ± 0.10 log no. of cells/mL for isolates SUK 1201, SUK 1205 and SUK 1207 respectively. This was accompanied with a gradual change in the colour of the medium which turned yellow to pale green with formation of visible green precipitates. Maximum precipitation was obtained with isolate SUK 1201

Table 4 - Morphological, physiological and biochemical characteristics of some selected bacterial isolates from chromite mine overburden.

Characters	Response		
	Bacterial isolate		
	SUK 1201	SUK 1205	SUK 1207
Morphological			
Colony	Round convex pale yellow	Round convex white	Round convex white
Gram nature	+ve	+ve	-ve
Shape	Rod - coccus cycle	Rod - coccus cycle	Rods
Arrangement	Single	Chain	Single
Endospore	-	-	-
Motility	-	+	+
Physiological			
Diffusible pigment	-	-	-
Range of pH for growth	5.0-9.0	5.0-9.0	4.0-8.0
Growth in Kings B	+	+	+
Growth in Mac Conkey agar	-	-	-
NaCl tolerance (%)	2.5	8.5	1.0
Siderophore production	+	+	+
Biochemical			
Citrate utilization	-	±	-
Lysine decarboxylase	-	-	-
Ornithine decarboxylase	-	-	-
Urease production	-	-	-
Nitrate reduction	-	-	-
H ₂ S production	-	-	-
Cellulase production	+	-	+
Lipase production	-	+	-
Gelatinase production	-	-	-
Methyl red test	-	-	-
Catalase production	+	+	+
Voges Proskaur test	-	-	-
Amylase production	-	-	+
Indole production	-	-	-
Oxidase production	+	+	+
Cytochrome oxidase	-	-	+
Arginine dihydrolase	-	+	+

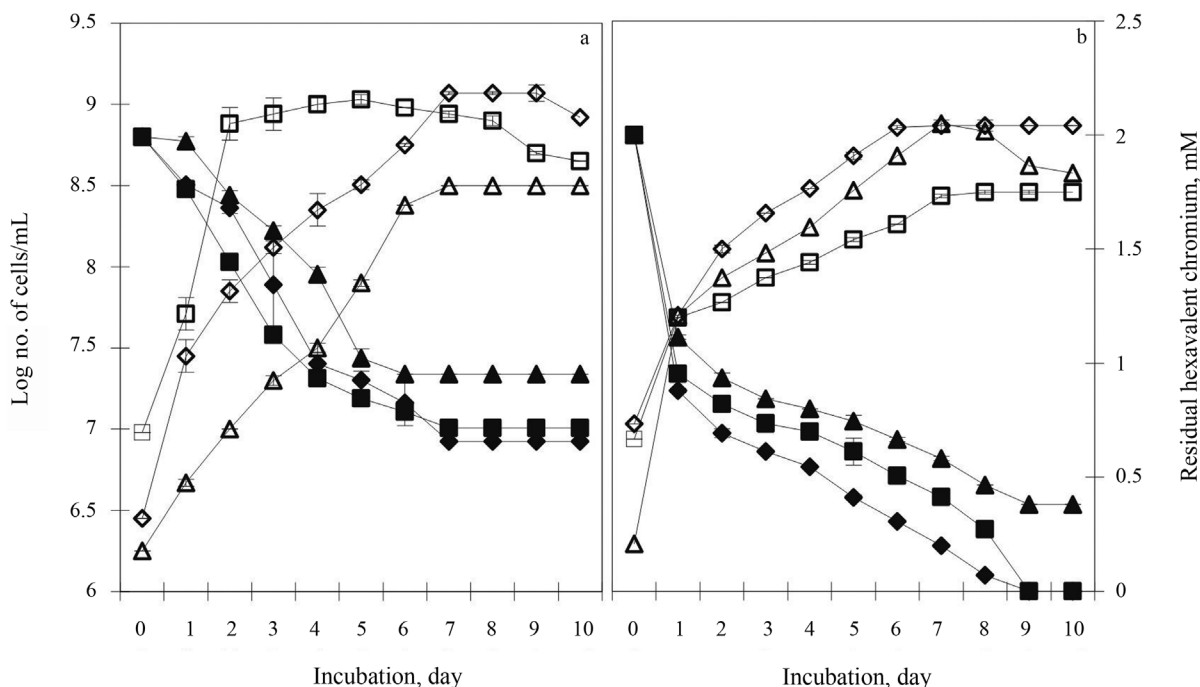


Figure 3 - Time course of growth and hexavalent chromium reduction by selected bacterial isolates in VB broth [a] and KSC medium [b] (Growth: -□- SUK 1201, -△- SUK 1205, -◇- SUK 1207; Cr⁺⁶ reduction: -■- SUK 1201, -▲- SUK 1205, -◆- SUK 1207).

where the Cr⁺⁶ level in the medium declined to 0.66 mM indicating 67% reduction (Figure 3a). In complex KSC medium containing 1% glucose, the chromate reduction was much more pronounced. Complete reduction of 2 mM Cr⁺⁶ in the medium was achieved with bacterial isolates SUK 1201 and SUK 1205, while SUK 1207 showed only 81% reduction. As in VB broth, there was also a gradual increase in cell number up to the 7th day of incubation (Figure 3b).

Growth associated reduction in mine seepage

Considering the efficient reduction potential in KSC medium and VB broth, bacterial isolates SUK 1201 and SUK 1205, were further evaluated for reduction of Cr⁺⁶ (60 μM) contained in natural mine seepage under 3 different experimental conditions. These include: i) direct use of mine seepage, ii) mine seepage supplemented with 0.1% glucose and iii) mine seepage supplemented with VB broth. Results show that isolates SUK 1201 and SUK 1205 could reduce 34 and 41% of 60 μM of Cr⁺⁶ in mine seepage water respectively. However, their reducing ability was increased to 58 and 68% when mine seepage was supplemented with 0.1% glucose (Figure 4 a, b). Interestingly, hexavalent chromium was completely reduced by both the isolates when mine seepage was supplemented with 2% VB concentrate.

Discussion

The present microbiological analysis clearly demonstrates that the chromite mine overburden samples which

are usually rich in mineral contents but poor in organic substances are not inferior in microbial density as well as diversity compared to other chromium contaminated soils (Table 1). Existence of such diverse type of microbiota in chromite mine overburden has previously been demonstrated (Acharya *et al.*, 1998). It was also evident that the microbial activity of the samples was positively correlated with total microbial count and the calculated *r* value was highly significant at *p* = 0.001. The microbial population was dominated by Gram-negative (58%) organisms contrary to the high frequency of Gram-positive bacteria in metal polluted areas (Viti *et al.*, 2003; Mishra *et al.*, 2010).

The bacterial isolates derived from the chromite mining environment showed a high level of tolerance to chromium, about 35 and 54% of isolates from Sukinda Valley and Baula - Nuasahi area respectively tolerated upto 8 mM chromium (Figure 1). Such an adaptation and tolerance to chromium was evident in indigenous microbiota of chromite mine soils (Dhal *et al.*, 2010) as well as in anthropogenic (Basu *et al.*, 1997; Viti *et al.*, 2003) and geogenous (Pal and Paul, 2004) environments. In addition, isolates like *Aeromonas* and *Pantoea* sp. (Alam and Ahmad, 2011) were found to tolerate upto 4.7 mM of Cr⁺⁶, whereas *Thermus scotocductus* isolated from mine ground water (Opperman and Heerden, 2007) tolerated 0.3 mM Cr⁺⁶.

The microbial cells in order to combat the metal stressed conditions have developed several resistance mechanisms such as exclusion of metals by permeability barrier, active transport of metal, intracellular sequestration of metal by binding of proteins, extracellular sequestration,

Table 5 - Utilization and fermentation of different carbon sources by some selected bacterial isolates from chromite mine overburden.

Characters	Response		
	Bacterial isolate		
	SUK 1201	SUK 1205	SKPD 1207
Carbon source			
Utilization of			
Glucose	+	+	+
Sucrose	+	+	+
Fructose	+	+	-
Maltose	+	+	+
Lactose	+	+	-
Galactose	+	+	-
Trehalose	+	+	-
Rhamnose	+	+	-
Raffinose	+	-	-
Mannose	-	+	-
Arabinose	-	+	-
Cellobiose	+	-	-
Xylose	+	+	-
Salicin	+	-	-
Adonitol	-	-	-
Mannitol	+	+	-
Glycerol	+	+	+
Fermentation of			
Glucose	+	+	+
Sucrose	+	+	-
Fructose	+	+	-
Lactose	-	-	-
Galactose	+	+	-
Rhamnose	-	-	-
Arabinose	±	+	-
Adonitol	-	-	+
Mannitol	-	-	-
Sorbitol	-	-	-
Generic identity <i>Arthrobacter</i> sp. <i>Arthrobacter</i> sp. <i>Pseudomonas</i> sp.			

enzymatic detoxification of metal ions to less toxic forms and reduction of metal sensitivity of cellular targets (Nies, 1999). Chromium tolerant microorganisms have been reported to develop strategies to detoxify the elevated levels of toxic chromate and dichromate. This is accomplished by transforming Cr^{+6} to less toxic Cr^{+3} via possible reduction systems or through intracellular or extracellular sequestration of metal ions making them unavailable to the ecosystem (Williams and Silver, 1984; Pattanapitpaisal *et al.*, 2002; Camargo *et al.*, 2003).

It was evident that the chromate reduction efficiency of bacterial isolates derived from two distantly separated

localities was significantly different and not correlated with their limit of chromium tolerance (Table 2). This supports the earlier observations that chromate reduction ability of bacterial isolates is not correlated with chromium resistance (Pattanapitpaisal *et al.*, 2001).

Like majority of the metal resistant bacteria, chromium resistant and reducing bacteria isolated from chromite overburden also exhibited the feature of multi-metal tolerance (Table 3) as well as resistance to a number of antibiotics (Figure 2). Such tolerance of the microbial communities to Ni^{+2} , Fe^{+3} , Cu^{+2} and Co^{+2} might be due to the selection of the pre-existing systems for metal tolerance or induction of the expression of selective gene or gene clusters. Similarly, co-existence of metal and antibiotic resistance in Cr^{+6} reducing bacteria has been reported by several others (Ganguli and Tripathi, 2002; Viti *et al.*, 2003; Pal *et al.*, 2005; Jain *et al.*, 2009). It may be presumed that antibiotic resistance in these bacteria might have been induced as a result of selective pressure created by the metal pollution and horizontal transfer of associated antibiotic resistance genes.

The selected bacterial isolates were characterized phenotypically and two of them, SUK 1201 and SUK 1205 were found to belong to *Arthrobacter* sp., while SUK 1207 was placed under *Pseudomonas* sp. (Tables 4 and 5). Actinomycetes like *Arthrobacter* capable of reducing chromate is not uncommon in chromium polluted waste water treatment plants (Molokwane *et al.*, 2008). Likewise, occurrence of pseudomonads was evident in metal polluted environments (Luli *et al.*, 1983; Camargo *et al.*, 2003) and chromite mine effluents (Lebedeva and Lyalikova, 1979; Dey and Paul, 2010).

Quantitative evaluation of Cr^{+6} reduction by the selected isolates during growth under shake-flask condition using chemically defined (Figure 3a) and complex organic media (Figure 3b) clearly established the superiority of isolates SUK 1201 and SUK 1205 over that of SUK 1207, which was not inferior. Interestingly, there was clear demonstration of formation of white to green precipitates of insoluble chromium species simultaneously with the transformation of Cr^{+6} in the medium.

Moreover, the most efficient *Arthrobacter* strains, SUK 1201 and 1205 completely reduced the hexavalent chromium of the mine seepage when supplemented with V. B. concentrate (Figure 4 a, b). Similar approaches made by Molokwane *et al.* (2008) with a consortium of indigenous microbiota isolated from Brits, South Africa showed 94% removal of Cr^{+6} in 24 h of incubation. The present observations, therefore, clearly demonstrate that the *Arthrobacter* strains indigenous to chromite overburden could find practical applications in the bioremediation of chromium pollutants in mining environments through the development of suitable microbial technology.

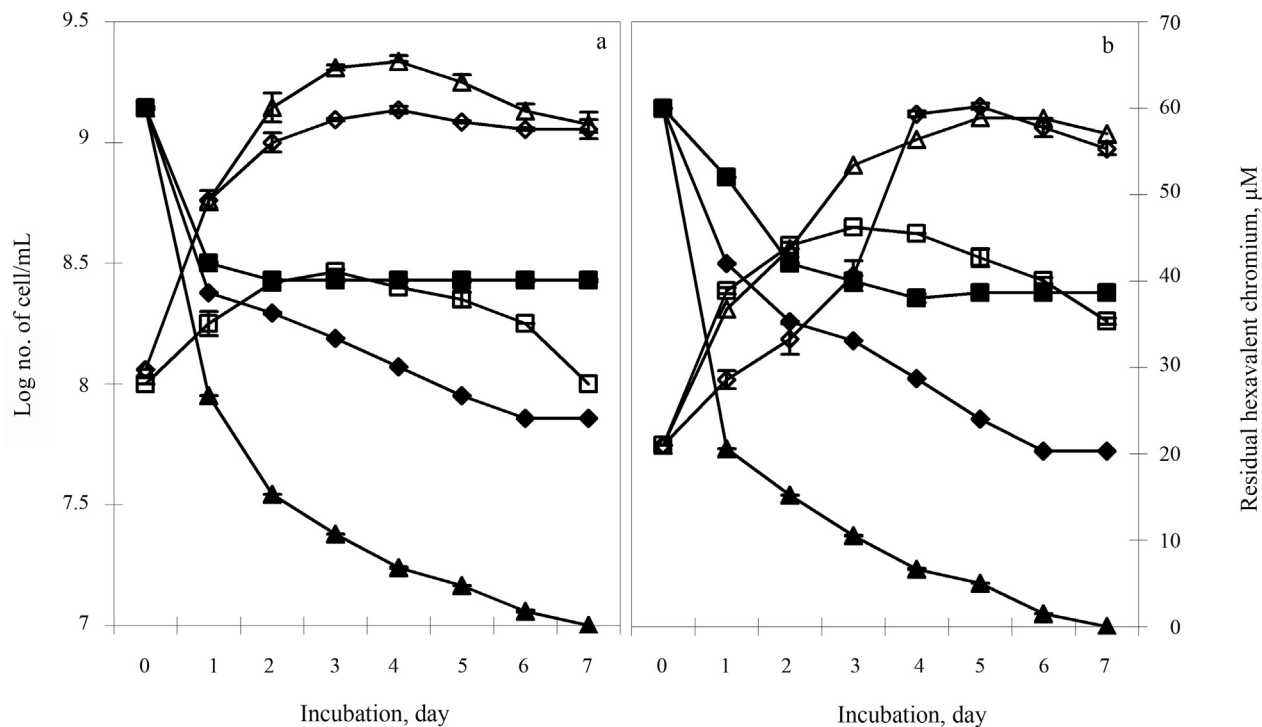


Figure 4 - Growth and reduction of hexavalent chromium in mine seepage water by bacterial isolates SUK 1201 [a] and SUK 1205 [b] under different experimental conditions (Growth: □- mine seepage, △- mine seepage + glucose, ◇- mine seepage + VB broth; Cr⁺⁶ reduction: ■- mine seepage, ▲- mine seepage + glucose, ◆- mine seepage + VB broth).

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