Journal of Hazardous Materials 213–214 (2012) 200–206

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Optimization of cultural conditions for growth associated chromate reduction by *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden

Satarupa Dey[1], A. K. Paul*

Microbiology Laboratory, Department of Botany, University of Calcutta, Kolkata 700019, India

ARTICLE INFO

Article history:
Received 24 October 2011
Received in revised form 24 January 2012
Accepted 25 January 2012
Available online 1 February 2012

Keywords:
Arthrobacter
Chromite mine overburden
Hexavalent chromium
Chromate reduction
Chromium bioremediation

ABSTRACT

Arthrobacter sp. SUK 1201, a chromium resistant and reducing bacterium having 99% sequence homology of 16S rDNA with *Arthrobacter* sp. GZK-1 was isolated from chromite mine overburden dumps of Orissa, India. The objective of the present study was to optimize the cultural conditions for chromate reduction by *Arthrobacter* sp. SUK 1201. The strain showed 67% reduction of 2 mM chromate in 7 days and was associated with the formation of green insoluble precipitate, which showed characteristic peak of chromium in to energy dispersive X-ray analysis. However, Fourier transform infrared spectra have failed to detect any complexation of end products of Cr(VI) reduction with the cell mass. Reduction of chromate increased with increased cell density and was maximum at 10^{10} cells/ml, but the reduction potential decreased with increase in Cr(VI) concentration. Chromate reducing efficiency was promoted when glycerol and glucose was used as electron donors. Optimum pH and temperature of Cr(VI) reduction was 7.0 and 35 °C respectively. The reduction process was inhibited by several metal ions and metabolic inhibitors but not by Cu(II) and DNP. These findings suggest that *Arthrobacter* sp. SUK 1201 has great promise for use in Cr(VI) detoxification under a wide range of environmental conditions.

1. Introduction

Microbial reduction of toxic hexavalent chromium [Cr(VI)] to comparatively less toxic trivalent form [Cr(III)] and its precipitation at near neutral pH is a cost effective green technology for the treatment of Cr(VI) contaminated wastes in contrast to the traditional physico-chemical methods [1,2]. Reduction of Cr(VI) has been achieved using a wide variety of bacterial isolates [2–4] and consortium [5,6] under both aerobic [7,8] as well as anaerobic conditions [9–11]. These bacterial isolates were derived primarily from anthropogenic sources [3,12–14], but their occurrence in naturally metal percolated environments is not uncommon [4].

Chromium resistant and reducing bacteria have been reported from chromite mining environments and have attracted increased interest for potential application in bioremediation of Cr(VI) polluted waste water [15,16]. *Arthrobacter* spp., the common Gram-positive bacteria having characteristics rod to cocci cell cycle during growth are no exception to these. They are not only capable of surviving in various metal stressed environments [2] such as tannery wastes [12], dichromate contaminated soil samples [2], waste water treatment plant [5] and other chromium contaminated sites [17–19], but are also able to reduce chromium during growth, by whole cells and by cell-free extracts. Asianti et al. [17] and Megharaj et al. [12] reported an *Arthrobacter* strain able to reduce nearly 35 and 30 μg/ml of Cr(VI) in 10 d and 46 h respectively. However, *Arthrobacter crystallopoites* ES 32 reported by Camargo et al. [2] showed much less reducing efficiency during growth. Molokwane et al. [5] reported an *Arthrobacter* sp., which in a consortium was able to reduce nearly 94.3% of 100 mg/l Cr(VI) in 24 h of incubation.

During the course of our survey for bacterial strains capable of tolerating and reducing high concentrations of Cr(VI) from metalliferous chromite resources, we have isolated a potent bacterium *Arthrobacter* sp. SUK 1201 from chromite mine overburdens of Orissa, India. The present study envisages to confirm the taxonomic identity and phylogenetic relation of the strain by 16S rDNA analysis and optimize the cultural conditions for Cr(VI) reduction under batch culture, which facilitates its in situ application for bioremediation of contaminated wastes.

2. Materials and methods

2.1. Source and maintenance of bacterial culture

The chromate reducing bacterium, *Arthrobacter* sp. SUK 1201 used in this study was isolated from metalliferous mine

* Corresponding author at: Microbiology Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, India.
Tel.: +91 033 2461 5445; fax: +91 033 2461 4849.
E-mail addresses: dey1919@gmail.com (S. Dey), amalk_paul@yahoo.co.in, akpbot@caluniv.ac.in (A. K. Paul).
[1] Microbiology Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, India.

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2012.01.078

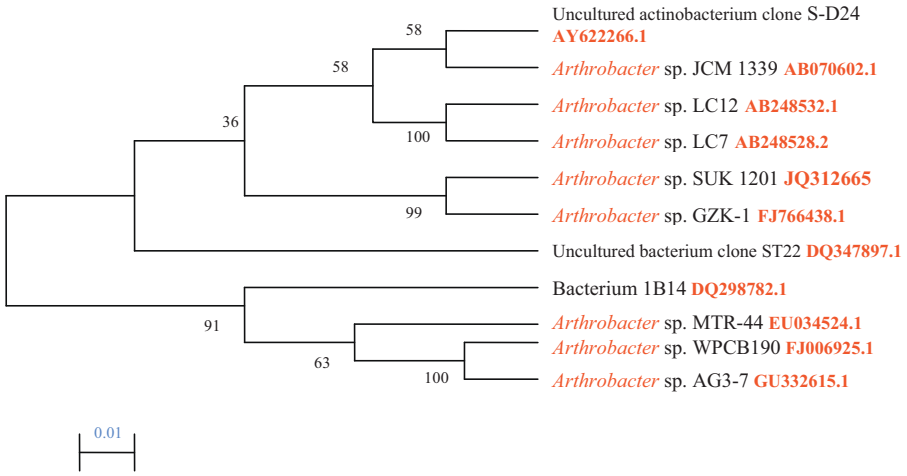

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence of SUK 1201. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site.

overburden collected from chromite mining areas of Orissa, India. The strain was routinely maintained on slopes of peptone, yeast extract and glucose (PYEG) agar medium [20] containing (g/l) peptone, 10.0; yeast extract, 5.0; glucose, 3.0 and agar agar, 20.0 (pH 7.0), supplemented with 2 mM Cr(VI) and stored at 4 °C.

2.2. Identification of the strain

The identity of the isolate SUK 1201 was confirmed based on 16S rDNA analysis. The DNA was isolated and purified by phenol/chloroform extraction and precipitated by adding 3 M potassium acetate and isopropanol.

PCR amplification was performed using the 8 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-TACGGYTACCTTGTTACGACTT-3′) as forward and reverse primers respectively. Reactions were carried out using BDT v 3.1 cycle sequencing kit. The reaction mixture consisted of 2 μl BDT v 3.1 (ABI, cat # 4337455), 1 μl sequencing buffer (ABI, cat # 4336697), 2 μl primer (@ 4 μM), 4 μl template and 1 μl PCR water. The 16S rRNA gene was amplified using a 26 cycle PCR (96 °C, for 10 s; annealing temperature, 55 °C, for 5 s; extension temperature, 60 °C for 4 min) and hold at 4 °C. The PCR amplification products were analyzed by electrophoresis on a 1% agarose gel and purified. DNA sequencing was performed using the dideoxy chain termination method with an ABI 3730 × 1 Genetic Analyzer. A consensus sequence of 1385 b.p. of 16S rDNA gene was generated from forward and reverse sequence data using aligner software.

The 16S rDNA gene sequence was analyzed using BLAST program with NCBI GenBank database. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4. The sequence was deposited at Gen Bank with accession No. JQ312665.

2.3. Reduction of Cr(VI) during growth

Reduction of chromium during growth of *Arthrobacter* sp. SUK 1201 was determined in Vogel Bonner (V. B.) broth. The V. B. broth contained 2% of sterile stock solution of V. B. concentrate containing (grams per liter) anhydrous K_2HPO_4, 500.0; $Na(NH_4)HPO_4{\cdot}4H_2O$, 175.0; citric acid, 100.0; $MgSO_4{\cdot}7H_2O$, 10.0 and 2.0% of 25% D-glucose 20.0 ml (pH 7.0) in 1 l distilled water [20]. The medium (20 ml/100 ml flask) was inoculated with overnight grown cultures and incubated at 35 °C under continuous shaking (120 rpm). Unless otherwise mentioned, the initial inoculum was maintained at 10^6 cells/ml in all experiments. In order to monitor any abiotic Cr(VI) reduction, cell-free controls were used and all experiments were done in triplicates. Residual hexavalent chromium was measured following the usual diphenyl carbazide method [21].

2.4. Fourier transform infrared (FTIR) spectroscopy

The cell mass along with the green precipitate formed during the course of Cr(VI) reduction was separated from the culture by centrifugation at 10,000 × *g* for 10 min at 4 °C. The pellet was washed thrice with distilled water to remove traces of water soluble Cr(VI) and subsequently lyophilized at −56 °C using a Secfroid LSL lyophilizer.

The lyophilized cell mass along with precipitate was powdered in mortar and pestle, mixed with 2% KBr and compressed into translucent sample discs using manual hydraulic press. The disc was then scanned between 500 and 4000 cm^{-1} in a Perkin Elmer FTIR spectrophotometer (Model No. RX-1). Biomass grown in chromium-free medium was used as control.

2.5. Determination 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 peptone yeast extract glucose (PYEG) agar medium and the number of colony forming units/ml was calculated after incubating at 35 °C for 2 d.

2.6. Estimation of chromium

Hexavalent chromium reduction in the culture filtrate was estimated by measuring the decrease of chromate at definite time interval. Cr(VI) was quantified following diphenyl carbazide method of Park et al. [21].

Total chromium was measured using a Varian Atomic Absorption Spectrometer (spectrAA-20Plus). Elemental analysis of chromate reduction products was carried out with energy dispersive X-ray (EDX) analysis. For EDX analysis the cell mass along with the green precipitate was separated from the culture by centrifugation at 10,000 × *g* for 10 min at 4 °C. The pellet was washed and subsequently lyophilized at −56 °C using a Secfroid LSL lyophilizer. The lyophilized cell mass along with precipitate was powdered in mortar and pestle and mounted on aluminum stubs. It was

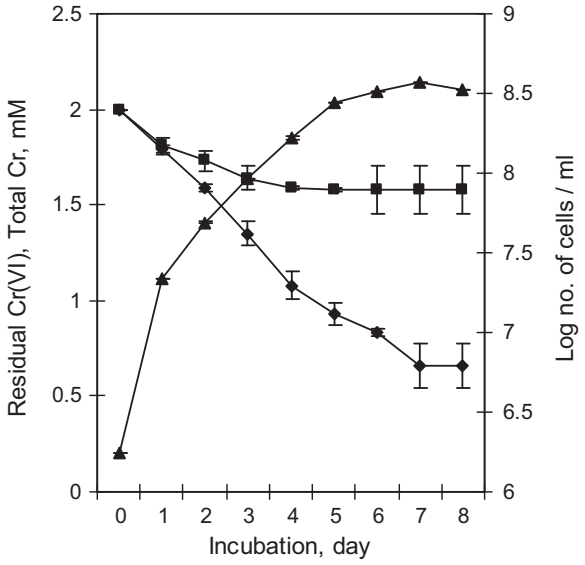

Fig. 2. Growth and reduction of hexavalent chromium by isolate *Arthrobacter* sp. SUK 1201 in Vogel Bonner broth under batch culture (-♦- residual hexavalent chromium, -■- total chromium, -▲- growth).

subjected to 90°A thick carbon coating in polaron Sc 7640 sputter coater for 10 min. Elemental analysis was carried out at 15 kV.

3. Results

3.1. Identification of the strain

The chromium resistant and reducing Gram-positive bacterial strain *Arthrobacter* sp. SUK 1201 isolated from overburden samples of chromite mine around Sukinda, Orissa, India showed a characteristics rod to cocci cycle during growth. The consensus sequence of 1385 b.p. of 16S rDNA gene was generated and used to carry out BLAST with database of NCBI GenBank. The results showed 99% sequence similarity with *Arthrobacter* sp. GZK-1. The phyllogenetic tree has been drawn using Neighbor-Joining program in MEGA 4 software between different members of the genus *Arthrobacter* and the present bacterial isolate SUK 1201 and are shown in Fig. 1.

3.2. Chromate reduction during growth

Chromate reduction by *Arthrobacter* sp. SUK 1201 as studied under batch culture in V. B. broth supplemented with 2 mM Cr(VI) showed gradual discoloration of the medium and formation of a light to deep green colored precipitate in the culture along with decrease in the Cr(VI) content (Fig. 2). However, there was no significant change in the total chromium content of the medium. EDX analysis carried out with washed lyophilized cells along with green colored precipitate confirmed the presence of chromium in the precipitate. The green precipitate was insoluble at neutral pH, but was highly soluble at pH below 5.5 and may possibly represent salts of Cr(III) (Fig. 3).

3.3. FTIR analysis

The FTIR spectra of biomass grown in chromium-free medium and those grown in medium containing 2 mM Cr(VI) were compared. No major change in the spectra was noticed (Fig. 4), which indicated that the end product of hexavalent chromium reduction so formed during the course of growth has not complexed with bacterial cell.

3.4. Effect of initial Cr(VI) concentration

Growth as well as chromate reduction was monitored at different initial chromium concentration ranging from 0.5 to 6.0 mM. Complete reduction of hexavalent chromium in the medium even failed to occur at the lowest concentration (0.5 mM), where nearly 92% of initial Cr(VI) was reduced. With increase in chromium concentration (1–6 mM), the reduction rate increased from 0.34 to 2.0 mM Cr(VI)/day during the first day of incubation. However, the extent of reduction decreased with prolonged incubation and at higher concentration. Virtually there was no reduction from 4th and 3rd day of incubation at 4 and 6 mM Cr(VI) respectively, although some degree of growth was evident (Fig. 5a and b).

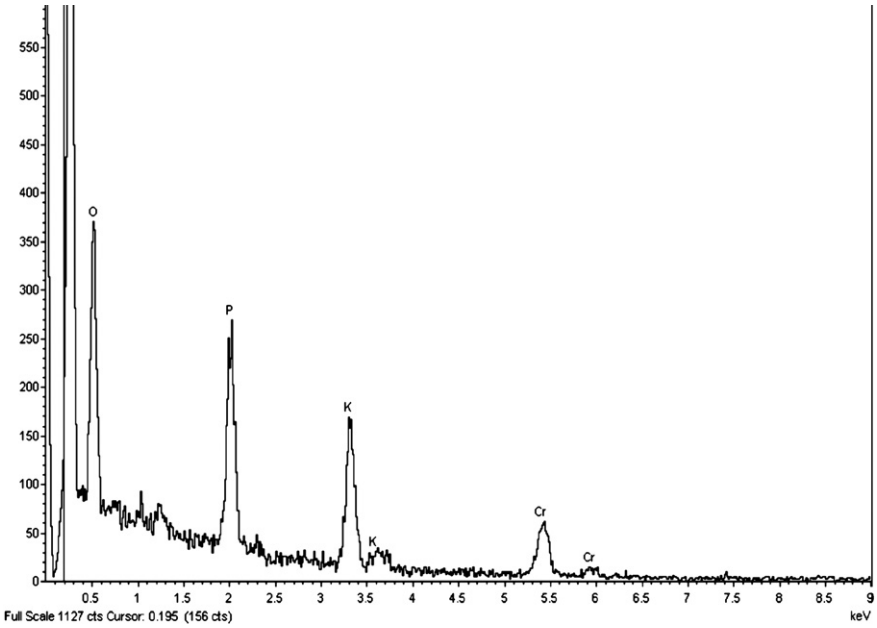

Fig. 3. EDX spectrum of the precipitate formed during the Cr(VI) reduction by *Arthrobacter* sp. SUK 1201 cells.

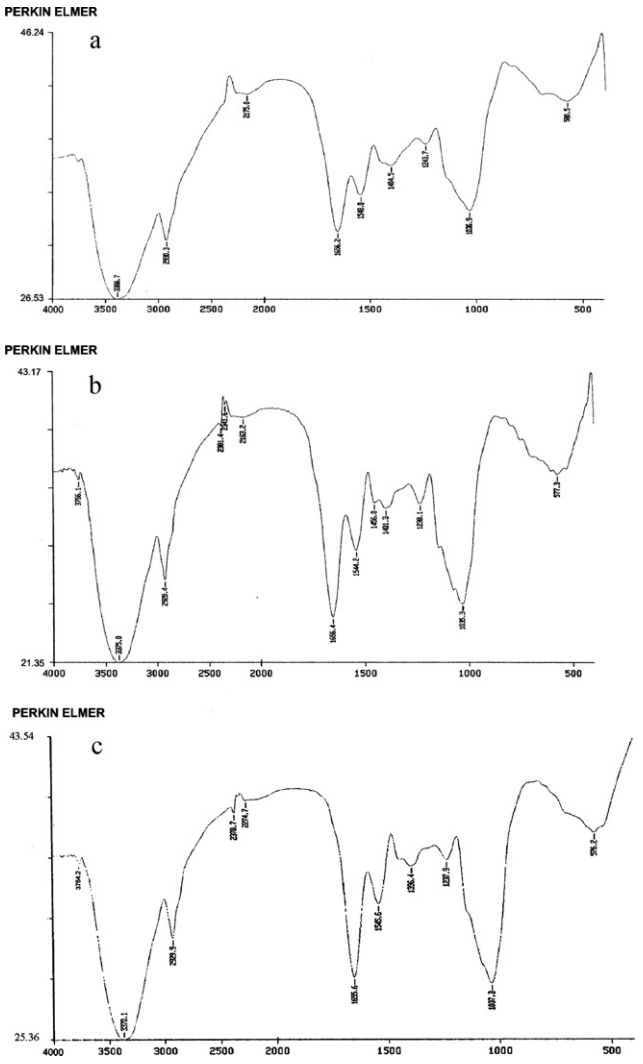

Fig. 4. FTIR spectrum of the cell mass during Cr(VI) reduction by *Arthrobacter* sp. SUK 1201, (a) control, (b) 2 days and (c) 4 days.

3.5. Effect of cell concentration on Cr(VI) reduction

The effect of initial cell density on Cr(VI) reduction was tested at a concentration of 10^5–10^{10} cells/ml. Cr(VI) reduction was found to increase proportionally with increase in cell density. At the highest cell density (10^{10} cells/ml, 2 mM of Cr(VI) declined to 0.2 mM in 8 days showing about 90% of initial Cr(VI) reduction, but at lower cell concentration (10^5 cells/ml) only 53% Cr(VI) was reduced (Fig. 6).

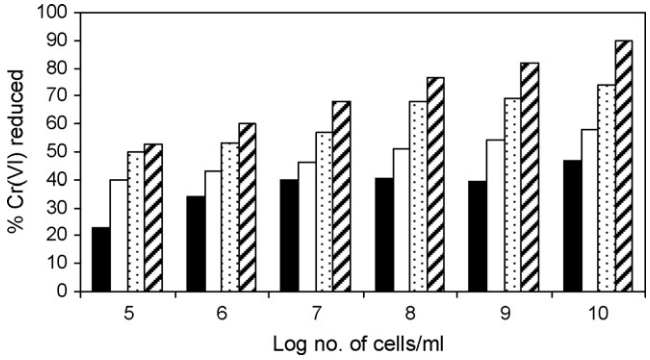

Fig. 6. Effect of cell density on Cr(VI) reduction by isolate *Arthrobacter* sp. SUK 1201 (■ 2 days, □ 4 days, ⬚ 6 days, ▨ 8 days).

3.6. Effect of electron donor

Several organic compounds viz. propionate, acetate, benzoate, glucose, sucrose, glycerol, propylene glycol, chlorophenol and cresol were supplemented at 0.1% (w/v) level in the V. B. broth and reduction of Cr(VI) during growth of *Arthrobacter* sp. SUK 1201 was studied. Complete reduction of initial 2 mM chromate was seen within 4 days of incubation when glycerol was used as electron donor. This was followed by glucose where 76% of initial Cr(VI) was reduced. Sucrose was the least effective electron donor followed by tryptone and acetate (Fig. 7a and b).

3.7. Effect of pH and temperature

Chromate reduction by isolate *Arthrobacter* sp. SUK 1201 occurred fairly well when the initial pH of the media was maintained between 4.0 and 8.0. Maximum reduction of hexavalent chromium (68.65%) occurred at pH 7.0, which was affected on either side of the pH scale (Fig. 8).

The chromate reduction efficiency of the isolate SUK 1201 was greatly influenced by the incubation temperature when tested over a temperature range of 30–40 °C. The optimum temperature for reduction was found to be 35 °C with 65.7% of chromate reduction.

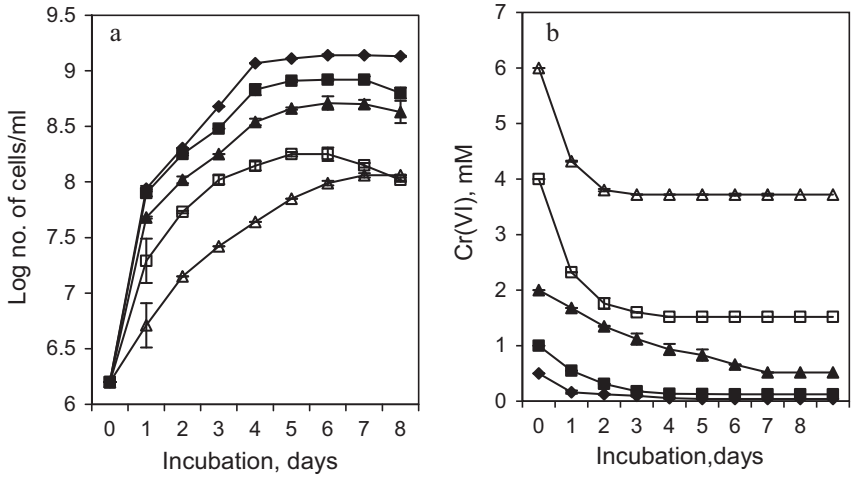

Fig. 5. Effect of concentration of Cr(VI) on growth (a) and Cr(VI) reduction (b) by isolate *Arthrobacter* sp. SUK 1201 (-♦- 0.5 mM, -■- 1.0 mM, -▲- 2.0 mM, -□- 4.0 mM, -△- 6.0 mM).

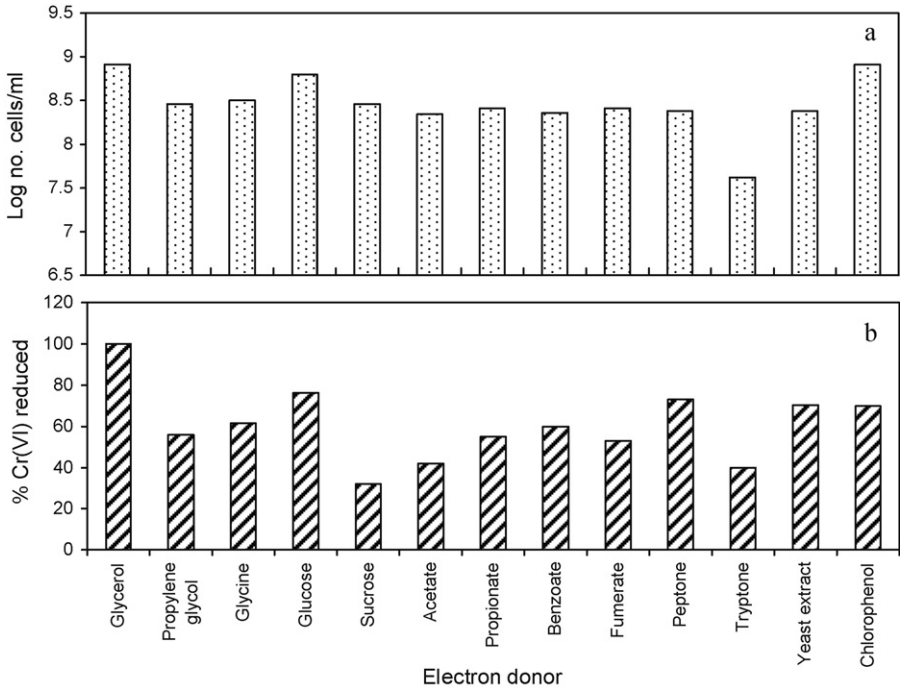

Fig. 7. Effect of electron donor on growth (a) and Cr(VI) reduction (b) by isolate *Arthrobacter* sp. SUK 1201.

However, the process of reduction was severely affected when the temperature was increased to 40 °C (Fig. 9).

3.8. Effect of metal ions

Effect of metal ions on chromate reduction was investigated using metals like Ni(II), Mn(II), Cu(II), Co(II), Zn(II) and Cd(II). Reduction of Cr(VI) by the isolate was maximum (73%) when 1 mM Cu(II) was supplemented in the medium along with Cr(VI) and this was much better than the control set containing only Cr(VI). Presence of Cu(II) however, retarded the growth to some extent. Cadmium appeared to be most toxic of all the metal tested, presence of which impaired growth as well as chromate reduction and was followed by Zn(II), Co(II) and Ni(II). Apparently, Cr(VI) reduction by the isolate (64%) was more or less unaffected by the presence of Mn(II) (Table 1).

3.9. Effect of inhibitor

Several inhibitors such as sodium azide (NaN_3), sodium fluoride (NaF), 2,4-di nitrophenol (DNP), N,N,-dicyclohexyl carboiimide (DCC) and carbonyl cyanide-m-chloro phenyl hydrazone (CCCP) were used to evaluate their influence on chromate reduction. Reduction was remarkably inhibited by most of the inhibitors except DNP when used at 1 mM level. Only 34–46% of chromate reduction was possible after 8 days of incubation. DNP appeared neither inhibitory nor promotive as it leads to the reduction of 68.5% of 2 mM Cr(VI) as against 67.3% in the control (Table 2).

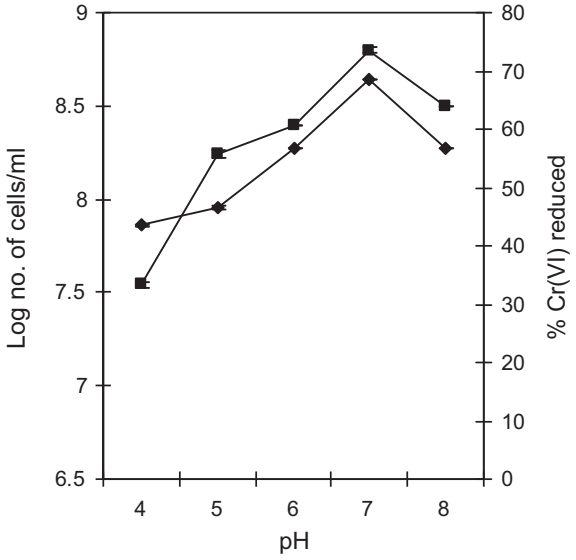

Fig. 8. Effect of pH on growth and chromate reduction during growth by isolate *Arthrobacter* sp. SUK 1201 (-■- growth, -♦- % Cr(VI) reduced).

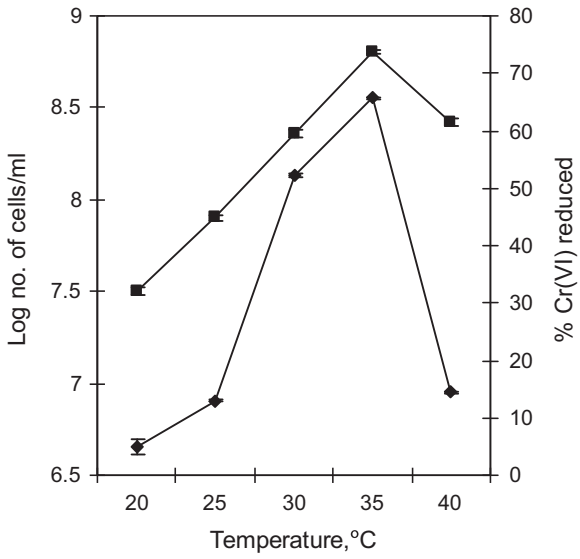

Fig. 9. Effect of temperature on growth and chromate reduction during growth by isolate *Arthrobacter* sp. SUK 1201 (-■- growth, -♦- % Cr(VI) reduced).

Table 1
Effect of metal ions on growth and Cr(VI) reduction by *Arthrobacter* sp. SUK 1201.

Metals	Incubation, days							
	2		4		6		8	
	A	B	A	B	A	B	A	B
Control (-Metal)	7.8 ± 0.06	19.0 ± 0.0	8.16 ± 0.0	49.75 ± 0.0	8.61 ± 0.0	59.0 ± 0.06	8.52 ± 0.0	67.0 ± 0.0
Ni(II)	7.90 ± 0.0	23.25 ± 0.0	8.04 ± 0.04	46.4 ± 0.0	8.74 ± 0.0	59.8 ± 0.06	8.28 ± 0.0	59.8 ± 0.06
Mn(II)	7.60 ± 0.0	46.4 ± 0.0	8.42 ± 0.02	59.8 ± 0.0	8.62 ± 0.0	63.15 ± 0.06	8.60 ± 0.0	63.15 ± 0.06
Cu(II)	6.89 ± 0.0	33.0 ± 0.0	8.25 ± 0.0	39.7 ± 0.0	8.55 ± 0.02	59.8 ± 0.0	8.38 ± 0.0	73.2 ± 0.0
Co(II)	7.20 ± 0.0	33.0 ± 0.09	8.03 ± 0.0	46.4 ± 0.0	8.55 ± 0.0	46.4 ± 0.0	8.26 ± 0.0	46.4 ± 0.0
Zn(II)	7.25 ± 0.08	30.0 ± 0.0	7.83 ± 0.01	33.0 ± 0.2	8.60 ± 0.02	39.7 ± 0.0	7.90 ± 0.0	39.7 ± 0.0
Cd(II)	7.38 ± 0.0	9.8 ± 0.0	7.71 ± 0.02	9.8 ± 0.0	8.38 ± 0.0	9.8 ± 0.0	7.68 ± 0.0	9.8 ± 0.0

A, growth, log no. of cells/ml; B, % Cr(VI) reduced. All metals were added to the chromate reduction medium at 1 mM level. Results represent mean ± standard error of triplicate sets.

Table 2
Effect of different inhibitors on growth and Cr(VI) reduction by *Arthrobacter* sp. SUK 1201.

Inhibitors	Incubation, days							
	2		4		6		8	
	A	B	A	B	A	B	A	B
Control (-Inhibitor)	7.72 ± 0.06	32.5 ± 0.0	8.26 ± 0.01	49.60 ± 0.6	8.52 ± 0.0	57.0 ± 0.6	8.60 ± 0.0	67.3 ± 0.0
CCCP	6.86 ± 0.02	19.8 ± 0.02	7.02 ± 0.0	23.5 ± 0.0	7.01 ± 0.0	31.0 ± 0.02	6.45 ± 0.0	34.0 ± 0.02
DCC	7.02 ± 0.04	21.0 ± 0.0	7.23 ± 0.02	33.0 ± 0.06	7.09 ± 0.06	39.7 ± 0.0	6.75 ± 0.02	39.7 ± 0.0
DNP	7.02 ± 0.04	30.0 ± 0.0	7.23 ± 0.0	37.0 ± 0.02	7.02 ± 0.0	68.5 ± 0.06	6.75 ± 0.02	68.5 ± 0.06
NaN_3	7.68 ± 0.04	37.5 ± 0.59	7.61 ± 0.06	40.0 ± 0.0	7.30 ± 0.05	46.75 ± 0.0	7.02 ± 0.0	46.75 ± 0.0
NaF	6.82 ± 0.02	26.8 ± 0.07	7.02 ± 0.06	30.0 ± 0.0	6.96 ± 0.0	33.0 ± 0.06	6.8 ± 0.0	34.24 ± 0.0

A, growth, log no. of cells/ml; B, % Cr(VI) reduced. All inhibitors were added to the chromate reduction medium at 1 mM level. Results represent mean ± standard error of triplicate sets. CCCP, carbonyl cyanide-m-chloro phenyl hydrazone; DCC, N,N,-dicyclohexyl carboiimide; DNP, 2,4-di nitrophenol; NaN_3, sodium azide; NaF, sodium fluoride.

4. Discussion

Ever since the findings of Camargo et al. [2] that *Arthrobacter* spp. could thrive better in metal stressed environments and could reduce hexavalent chromium, several reports have been published on chromate reduction by members of this actinomycetes genus isolated from soil contaminated with tannery effluent [12], basalt rocks [17], activated sludge [5] and landfarming process soil sample [19]. Moreover, *Arthrobacter* spp. have attracted the attention for its potential applications in bioremediation of Cr pollutant in culture [12], using free and immobilized cell-free extract [18] and biofilm packed bed reactors [19].

The bacterial isolate SUK 1201, obtained from metalliferous chromite mine overburden of Orissa, India was identified as *Arthrobacter* sp. SUK 1201 (MTCC 8728). The taxonomic identity of strain as been confirmed following analysis of 16S rDNA sequence homology, which showed 99% homology with *Arthrobacter* sp. GZK-1, previously isolated from sugarcane cultivated soils in Kenya [22]. The strain GZK-1 was reported to mineralize herbicides like atrazine but were unable to resist and reduce Cr(VI). Strikingly, *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden has shown resistance to heavy metals including Cr and developed the property of reducing hexavalent chromium as an adaptive feature in withstanding the toxic mining environment enriched with different metallic ions.

Arthrobacter sp. SUK 1201 reduced nearly 67% of 2 mM Cr(VI) in V. B. broth (Fig. 2) in 7 days, where low level of organic substances minimized the possible complexation of Cr(VI) with media constituents [12] and Cr(VI) reducing capability of the isolate was more or less accurately assessed. This corroborates the findings of Megharaj et al. [12] where Cr(VI) reduction was assessed using synthetic media. Chromate reduction by SUK 1201 during growth resulted in the formation of characteristics extracellular green precipitates. Wang et al. [9] and Carlos et al. [23], reported the formation of white colloid precipitate as a result of Cr(VI) reduction by *Enterobacter cloacae* and *Bacillus subtilis* respectively, while similar extracellular blue precipitate formation during Cr(VI) reduction was also demonstrated in *Achromobacter* [24] and *Leucobacter* sp. [25].

With increase in Cr(VI) concentration, chromate reduction rate increased (Fig. 5) as presence of Cr(VI) and the cell density stimulated Cr(VI) reduction activity [7]. However, the reduction rate decreased with incubation due to Cr(VI) toxicity and reduction in cell number as was evident in *Bacillus* sp. [20].

Chromate reducing organisms in general may utilize a variety of organic compounds as electron donors for Cr(VI) reduction [26,27]. The isolate SUK 1201 was able to utilize a wide variety of sugar metabolites and nitrogenous components but glycerol was most effective (Fig. 7). Pal and Paul [4] found that glycerol promoted Cr(VI) reduction by *Bacillus sphaericus*. While a considerable increase in Cr(VI) reduction by *Bacillus* sp. FM1 was achieved in presence of glucose [28].

Arthrobacter sp. SUK 1201 effectively reduced chromite in a narrow range of pH with an optimum of pH 7.0 (Fig. 8) similar to that of Camargo et al. [29], who also reported that the optimal pH for Cr(VI) reduction ranged between 7 and 8. Thacker and Madamwar [30] reported that maximum growth and chromate reduction by *Ochrobactrum* sp. occurred at pH 7.0. However, Masood and Malik [28] reported the optimum pH for Cr(VI) reduction by *Bacillus* sp. to be 8.

Temperature is an important factor that affect microbial Cr(VI) reduction. The optimum temperature for chromate reduction by SUK 1201 was found to be 35 °C (Fig. 9) characteristic of mesophilic organism. Similar optimum temperature for Cr(VI) reduction was also reported in isolate *Ochrobactrum intermedium* SDCr-5 [7] and in *Bacillus* sp. [28]. Optimum temperature for growth and Cr(VI) reduction was found to range between 35 and 40 °C for *Staphylococcus aureus* and *Pediococcus pentosaceus* [31].

Though *Arthrobacter* sp. SUK 1201 was tolerant to a number of heavy metals, the Cr(VI) reduction efficiency was significantly affected by metals like Cd(II) and Zn(II). Similar inhibitory effect was also evident with different strains of *Bacillus* [28,32]. The

stimulatory effect of Cu(II) on the chromate reductase activity of *Arthrobacter* sp. SUK 1201 (Table 1) may probably be due to the fact that Cu is a prosthetic group for many reductase enzymes and acts as an electron redox center and help in the shuttle of electron between subunits. Similar enhancement of Cr(VI) reduction potential was also evident with *Bacillus* sp. [29] and *O. intermedium* [7].

Chromate reductase activity of SUK 1201 was severely affected by the metabolic inhibitors like CCCP, NaF and DCC (Table 2) due to disruption of chemiosmotic gradient, disruption of enolase activity [11] and inhibition of ATPase activity respectively. Like *Arthrobacter* sp. SUK 1201, inhibition of Cr(VI) reduction was also evident with *Stenotrophomonas maltophilia*, *Staphylococcus gallinarum*, *Pantoea* sp. and *Aeromonas* sp. in presence of NaN_3 [33]. DNP, the uncoupler might have accelerated the respiratory chain linked electron transport mechanism [34] and thereby showed chromate reductase activity more or less similar to control. Enhancement of Cr(VI) reduction by DNP has also been reported in *Burkholderia cepacia* [34] and *Staphylococcus gallinarum* [33].

5. Conclusion

The optimization of cultural and physico-chemical features of Cr(VI) reduction by *Arthrobacter* sp. SUK 1201 clearly established the biotechnological potential of transformation of Cr(VI) to less toxic Cr(III) and thus could be useful in detoxification of chromium pollutants particularly in aquatic systems.

Acknowledgments

The authors acknowledge the financial support from the Department of Biotechnology, Ministry of Science and Technology, Government of India vide Sanction number BT/PR/5766/NDB/51/061/2005.

References

[1] Y. Wang, Microbial reduction of chromate, in: D.R. Lovley (Ed.), Environmentally Microbe Metal Interaction, American Society for Microbiology Press, Washington, DC, 2000, pp. 225–235.
[2] F.A.O. Camargo, F.M. Bento, B.C. Okeke, W.T. Frankenberger, Hexavalent chromium reduction by an actinomycetes, *Arthrobacter crystallopoites* ES 32, Biol. Trace Elem. Res. 97 (2004) 183–194.
[3] M. Basu, S. Bhattacharya, A.K. Paul, Isolation and characterization of chromium-resistant bacteria from tannery effluents, Bull. Environ. Contam. Toxicol. 58 (1997) 535–542.
[4] A. Pal, A.K. Paul, Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil, Microbiol. Res. 159 (2004) 347–354.
[5] P.E. Molokwane, C.K. Meli, M.N. Evans Chirwa, Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa), Water Res. 42 (2008) 4538–4548.
[6] L.M. Barreva, E.C. Urbina, Biological reduction of hexavalent chromium: a potential strategy for the bioremediation of Cr(VI) polluted waste water, World Environment Foundation, 2006, pp. 4984–4989.
[7] S. Sultan, S. Hasnain, Reduction of toxic hexavalent chromium by *Ochrobactrum intermedium* strain SDCr-5 stimulated by heavy metals, Bioresour. Technol. 98 (2007) 340–344.
[8] U. Thacker, R. Parikh, Y. Shouche, D. Madamwar, Hexavalent chromium reduction by *Providencia* sp., Process Biochem. 41 (2006) 1332–1337.
[9] P.C. Wang, T. Mori, K. Komori, M. Sasatsu, K. Toda, H. Ohtake, Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions, Appl. Environ. Microbiol. 55 (1989) 1665–1669.
[10] T. Srinath, S. Khare, P.W. Ramteke, Isolation of hexavalent chromium-reducing facultative anaerobes from tannery effluent, J. Gen. Appl. Microbiol. 47 (2001) 307–312.
[11] D.J. Opperman, E. van Heerden, Aerobic Cr(VI) reduction by *Thermus scotoductus* strain SA-01, J. Appl. Microbiol. 103 (2007) 1907–1913.
[12] M. Megharaj, S. Avudainayagam, R. Naidu, Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste, Curr. Microbiol. 47 (2003) 51–54.
[13] P. Viti, A. Pace, L. Giovannetti, Characterisation of chromium-resistant bacteria isolated from chromium-contaminated soil by tannery activity, Curr. Microbiol. 46 (2003) 1–5.
[14] A. Ganguli, A.K. Tripathi, Inducible periplasmic chromate reducing activity in *Pseudomonas aeruginosa* from a leather tannery effluent, J. Microbiol. Biotechnol. 11 (2001) 355–361.
[15] E.V. Lebedeva, N.N. Lyalikova, Reduction of crocoite by *Pseudomonas chromatophila* sp. nov., Mickobiologia 48 (1979) 517–522.
[16] B. Dhal, H. Thatoi, N. Das, B.D. Pandey, Reduction of hexavalent chromium by *Bacillus* sp. isolated from chromite mine soils and characterization of reduced product, J. Chem. Technol. Biotechnol. 85 (2010) 1471–1479.
[17] N.V. Asianti, M.K. Abuladze, T.M. Kartvelishvili, N.G. Bakradze, N.A. Sapojnikova, N.Y. Tsibakhashvili, L.V. Tabatadze, L.L. Asanishvili, H. Holman, Effect of chromium (VI) action on *Arthrobacter oxydans*, Curr. Microbiol. 49 (2004) 321–326.
[18] R. Elangovan, L. Philip, K. Chandraraj, Hexavalent chromium reduction by free and immobilized cell-free extract of *Arthrobacter rhombi*-RE, Appl. Biochem. Biotechnol. 160 (2010) 81–97.
[19] A. Cordoba, P. Vargas, J. Dussa, Chromate reduction by *Arthrobacter* CR47 in biofilm packed bed reactors, J. Hazard. Mater. 151 (2008) 274–279.
[20] Y.T. Wang, C. Xiao, Factors affecting hexavalent chromium reduction in pure cultures of bacteria, Water Res. 29 (1995) 2467–2474.
[21] C.H. Park, B. Keyhan, B. Wielinga, S. Fendorf, A. Matin, Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase, Appl. Environ. Microbiol. 66 (2000) 1788–1795.
[22] Z. Getenga, U. Dörfler, A. Iwobi, M. Schmid, R. Schroll, Atrazine and terbuthylazine mineralization by an *Arthrobacter* sp. isolated from a sugarcane cultivated soil in Kenya, Chemosphere 77 (2009) 234–239.
[23] G. Carlos, A. Itziar, J.L. Maria, L.S. Juan, Aerobic chromate reduction by *Bacillus subtilis*, Biodegradation 9 (1998) 133–141.
[24] W. Zhu, L. Chai, Z. Ma, Y. Wang, H. Xiao, K. Zhao, Anaerobic reduction of hexavalent chromium by bacterial cells of *Achromobacter* sp. Strain Ch1, Microbiol. Res. 163 (2006) 616–623.
[25] W. Zhu, Z. Yang, Z. Ma, L. Chai, Reduction of high concentrations of chromate by *Leucobacter* sp. CRB1 isolated from Changsha, China, World J. Microbiol. Biotechnol. 24 (2008) 991–996.
[26] L. Philip, C. Venkobachar, Cr(VI) reduction by *Bacillus coagulans* isolated from contaminated soils, J. Environ. Eng. 124 (1998) 1165–1170.
[27] Y.G. Liu, W.H. Xu, G.M. Zeng, Experimental study on reduction by *Pseudomonas aeruginosa*, J. Environ. Sci. 16 (2004) 795–801.
[28] F. Masood, A. Malik, Hexavalent chromium reduction by *Bacillus* sp. strain FM1 isolated from heavy-metal contaminated soil, Bull. Environ. Contam. Toxicol. 86 (2011) 114–119.
[29] F.A.O. Camargo, B.C. Okeke, F.M. Bento, W.T. Frankenberger, In vitro reduction of hexavalent chromium by a cell-free extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}, Appl. Environ. Microbiol. 62 (2003) 569–573.
[30] U. Thacker, D. Madamwar, Reduction of toxic chromium and partial localization of chromium reductase activity in bacterial isolate DM1, World. J. Microbiol. Biotechnol. 21 (2005) 891–899.
[31] M. Ilias, I.M. Rafiqullah, B.C. Debnath, K.S.B. Mannan, M.M. Hoq, Isolation and characterization of chromium(VI)-reducing bacteria from tannery effluents, Indian J. Microbiol. 51 (2011) 76–81.
[32] C. Desai, K. Jain, D. Madamwar, Evaluation of in vitro Cr(VI) reduction potential in cytosolic extracts, Bioresour. Technol. 99 (2008) 6059–6069.
[33] M.Z. Alam, S. Ahmad, Toxic chromate reduction by resistant and sensitive bacteria isolated from tannery effluent contaminated soil, Ann. Microbiol. (2011), doi:10.1007/s13213-011-0235-4.
[34] R. Wani, K.M. Kodam, K.R. Gawai, P.K. Dhakephalkar, Chromate reduction by *Burkholderia cepacia* MCMB-821 isolated from the pristine habitat of alkaline crater lake, Appl. Microbiol. Biotechnol. 75 (2007) 627–632.