Research Article

# Reduction of Cr(VI) by *Bacillus megaterium* Isolated from Sewage Treatment Plant

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**Abstract**

A new strain was isolated from raw sewage of coimbatore corporation sewage treatment plant and identified as *Bacillus megaterium* using 16s r RNA sequencing. Isolated bacterium tolerated up to 250 ppm and reduced about 80% of 100 ppm Cr(VI) in 72 hours. It also reduced 100% of 25 ppm of Cr(VI) in 30 hrs and 50 ppm in 54 hours. Chromate reducing efficiency of isolate was maximum at pH 6.5 in presence of 0.5% glucose. Heavy metals Cd, Ni and Pb are found to have inhibitory effect on chromate reduction capacity of bacteria whereas Cu did not affect the growth and chromate reduction. The higher chromate reductase activity of 0.896 µmoles/min/mg of protein was observed in 50 µM Cr (VI) concentrations. The chromate reductase activity was found to be higher at pH 7.0.

**Keywords:** *Bacillus megaterium*, chromate reductase, chromium toxicity, sewage, enzyme activity

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## Introduction

Chromium is widely used in many industrial processes like tanning, electroplating, dyeing, paints and pigments, ceramics, textile processing, metal finishing, steel manufacturing and others. These industries pump out effluent which contains chromium either in trivalent or hexavalent forms that are the most dominant forms existing in environment. Hexavalent chromium in environment is soluble, and the most toxic species usually found as oxyanions, whereas trivalent chromium is hundred times less toxic, less soluble and less mobile, mostly found as oxides, hydroxides or sulfates, generally binds with organic matter [1]. Hexavalent chromium as a priority pollutant attributes strong oxidizing agents, mutagens, teratogens and is listed as Class A (human carcinogen) by the US Environmental Protection Agency [2]. Chromate oxyanions, analogous in structures with sulfate and phosphate ions, can readily permeate through biological membranes and their intracellular reduction of Cr(VI) generates Cr(V), Cr(III) valence states and reactive oxygen species (ROS). Chromium exists in various oxidation states ranging from 2+ to 6+, in which $Cr^{3+}$ and $Cr^{6+}$ are more stable in environment and differ markedly in their properties [3]. The molecular mechanisms of mutagenesis involve the formation of ternary adducts of intracellular Cr(III) with DNA, proteins and oxidative damage of DNA by Cr(V) and ROS [4].

Microorganisms are wonderful creatures that can accommodate everywhere, even in places where the higher organisms could not survive. Those microorganisms can be effectively used for bioremediation of chromium through biosorption, chromate reduction, chromate efflux and bioaccumulation. Chromium transformation studies have been carried out in several bacterial species and *Pseudomonas* is the first isolated strain from sewage sludge for chromium transformation under anaerobic conditions [5]. Biological reduction of hexavalent chromium has been demonstrated in several bacterial species including *Pseudomonas sp*. [6], *Shewanella oneidensis* [7], *Bacillus thuringiensis*, *Bacillus subtilis* [8] and *Bacillus methylotrophicus* [9].

## Methods

### *Bacterial isolation and chromate tolerance*

The organism was isolated from raw sewage collected from Ukkdam municipality sewage treatment plant containing 12 ug/mL of total chromium. The strains were isolated using nutrient agar medium and screened for chromium tolerance ranging from 10-1000 ug/mL. Chromium resistant strains were isolated and repeatedly streaked on nutrient agar containing 100 ug/mL of $K_2Cr_2O_7$. The 16s rRNA gene segment of isolated strain was amplified by PCR using the genomic DNA as template and the primer sequence 27-F and 149-R.

***Cr(VI) reduction and growth of bacteria***

Bacterial cultures were grown for overnight to an A660 of 1.0 in sterile broth. Time-course Cr(VI) reduction and growth was monitored in 100 mL sterile Minimal broth [10] containing filter sterilized 25, 50, 75 and 100 ug/mL of Cr(VI) concentrations as potassium chromate ($K_2Cr_2O_7$) and incubated in shaker at 100 rpm. Pre-grown inoculum (5%) was used for Cr transformation studies and uninoculated sterile broth served as control. Samples (2mL) were drawn for every 3 hours upto 72 hours and centrifuged at 6000 rpm for 10 minutes and the supernatants were analysed for remaining Cr(VI) following 1,5-diphenyl carbazide method by reading absorbance at 540 nm using spectrophotometer. The growth was monitored after every 3 h by measuring absorbance at 660 nm.

*Effect of pH on chromium reduction*

Effect of different pH (5.0-8.0), on the chromium reducing ability of the bacterial cells was determined in minimal media supplemented with 25 ppm of Cr (VI) as $K_2Cr_2O_7$.

*Effect of electron donors and other metal ions on chromium reduction*

Chromium reduction by isolated strains in presence of various electron donors (0.5%), such as glucose, acetate, peptone, sucrose, yeast extract, and tryptone were studied. Inhibitory or compatibility effect of other metals on chromium reduction, by isolates was performed using Cu, Ni, Pb and Cd (5 ppm).

*Chromate reductase activity*

The reaction system of 1 mL used, contained varying Cr(VI) final concentrations (25-200 µM) made up with potassium phosphate buffer, added with 0.2 mL of enzyme extract for chromate reduction. Reaction was kept constant with a reaction time of 30 min. at 30˚C. Unit enzyme activity for chromate reductase was derived as amount of enzyme that reduces 1 µM Cr (VI) per minute at 30˚C. Specific activity was defined as unit chromate reductase activity per milligram protein concentration in the cell-free extract. The amount of residual Cr(VI) was quantified by the diphenylcarbazide (DPC) method [11].

*Expression of chromate reductase*

The constitutive and induced expression of chromate reductase was determined in the absence of chromate (constitutive) and in the presence of 100 µM Cr (VI) (induced) by addition at 0 hr ($I_0$) and at 12 hrs ($I_{12}$) of culture growth. Chromate reducatse activity was expressed as µmoles/min/mg of protein.

*Effect of pH on chromate reductase activity*

The pH of assay mixture was adjusted from pH 2.0 to pH 9.0 using 0.1N HCl and 0.1N NaOH and inoculated with 12 hrs grown culture in 25 µM Cr (VI). The chromate reductase activity was determined and expressed as µmoles/min/mg of protein.

## Results and Discussion

Chromium reducing bacterium has been isolated and identified as *Bacillus megaterium* using 16s r RNA sequencing. Isolated *Bacillus megaterium* tolerated up to 250 ppm and reduced about 80% of 100 ppm Cr(VI) in 72 hours. It also reduced 100% of 25 ppm of Cr(VI) in 30 hrs and 50 ppm in 54 hours (**Figure 1**). Similar trends in growth and reduction of hexavalent chromate by immobilized cells of *Arthrobacter sp*. SUK 1205 was reported by [12]. Chromate reducing efficiency of the isolate was maximum at pH 6.5 in presence of 0.5% glucose as electron donor. Heavy metals Cd, Ni and Pb found to have inhibitory effect on chromate reduction capacity of bacteria whereas Cu did not affect the growth and chromate reduction. Camargo *et al*. (2003) [13] reported that maximum growth and chromate reduction by *Bacillus* sp. has occurred at pH 7.0. He has also reported that Cu(II) and Mn(II) stimulated the chromate reduction.

The higher chromate reductase activity of 0.896 µmoles/min/mg of protein was observed in 50 µM Cr (VI) concentrations (**Figure 2**). Similar trend of results were obtained in previous studies on Cr(VI) reduction by *Ochrobacterium intermedium* [14]. The expression of chromate reductase production was found in assay mixture induced with chromium and inoculated with 12 hrs grown culture (**Figure 3**). Polti *et al* (2010) [15] have reported

inducible expression of chromate reductase at 24 hours, while this study obtained maximum specific activity at 12 hours. Constitutive expression occurs when enzymatic and non-enzymatic pathways participate in the reduction of chromate analogous to the sulfate-reducing pathway [16]. The optimum pH required for the chromate reductase activity is 7 (**Figure 4**). Inducible chromate reductase exhibiting extracellular activity in *Bacillus methylotrophicus* exhibited optimum pH at 7.0 [9].

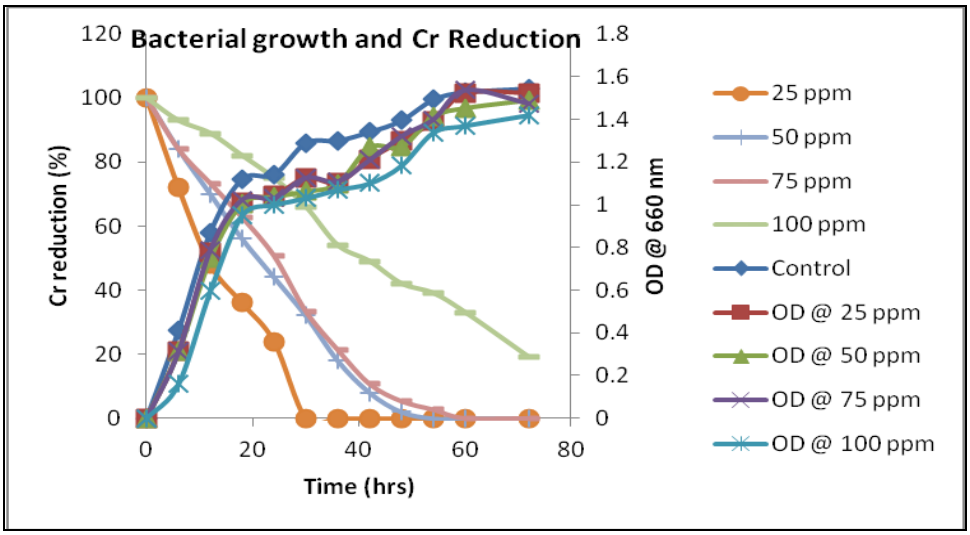


**Figure 1** Kinetics of bacterial growth and Cr reduction

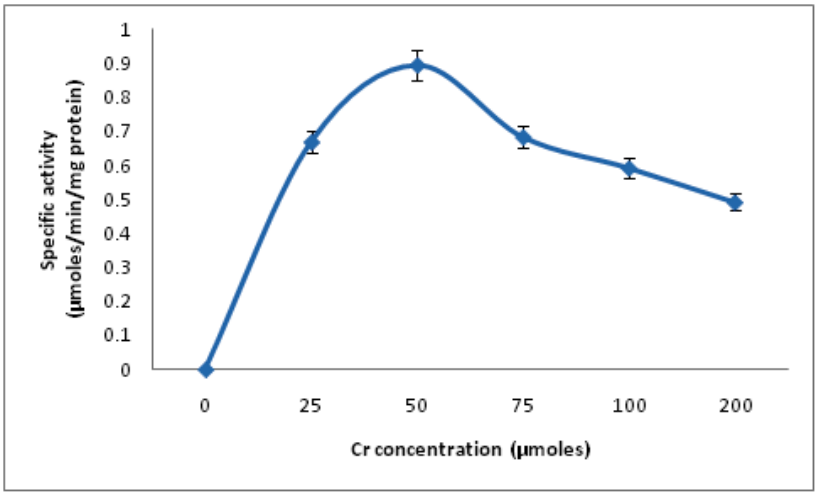


**Figure 2** Effect of chromate concentration on chromate reductase activity in *Bacillus megaterium*

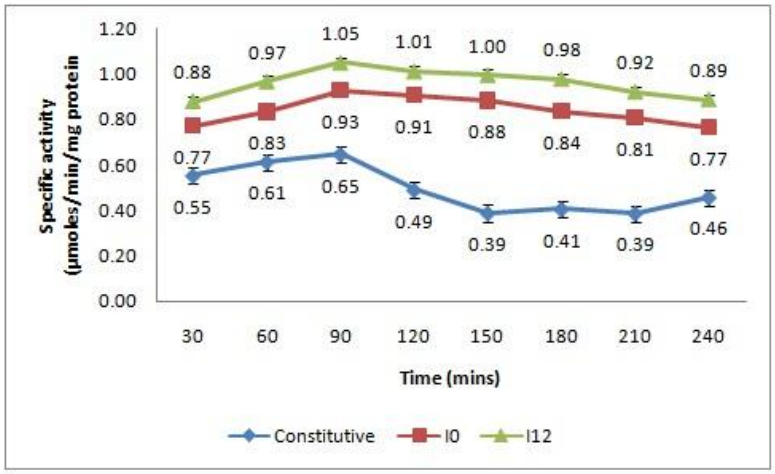


**Figure 3** Expression of chromate reductase in *Bacillus megaterium*

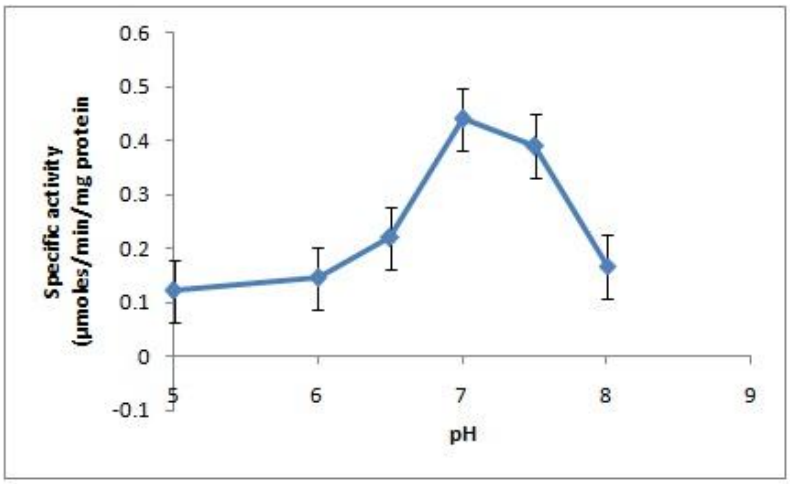


**Figure 4** Effect of pH on chromate reductase activity

## Conclusion

*Bacillus megaterium* a Cr(VI) reducing bacterium, was isolated from sewage and studies were conducted for its chromium reducing capabilities in presence of different electron donors and in varied pH ranges from 5.0 to 8.0. From the studies, it is evidenced that this bacterium could be potentially used for removal of chromium contamination at the pH of 7.0 especially in sewage systems.

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## Supplementary Information

***Sequencing information for Bacillus megaterium (FASTA)***

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