



Research article

Assessment of heavy metal tolerance and hexavalent chromium reducing potential of *Corynebacterium paurometabolum* SKPD 1204 isolated from chromite mine seepage

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Abstract: *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730), a heavy metal tolerant and chromate reducing bacterium isolated from chromite mine seepage of Odisha, India has been evaluated for chromate reduction under batch culture. The isolate was found to tolerate metals like Co(II), Cu(II), Ni(II), Mn(II), Zn(II), Fe(III) and Hg(II) along with Cr(VI) and was resistant to different antibiotics as evaluated by disc-diffusion method. The isolate, SKPD 1204 was found to reduce 62.5% of 2 mM Cr(VI) in Vogel Bonner broth within 8 days of incubation. Chromate reduction capability of SKPD 1204 decreased with increase in Cr(VI) concentration, but increased with increase in cell density and attained its maximum at 10^{10} cells/mL. Chromate reducing efficiency of SKPD 1204 was promoted in the presence of glycerol and glucose, while the highest reduction was recorded at pH 7.0 and 35 °C. The reduction process was inhibited by divalent cations Zn(II), Cd(II), Cu(II), and Ni(II), but not by Mn(II). Anions like nitrate, phosphate, sulphate and sulphite was found to be inhibitory to the process of Cr(VI) reduction. Similarly, sodium fluoride, carbonyl cyanide m-chlorophenylhydrazone, sodium azide and N, N,-Di cyclohexyl carboiimide were inhibitory to chromate reduction, while 2,4-dinitrophenol appeared to be neither promotive nor inhibitory to the process.

Keywords: *Corynebacterium paurometabolum*; mine seepage; metal tolerance; antibiotic resistance index; Cr(VI) bioremediation

Abbreviation

ARI	Antibiotic resistance index
CCCP	Carbonyl cyanide m-chloro phenylhydrazone
Cr(III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
DCC	N, N-dicyclocarbodiimide
DPC	1,5-diphenylcarbazine
2,4-DNP	2,4-dinitrophenol
g/L	Gram per litre
IMTECH	Institute of Microbial Technology
MIC	Minimum inhibitory concentration
mM	Millimolar
µg/disc	Microgram per disc
MTCC	Microbial Type Culture Collection
PYEG	Peptone yeast extract glucose medium
rpm	Rotations per minute
S.D.	Standard deviation
V.B. broth	Vogel Bonner broth

1. Introduction

Chromium in its trivalent form [Cr(III)] is mostly found in nature as chromite ores. In India, chromite ores are mainly located in Sukinda Valley (21°0'-21°5'N: 85°43'-86°0'E) of Jajpur and Baula-Nuasahi belt (21°10'-21°15'N: 86°18'-86°22'E) of Keonjhar districts of Odisha, India. This area is one of the most important craton where nearly 95% of the Indian chromite deposits are located [1].

Both opencast and underground mining operations for chromite ores in these localized areas of Odisha, India often generate huge amount of mine overburden and mine seepage effluents loaded with toxic metals including chromium. The inert chromites undergo oxidation to generate toxic hexavalent chromium [Cr(VI)] which are mutagenic and carcinogenic in nature and are mobilized into the water bodies [2] causing extensive chromium pollution in and around the mining areas. The toxicity of Cr(VI) is mainly attributed to the process of reduction of Cr(VI) to lower oxidation state, as the process results in the production of free radicals which leads to oxidative stress, DNA damage and ultimately results in altered gene expression [3,4].

Removal of toxic Cr(VI) pollutants often involves conventional chemical reduction of Cr(VI) to Cr(III) and its precipitation following adjustment of pH (usually addition of lime). Such precipitation methods generate large quantities of solid waste for disposal. Other methods of chromate removal by ion exchange and adsorption are not cost effective [5]. Moreover, the huge secondary waste materials also pose serious threat to the environment. On the other hand transformation of Cr(VI) to Cr(III)

and its subsequent precipitation by indigenous microbiota (bacteria, fungi, yeast and algae) has been identified as a cost effective green strategy for removal of Cr-pollutants [6].

Several bacterial strains have been isolated and reported from chromite mining areas of Sukinda valley, Odisha by different groups, which include *Arthrobacter* sp. SUK 1201 [7], *Bacillus* sp. [8], *Bacillus subtilis*, *Acinetobacter junii*, and *Escherichia coli* [9]. However, as far as we are aware, chromate reducing bacteria belonging to the genus *Corynebacterium* has not been reported so far. The present study concentrates on the assessment of Cr(VI) reducing ability of a heavy metal resistance bacterium, *Corynebacterium paurometabolum* SKPD 1204 during growth and optimization of the environmental factors responsible for such Cr(VI) reduction.

2. Materials and Methods

2.1. Source and maintenance of bacterial culture

Chromate reducing bacterium, *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730) isolated from chromite mine seepage of Odisha, India was used throughout this study. The bacterium was grown and maintained on slopes of peptone yeast-extract and glucose (PYEG) agar medium [10] 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).

2.2. Reduction of Cr(VI) during growth

Chromium reduction studies during growth of *C. paurometabolum* SKPD 1204 were undertaken in Vogel Bonner (V. B.) broth which contained 2% of sterile stock solution of V. B. concentrate containing (g/L): 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 20.0 mL of 25% D-glucose (pH 7.0) in 1 litre distilled water [10]. The 25 mL medium in 100 mL flask was inoculated with overnight grown cultures in peptone yeast extract (PYEG) medium and incubated at 35 °C under continuous shaking (120 rpm). Unless otherwise mentioned, the initial inoculum dose was maintained at 10^6 cells/mL in all experiments. In order to observe any abiotic Cr(VI) reduction, cell-free controls were used. The growth of the isolate was determined by viable cell count method, while the residual hexavalent chromium was measured following the usual diphenyl carbazide (DPC) method [11]. The rate of Cr(VI) reduction was measured and expressed as mM Cr(VI)/mg of cell/h. Specific activity of chromate reductase was expressed as U/mg of protein where one unit (U) of Cr(VI) reductase activity was defined as the amount of enzyme that convert 1.0 mM Cr(VI) per h at 35 °C.

The total chromium in the culture filtrate was measured using Varian Atomic Absorption Spectrophotometer (SpectrAA-20Plus). Total protein was estimated by Folin-phenol reagent [12].

2.3. Bacterial tolerance to heavy metals

Heavy metal tolerance of *C. paurometabolum* SKPD 1204 including Cr(VI) was evaluated by broth dilution method of Calomiris et al., [13]. The V. B. broth and PYEG broth (5mL/tube)

supplemented with increasing concentration of heavy metals was inoculated with 0.2 mL of overnight grown cultures and incubated at 35 °C for 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.

2.4. Antibiotic susceptibility

Susceptibility of *C. paurometabolum* SKPD 1204 to a number of different antibiotics was evaluated by disc-diffusion method. Antibiotic impregnated discs (6 mm, dia. HIMEDIA) were placed on freshly prepared lawns of the bacterial isolate on PYEG agar medium and incubated at 35 °C for 24 h. The diameter of inhibition zone was measured to nearest mm and the isolates were classified as sensitive, resistant and intermediate according to standard antibiotic sensitivity testing method. 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), polymyxin 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), trimethoprim (30 µg/disc), amoxycillin (30 µg/disc), novobiocin (30 µg/disc) and norfloxacin (30 µg/disc). The antibiotic resistance index (ARI) of the organism was expressed as the ratio of the number of resistant antibiotic to the total number of antibiotics tested.

2.5. Kinetics of Cr(VI) reduction

The rate of Cr(VI) reduction will be evaluated by determining the kinetics of Cr(VI) reduction at different Cr(VI) concentrations. The kinetics of Cr(VI) reduction was calculated in the following equation [14]:

$$y = a.e^{-kt}$$

$$C/C_0 = a.e^{-kt}$$

Linearized form becomes: $\ln C/C_0 = \ln a - kt$

where a is constant, y , C/C_0 is the fraction of Cr(VI) reduction at time t , C is the concentration of Cr(VI) at time t , C_0 is the original Cr(VI) concentration, and k is the rate constant.

2.6. Statistical analysis

All experiments were carried out in triplicates and the results were expressed as mean \pm S.D.

3. Results

The chromium resistant and reducing Gram-positive strain *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730) was isolated from chromite mine drainage water of Sukinda, Odisha. The

isolate was also screened for its tolerance to Cr(VI) along with other heavy metals such as Ni(II), Fe(III), Cu(II), Co(II), Mn(II), Zn(II), Cd(II) and Hg(II). It showed a high degree of tolerance to chromium (MIC 16 mM) in synthetic V. B. broth where as tolerance for Co(II), Cu(II), Ni(II), Mn(II), Zn(II) and Fe(III) were 4.6, 4.2, 3.2, 2.9, 2.4 and 1.8 mM respectively (Figure 1). However, the isolate was sensitive to Cd(II) (MIC 1.2 mM) and Hg(II) was most toxic to the isolate (MIC 0.001 mM). The level of tolerance to these metal cations was increased in the PYEG medium. The metal sensitivity profile of the isolate could be represented in the following order: Hg (0.1 mM) > Cd (2.24 mM) > Co (4.4 mM) > Zn (6 mM) > Ni (6.25 mM) > Cu (6.8 mM) > Fe (7 mM) > Mn (11 mM).

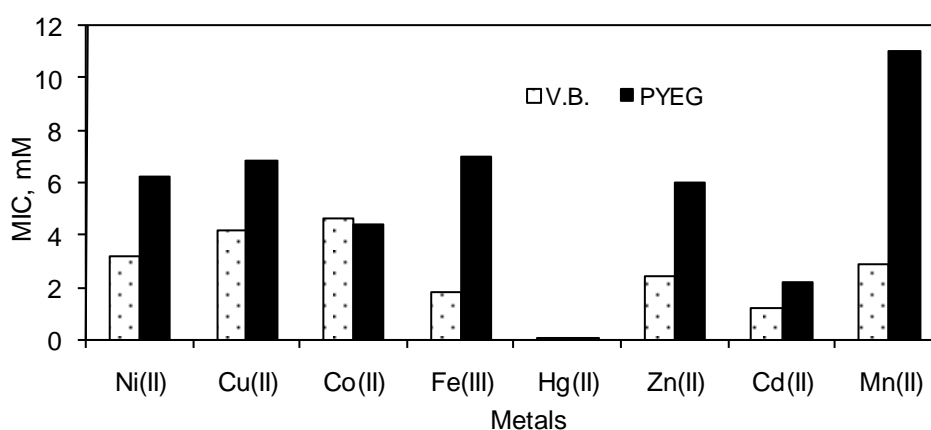


Figure 1. Heavy metal tolerance of *Corynebacterium paurometabolum* SKPD 1204. [Minimum inhibitory concentration of heavy metals was determined in V. B. broth and PYEG medium following broth dilution assay (Calomiris et al., 1982). All metals were used as chloride salts].

Table 1. Antibiotic sensitivity profile of the bacterial isolate *Corynebacterium paurometabolum* SKPD 1204.

Response	Antibiotics
Sensitive	Tetracycline, gentamycin, doxycycline, streptomycin, nalidixic acid, rifampicin, trimethoprim
Resistant	Erythromycin, methicillin, neomycin, kanamycin, doxycycline, ampicillin, amoxycillin, polymyxin B, penicillin G, netilin, novobiocin, norfloxacin

[Antibiotic sensitivity of the isolate was determined by disc-diffusion method.]

Antibiotic sensitivity profile of the isolate clearly indicated that out of 19 different antibiotics tested, the isolate was resistant to at least 12 antibiotics including erythromycin, methicillin, neomycin, kanamycin, doxycycline, ampicillin, amoxycillin, polymyxin B, penicillin G, netilin, novobiocin and norfloxacin (Table 1). However, it was sensitive to tetracycline, gentamycin,

doxycycline, streptomycin, nalidixic acid, rifampicin and trimethoprim. The antibiotic resistance index (ARI) of the organism was found to be 0.631.

During growth, the isolate SKPD 1204 reduced 62.5% of 2 mM Cr(VI) (Figure 2A) in V. B broth followed by gradual discolouration of the medium along with decrease in Cr(VI) content. The Cr(VI) reduction rate was reduced from 0.075 to 0.0037 mM Cr(VI)/mg of cell/h after 8 days of incubation (Figure 2A), while the specific activity of the chromate reductase enzyme was found to decline from 1.03 on 2nd day to 0.042 U/mg on the 8th day of incubation (Figure 2B). However, a gradual increase in growth was also evident from the increase in dry wt. of cells from 0.018 to 0.056 mg/mL of culture.

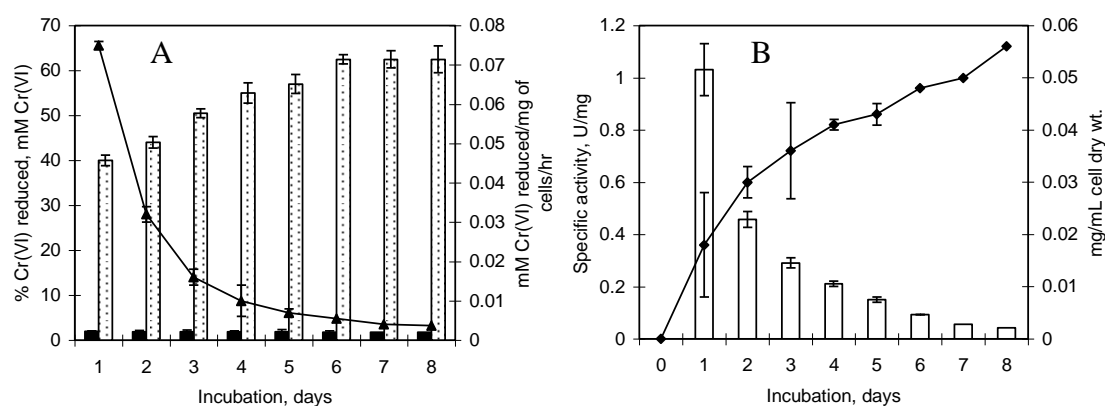


Figure 2. Growth associated reduction of hexavalent chromium by *Corynebacterium paurometabolum* SKPD 1204 in Vogel Bonner broth under batch culture showing [A] total Cr(VI), mM ■, % Cr(VI) reduced ▤, mM Cr(VI) reduced/mg of cells/h -▲- and [B] showing increase in cell dry wt. -◆- and specific activity U/mg □ of the chromate reductase enzyme. [One unit (U) of Cr(VI) reductase activity was defined as the amount of enzyme that convert 1.0 mM Cr(VI) per h at 35 °C.]

The influence of Cr(VI) concentration on its reduction by SKPD 1204 was tested in the range of 0.5 to 6.0 mM (Figure 3). At the lowest concentration (0.5 mM), only 75% of Cr(VI) in the medium could be reduced in 8 days of incubation. At higher chromium concentrations (4 and 6 mM), complete reduction of Cr(VI) could not be achieved, which was also accompanied with an decrease in log no. of cells/mL.

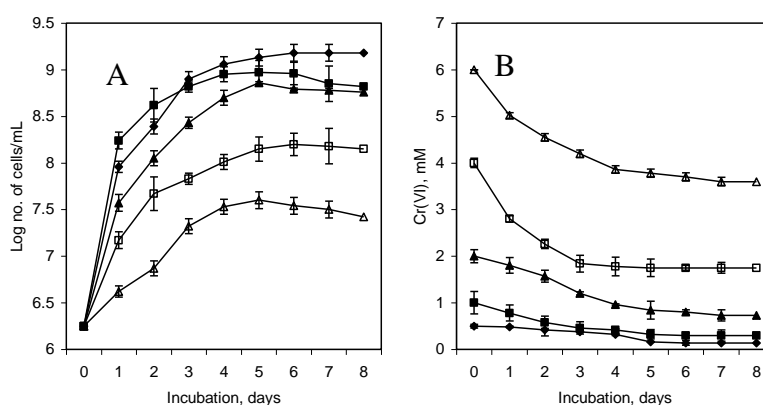


Figure 3. Effect of Cr(VI) on growth (A) and Cr(VI) reduction [expressed as the residual Cr(VI), mM] (B) by *Corynebacterium paurometabolum* SKPD 1204 (-◆- 0.5 mM, -■- 1.0 mM, -▲- 2.0 mM, -□- 4.0 mM, -△- 6.0 mM).

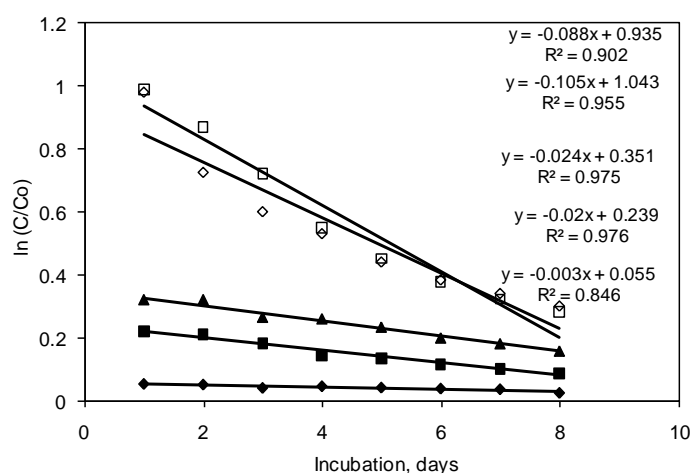


Figure 4. Rate of Cr(VI) reduction by *Corynebacterium paurometabolum* SKPD 1204 at different Cr(VI) concentration levels (-◆- 0.5 mM, -■- 1.0 mM, -▲- 2.0 mM, -□- 4.0 mM, -◇- 6.0 mM).

The results of Cr(VI) reduction at different (0.5–6 mM) Cr(VI) concentrations fit well with exponential decay. In the kinetics study, the time course reduction data fitted well ($R^2 > 0.97$) with the linearized form of exponential rate of equation when the initial Cr(VI) concentration was 1 mM (Figure 4). The rate of Cr(VI) reduction was found to increase with increase in Cr(VI) concentration.

The chromate reduction efficiency of this isolate was much sensitive to the incubation temperature tested over a range of 20 to 40 °C (Figure 5A). Maximum (62.5%) chromate reduction was recorded at 35 °C showing gradual decrease in Cr(VI) reducing ability at 40 °C, which showed nearly 56% Cr(VI) reduction. The Cr(VI) reducing ability of this isolate was much less (21% reduction) at lower temperature (20 °C). Chromate reduction by SKPD 1204 also appeared to vary

greatly due to the influence of the initial pH (4.0 to 8.0) of the growth medium. Cr(VI) reduction was maximum (60%) at pH 7.0 (Figure 5B).

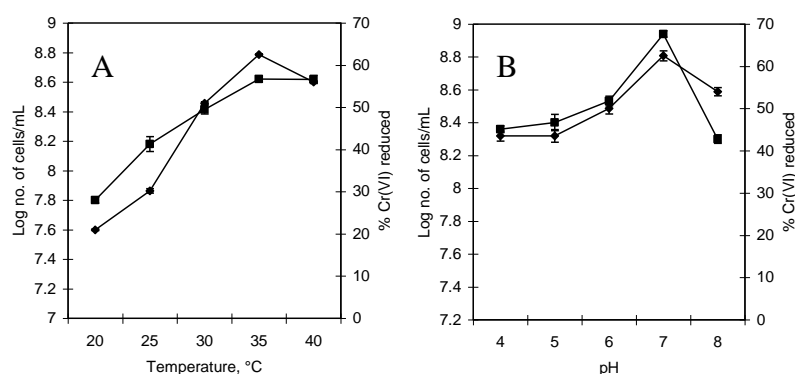


Figure 5. Effect of temperature [A] and pH [B] on chromate reduction during growth of *Corynebacterium paurometabolum* SKPD 1204.

The effect of inoculum density of the bacterial isolate was tested on the growth and chromate reduction by adding freshly prepared inoculum ranging from 10^5 – 10^{10} cells/mL. It was evident that with increase in cell density, the reduction capability of SKPD 1204 gradually increased. However, even at highest cell density i.e. at 10^{10} cells/mL complete reduction of initial 2 mM Cr(VI) failed to occur (showing 82% reduction) and very little cell growth was observed. At lower cell concentration (10^5 cells/mL) nearly 43% of initial Cr(VI) was reduced (Figure 6).

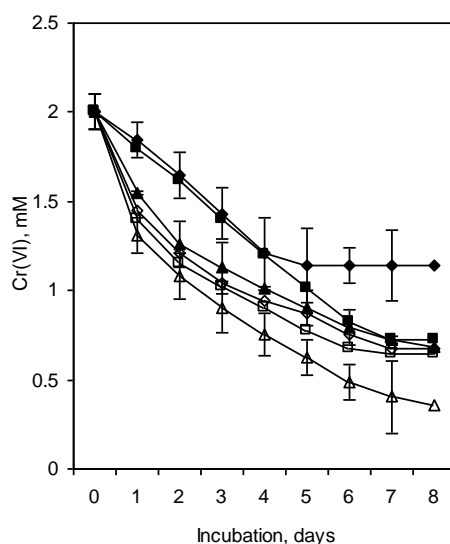


Figure 6. Effect of cell density on Cr(VI) reducing [expressed as the residual Cr(VI), mM] ability of *Corynebacterium paurometabolum* SKPD 1204 (-◆- 10^5 , -■- 10^6 , -▲- 10^7 , -□- 10^9 , -△- 10^{10} cells/mL).

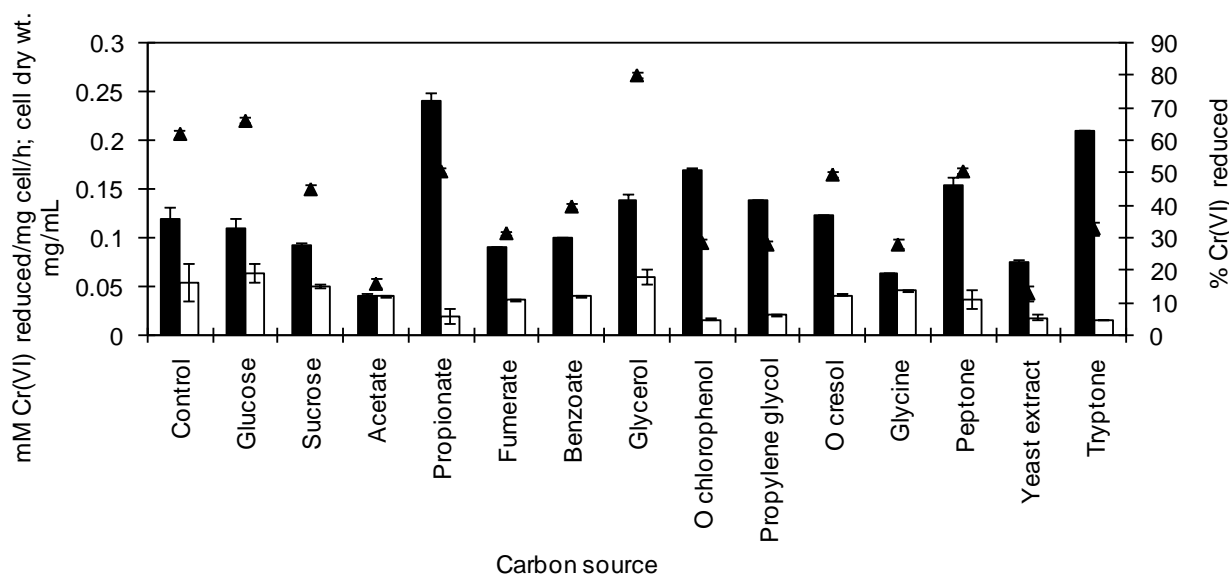


Figure 7. Effect of carbon source on growth (expressed as cell dry wt. mg/mL ■), % Cr(VI) reduced [▲], and rate of reduction (mM Cr(VI)/mg of cell/h □) by *Corynebacterium paurometabolum* SKPD 1204.

Reduction of Cr(VI) during growth of SKPD 1204 was studied in presence of propionate, acetate, benzoate, fumerate, glucose, sucrose, glycerol, propylene glycol, chlorophenol, glycine, yeast extract, tryptone, peptone and cresol as carbon sources (Figure 7). Glycerol followed by glucose was found to be the most efficient carbon source reducing 80% and 66% of initial 2 mM chromate during 8 days of incubation respectively. In addition, the isolate was able to utilize a wide variety of carbon sources including sugar, sugar alcohols, amino acids and organic acids with considerable variation of reduction efficiency. Yeast extract was the least efficient carbon source for SKPD 1204 showing nearly 13% chromate reduction. However, the rate of Cr(VI) reduction was high in case of propionate and tryptone showing a value of 0.24 and 0.21 mM Cr(VI) reduced/mg of cell/h although they reduced only 50 and 33% of initial 2 mM Cr(VI) respectively.

Metal cations like Ni(II), Co(II), Zn(II), Cu(II) and Cd(II) in the growth medium in general resulted in inhibition of chromate reduction (Table 2). However, presence of Mn(II) was found to be neither inhibitory nor promotive in nature as it resulted in almost 60% reduction of 2 mM Cr(VI) in the control set without additional metal supplementation. The presence of Zn(II) was found to be most inhibitory showing only 5.5% reduction of Cr(VI) with significant impairment of growth.

Effects of anions on Cr(VI) reduction revealed that most anions tested were inhibitory to the process of Cr(VI) reduction. Nitrate was found to be most inhibitory in nature showing nearly 37% reduction of initial 2 mM Cr(VI) and sulphite was least inhibitory showing 46.5% reduction as against 62.5% reduction in the control (Table 2).

Table 2. Effect of cations and anions on Cr(VI) reduction during growth of *Corynebacterium paurometabolum* SKPD 1204 under batch culture.

(Cation/Anions)*	Incubation, days							
	2		4		6		8	
	A	B	A	B	A	B	A	B
Control	7.75 ±0.06	36.5 ±0.1	8.38 ±0.01	48.00 ±0.6	8.98 ±0.0	56.0 ±0.6	8.92 ±0.0	62.5 ±0.0
Cations								
Ni(II)	8.23 ±0.04	38.5 ±0.12	8.98 ±0.02	39.5 ±0.06	9.19 ±0.06	35.0 ±0.0	8.60 ±0.02	35.6 ±0.0
Mn(II)	8.54 ±0.04	13.50 ±0.34	9.04 ±0.0	46.5 ±0.02	9.09 ±0.0	50.5 ±0.06	9.09 ±0.02	59.5 ±0.06
Cu(II)	8.25 ±0.04	25.7 ±0.39	8.6 ±0.06	26.5 ±0.0	8.98 ±0.05	26.5 ±0.0	8.30 ±0.0	26.5 ±0.0
Co(II)	8.14 ±0.02	33.5 ±0.07	8.60 ±0.06	42.0 ±0.0	8.80 ±0.0	43.5 ±0.06	8.36 ±0.0	43.5 ±0.06
Zn(II)	8.28 ±0.03	5.5 ±0.27	8.64 ±0.04	5.5 ±0.27	8.86 ±0.04	5.5 ±0.2	8.68 ±0.02	5.5 ±0.13
Cd(II)	7.42 ±0.02	9.8 ±0.54	7.58 ±0.04	9.8 ±0.64	7.38 ±0.06	10.8 ±0.54	7.16 ±0.09	10.8 ±0.2
Anions								
Sulphate	7.65 ±0.16	33.5 ±0.14	7.76 ±0.36	36.5 ±0.15	7.75 ±0.16	40.5 ±0.15	7.45 ±0.16	40.5 ±0.15
Sulphite	7.67 ±0.08	36.5 ±0.04	7.50 ±0.31	40.0 ±0.13	7.45 ±0.16	46.5 ±0.91	7.35 ±0.16	46.5 ±0.53
Phosphate	7.66 ±0.18	30.5 ±0.04	7.64 ±0.1	39.7 ±0.18	7.05 ±0.16	39.7 ±0.58	7.05 ±0.06	39.7 ±0.58
Nitrate	7.88 ±0.08	32.5 ±0.04	7.50 ±0.01	34.0 ±0.09	7.45 ±0.16	36.5 ±0.65	7.35 ±0.16	36.5 ±0.65

A= Growth, log no. of cells/mL; B =% Cr(VI) reduced

* All cations /anions were added to the chromate reduction medium at 1 mM level.

Results represent mean of triplicate sets ±SD.

Among the different inhibitors used, all were inhibitory to Cr(VI) reduction. Sodium fluoride was most inhibitory followed by CCCP showing 36.25% and 42% reduction of initial 2 mM Cr(VI) respectively (Figure 8). DNP was found to be least inhibitory in nature showing reduction almost comparable to the control.

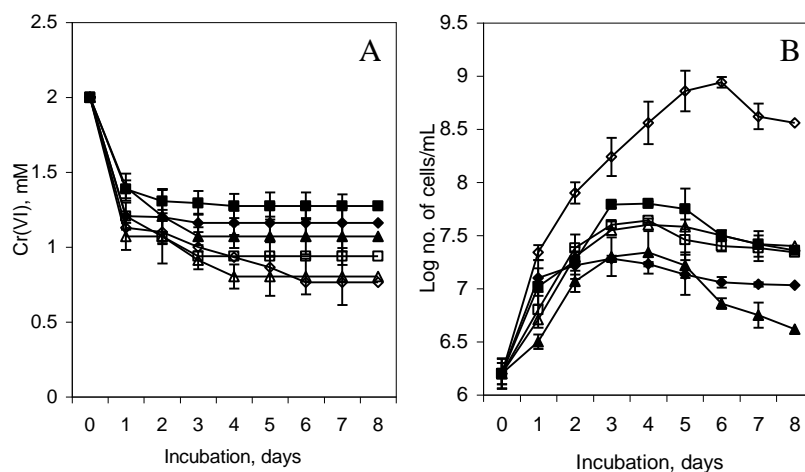


Figure 8. Effect of metabolic inhibitors on chromate reduction [expressed as the residual Cr(VI), mM] [A] and growth [B] by *Corynebacterium paurometabolum* SKPD 1204 (-◇ Control, -◆- CCCP, -■- Sodium fluoride, -▲- Sodium azide, -□- DCC, -△- DNP).

4. Discussion

The bacterium, *C. paurometabolum* SKPD 1204 (MTCC 8730) isolated from the mine seepage water of Sukinda chromite mines of Odisha, India has been reported previously from this laboratory [15] and deposited in the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India with an Accession number of MTCC 8730. The isolate has also been shown to tolerate up to 16 mM of Cr(VI) [16]. Steinhaus (1941) [17], though recorded *C. paurometabolum* from the mycetomes and ovaries of bed bugs (*Cimex lectularius*) but the ability of the isolate to tolerate and reduce Cr(VI) has not been reported elsewhere. However, members of the genus *Corynebacterium* isolated from tannery samples were capable of reducing Cr(VI) [18]. Since the chromium contaminated environments often contain different metal cations and anions [19], the present *Corynebacterium* isolate, SKPD 1204 showed a higher tolerance to chromium and other heavy metals (Figure 1) as an adaptive feature of continuous exposure to an environment containing high concentration of metal ions. The isolate was highly sensitive to Hg(II), which corroborates the findings of Tahri Joutey et al., [19] and Kang et al., [20] with those of *Serratia proteamaculans* and *Pseudomonas aeruginosa* AB93066 respectively. Similarly, *Bacillus* sp.ev3 [21] showed resistance to several metal ions and the order of resistance was found to be Ni>Pb>Zn>Cu>Cd>Hg. The main resistance mechanisms are claimed to be related with the membrane-potential dependent efflux of metals through different membrane transporters [22].

In addition to metal, the isolate SKPD 1204 also showed a high antibiotic resistance index indicating resistance to a number of antibiotics tested (Table 1). Similar, co-existence of metal and antibiotic resistance in Cr(VI) tolerant and reducing bacteria has been reported by several others [23,24]. It may be assumed that antibiotic resistance in these bacteria might have been resulted due to the selective pressure created by the metal-pollution and horizontal transfer of associated antibiotic resistance genes. The isolate was found to be sensitive to a number of antibiotics such as tetracycline, doxycycline, streptomycin, gentamycin, rifampicin, nalidixic acid and trimethoprim, which indicate that the isolate was most affected by the protein synthesis inhibitors along with inhibitors of nucleic acid metabolism.

The bacterium *C. paurometabolum* SKPD 1204 was able to reduce nearly 62.5% of initial 2 mM Cr(VI) within 8 days of incubation (Figure 2), however no visible characteristic green precipitate of Cr(III) was found as reported earlier with isolates like *Arthrobacter* sp. SUK 1201 [7].

The ability of this isolate to reduce Cr(VI) was probed under different Cr(VI) concentrations, pH and temperature. With increase in Cr(VI) concentration the reduction capability of the isolate decreased as higher concentration of Cr(VI) was found to be inhibitory to the cell growth and reductase enzyme activity (Figure 3). Similar reduction in the Cr(VI) reducing efficiency with increase in initial Cr(VI) was also noted in *Streptomyces sphaericus* [25] and *Bacillus amyloliquefaciens* [26].

Temperature is also an important factor that controls the Cr(VI) reducing efficiency. In the present study Cr(VI) reduction was adversely affected at both lower (20 °C) as well as higher temperatures (40 °C) (Figure 5A) mainly due to loss of enzymatic and metabolic activities of the cells due to prolonged incubation at higher temperature [27]. Cr(VI) reduction efficiency was found to be optimum at pH 7 (Figure 5B). An optimum pH of 6–8 for Cr(VI) reduction by *Acinetobacter* sp. B9 and *P. aeruginosa* AB93066 were reported by Bhattacharya and Gupta [28] and Kang et al., [20].

Microbial reduction of chromium is significantly influenced by different carbon sources. In the present study Cr(VI) reduction process of SKPD 1204 was positively influenced by the presence of glucose and glycerol (Figure 7). Glycerol micelles are considered to mimic the cellular membranes and enhance Cr(VI) reduction [29], while glucose has been previously reported by many authors [26,28] as the most suitable carbon source. The influence of citrate as a constituent of V. B. broth on growth as well as chromate reduction might have exerted uniform effect in all experimental sets.

Presence of different anions, cations and heavy metals in mine and industrial effluents are likely to influence the Cr(VI) reducing ability of the microbes in such environments. Here in this study, Cr(VI) reducing ability of the isolate SKPD 1204 was influenced adversely in presence of most anions and metal cations (Table 2). Inhibition of Cr(VI) reduction by SKPD 1204 by sulphates are most likely as it acts as a competitive inhibitor of chromate transport. However, Poopal and Laxman [25] and Javaid and Sultan [27] have reported that sulphate and nitrate had no effect on Cr (VI) reduction by *Streptomyces griseus*. Presence of metal cations such as Co(II), Ni(II), Cu(II) and Zn(II) were inhibitory to the process of reduction and were also found to adversely affect the growth of cells. Mn(II) was not inhibitory to the reduction process, but promoted the growth possibly as micronutrient. The inhibitory effect of Cd(II) was possibly due to formation of a mercaptide bond

with sulfhydryl groups of enzyme molecule resulting in alteration of the protein structure and the activity of the enzyme. Though Zn(II) was found to be promotive for reduction of Cr(VI) in many bacterial strains [30], here in this study it was inhibitory to chromate reduction, but not to the growth of *C. paurometabolum* SKPD 1204. The inhibitory effect of Zn on a number of enzymes has been explained by its interaction with the active sites containing zinc-binding ligands [31].

Chromate reductase activity of SKPD 1204 was severely affected by the metabolic inhibitors like NaF, CCCP and DCC due to disruption of enolase activity [32] and chemiosmotic gradient and inhibition of ATPase activity respectively. The growth of the isolate was also inhibited in presence of these inhibitors. Similar inhibition of Cr(VI) reduction was also evident with *Arthrobacter* sp. SUK 1201 [7]. DNP, an uncoupler has been reported to accelerate the respiratory chain linked electron transport mechanism and thereby showed chromate reductase activity more or less similar to control (Figure 8). Enhancement of Cr(VI) reduction by DNP has also been reported in *Burkholderia cepacia* [33] and *Staphylococcus gallinarum* [34].

5. Conclusion

Search for microbes capable of detoxifying Cr(VI) in chromite mine effluent has resulted in the isolation of heavy metal tolerant and chromate-reducing bacterium, *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730). The chromate reducing ability of the isolate, apparently a new record has been optimized under laboratory conditions with reference to a number of nutritional and environmental factors and found to be efficient compared to other strains reported so far. The biotechnological potential of such transformation of Cr(VI) to less toxic Cr(III) by *C. paurometabolum* SKPD 1204 could therefore, be an useful tool in detoxification of chromium pollutants.

Conflict of Interest

All authors declare no conflict of interest in this paper.

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