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ORIGINAL RESEARCH ARTICLE

# Characterization of a Hexavalent Chromium Reducing Bacterial strain isolated from Tannery Effluents of Kolkata

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**ABSTRACT**

A Gram-negative chromate reducing bacteria (PM 08) was isolated from the contaminated sites of tannery soil, Kolkata. Study revealed that PM 08 has a great potential for bioremediation of Cr (VI) . This bacterial isolate could grow and reduce chromate to concentration ranging from 100–500 mg/l at pH 6 and temperature 37°C and showed 35% reduction of hexavalent chromium within72 hrs. It also exhibited multiple heavy metal (Sn, Hg, Pb, Cd, Zn) tolerance.

**Key words**: Gram negative bacteria; Chromate reduction; Hexavalent chromium, Tannery effluent.

## 1. INTRODUCTION

Tanneries in the Greater Kolkata, West Bengal, still exclusively dependent on a traditional, unmodified and cheap chromium-based tanning procedure use to discharge a chromium-rich (80-250mg/L) effluent per day[1], simply through some narrow, non cemented, inland canals, without any waste treatment and thereby polluting all neighboring water-bodies. As the safe recommended value for discharge is less than 5ml/L, this excess chromium persists indefinitely accumulating in living tissues throughout the food chain [2]

The Cr (VI) has been recognized as one of the most dangerous environmental pollutants due to its ability to cause corrosion of the skin and respiratory tract, irritation of mucus membranes and skin, tubular necrosis of the kidneys, chronic bronchitis, mutations and lung carcinoma in humans. [3], [4]. Hence the removal of hexavalent chromium becomes the need of the hour.

The remediation of chromium contaminated sites poses a number of unique challenges, of which the conventional methods are expensive and lack specificity [5]. But bioremediation is one of the promising technologies that is expected to play an important role in waste to detoxify Cr (VI) in the soil to reduce it to Cr(III), so that it gets immobilized in the soil matrix.[6]

Many microbes were reported to reduce Cr(VI) under aerobic and anaerobic conditions involving *Bacillus* spp and *Staphylococcus* spp [7] *Streptomyces griseus* [8] *Pseudomonas* species, *Aeromonas* species, *Bacillus* species, *Micrococcus* species and *Microbacterium* sp [9] *Trichoderma viride*.[10] *Fusarium solani* [11]. The main advantages of using bacteria for Cr(VI) reduction are its rapid rate of growth, lesser demand for high energy input, and convenience in genetic manipulation for obtaining mutant strains with better efficiency in the bioremediation of hexavalent chromium from industrial effluents.

The present study deals with the screening, isolation, preliminary characterization of a novel bacterial strain from tannery effluent and evaluation of its efficacy for reduction of hexavalent chromium.

## 2. MATERIALS AND METHODS

Soil samples from tannery effluents (Beleghata Tannery, West Bengal, Kolkata) were collected with sterile containers and transported to the laboratory immediately for analysis.

### Isolation of organisms

The effluent samples were serially diluted (10 fold) before plating on sterile nutrient agar plates and for the selective isolation of chromium tolerant bacteria, subcultured on 50mg/ml of chromium (VI) supplemented nutrient agar

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medium. All the inoculated plates were incubated for 24 to 48 hrs at 37 ºC. A number of morphologically different colonies were randomly selected and sequentially cultivated for purification on the same medium.

**Cultivation of bacterial strain**

The strain was cultivated in 100 ml Erlenmeyer flasks each containing 20 ml Basal Medium (BM) composed of (gl-1): peptone 0.9; $(NH_4)_2HPO_4$ 0.4; KCl 0.1; $MgSO_4.H_2O$ 0.1and glucose 0.25 (pH 6) supplemented with $K_2Cr_2O_7$ 0.1 at 37°C for 24 hrs.

**Growth measurement of bacterial culture**

10 ml of samples were withdrawn at regular interval of time at room temperature and turbidity is measured by Spectrophotometer (Schimadzu, Japan) at 650 nm [12].

**Photomicrographic Study**

Both the unstained strain (phase contrast) and acridine orange stained strain was visualized under Axioscop-40 (Zeiss) microscope at 100X.

**Tolerance to Cr (VI)**

The effect of different concentrations of Cr (VI) on the growth of the isolate was determined by incubating the isolates in 50 ml nutrient broth contained in 250 ml Erlenmeyer flasks. The medium was amended with different concentrations of Cr (VI), namely, 50,100,150, 200 and 500 µg/ml.

**Analysis of Cr (VI) reducing ability**

The bacterial culture was centrifuged at 10,000 RPM for 5 minutes and the chromium reducing activity was estimated by the decrease in chromium concentration in supernatant with time using hexavalent chromium specific colorimetric reagent S-diphenyl carbazide (DPC) 0.25% (w/v) prepared in acetone (AR) to minimize deterioration. The reaction mixture was set up in 10 ml volumetric flask as follows: 200 ml or 400 ml sample or standard $K_2Cr_2O_7$ (10mg/l) volume was made to 1 ml using glass distilled water followed by addition of 330 ml of 6M $H_2SO_4$ and 400 ml of DPC and final volume was made to 10 ml using glass distilled water Pattanapipitpaisal [13]. The residual chromium was spectrophotometrically measured at 540 nm.

**Effect of temperature, pH on the growth**

The strain was grown in different flasks containing media with various initial pH (4-9) at 37°C and at various cultivation temperature (4-50°C) maintaining initial pH of the medium at 6.0 to check the optimum pH and temperature for growth respectively.

**Evaluation of Heavy metal tolerance**

The strain was grown in basal medium (BM) supplemented with various metal ions ( 0.1 g/L) to check the tolerance of the bacterial isolate to various heavy metals .

**Chemicals**

All the chemicals used are of analytical grade.

All the above experiments were done in triplicate and average value was considered.

## 3. RESULTS AND DISCUSSION

After preliminary screening, a total of 5 chromium tolerant bacterial isolates representing morphologically different bacterial colony were able to grow on chromium containing plates. Out of which a motile, Gram negative, catalase positive, coccus with ability to utilize glucose was found to tolerate 200 mg/l chromium and was selected as the working strain and was designated as PM 08 (**Fig 1A & B**).

The strain PM 08 although could grow well at a temperature ranging between 25- 40°C and preferred to grow at acidic media, the most suitable temperature and pH were found to be was 37°C and 6.0 respectively (**Fig 2 A & B**).

The strain PM 08 showed tolerance to various heavy metals. viz. $Hg^{2+}$, $Cd^{2+}$ $Sn^{2+}$,Li+,$Pb^{2+}$, $Zn^{2+}$ ,$Co^{2+}$, but highest growth was shown in presence of hexavalent Chromium (**Table 1**). As other metals were also remaining present in the industrial effluents, resistance of the isolate to various metallic salts might be proved effective for their survival and successful bioremediation. The multiple metal resistances of PM 08 was similar to that reported *Ochrobactrum tritici* 5bv11. [14]

The strain could tolerate about 500mg/l of hexavalent Chromium in the medium (**Table 2**), which was similar to those bacterial strains *Arthrobacter crystallopoites* [15], *Pseudomonas* sp. CRB5 [16], consortia of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae*. [17] lesser than *Bacillus maroccanu s*[18], *Bacillus* sp. ES29 [19],*Bacillus cereus* ES04 [19], *Corynebacterium hoagii* ChrB20 [18] but higher than, *Bacillus megaterium* [20] *Frankia* strains [21],*Ralstonia metallidurans* AE [22], ,*Pseudomonas stutzeri* [23],*Escherichia coli* [24] , *Arthrobacter* sp. and *Bacillus* sp. [25]

Addition of Cr (IV) at early stage of log phase resulted into retarded growth while significant survival rate was found when added in the late log phase. Similarly no effect was observed when Cr (IV) was added in the stationary or lag phase (**Fig 3**).





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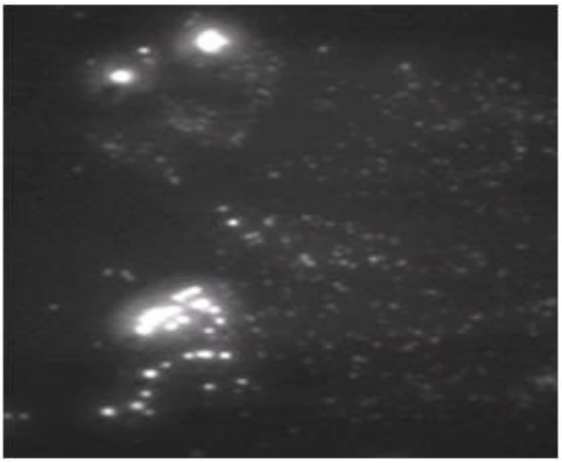
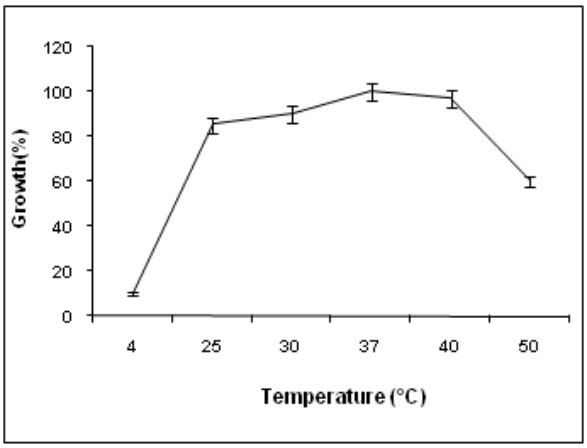
Chromate reduction as monitored at different initial chromium concentration ranging from 100 to 400 mg/l in aerobic conditions at pH 6 and 37°C showed that 100 mg/l was reduced to 65% in 72 h (**Fig 4**) The effects of different concentrations of Cr (VI) on its reduction by the isolate PM 08 showed that extent of Cr (VI) reduction was accomplished by some specific chromium reductase. [26] and due to the phenomenon of enzyme limitation, the rate of chromium reduction decreased with increasing chromium concentration.

**(A)**

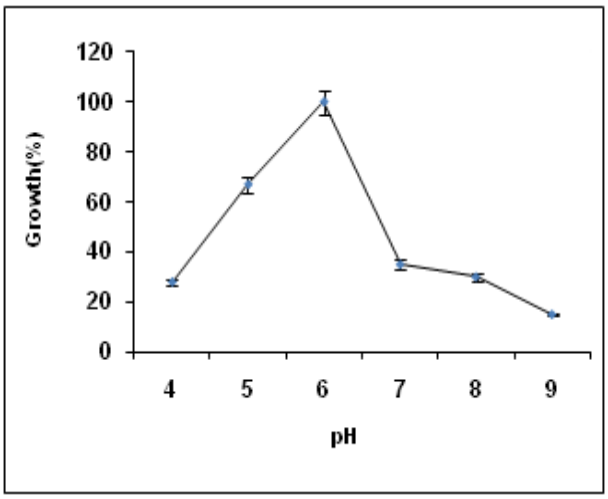


**(B)**

**Fig 1:Phase Contrast Microscopic view (A) and Fluirescent Microscopic view (B) of the bacterial isolate PM 08**




**(A)**




**(B)**

**Fig 2: Optimum temperature (A) and pH (B) for the growth of bacterial isolate PM 08**

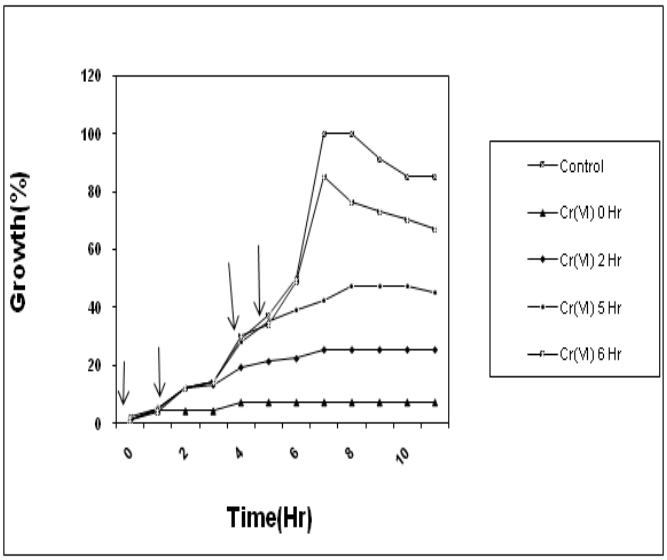



**Fig 3: Growth curve of PM 08 in presence of Cr(VI) added at different phases of growth (Glucose concentration-1%, pH6, temperature 37°C)**

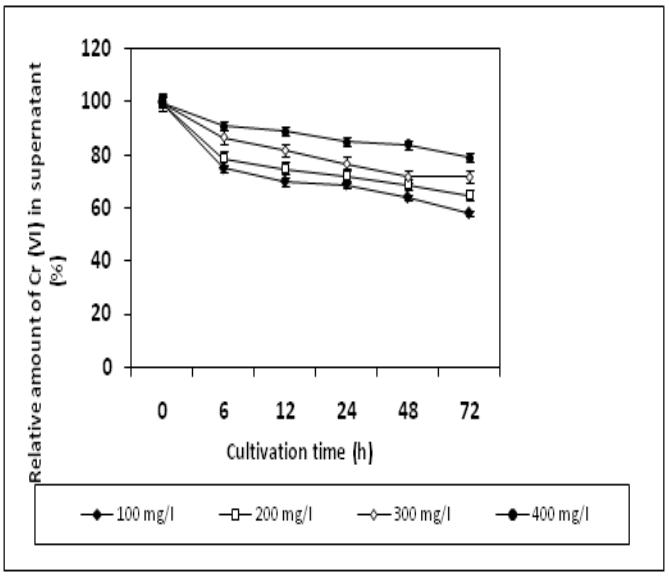



**Fig 4: Effect of time and Cr (IV) concentration on chromate reduction in PM 08**





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**Table 1: Relative tolerance of bacterial isolate PM 08 against various metal ions**

| Additives (100mg/l) | Growth (%) |
|---|---|
| Control | 100 |
| $Cr^{6+}$ | 95±2 |
| $Hg^{2+}$ | 60±1.8 |
| $Cd^{2+}$ | 75±1.5 |
| $Sn^{2+}$ | 50±2.8 |
| $Ca^{2+}$ | 78±3.1 |
| $Pb^{2+}$ | 78±2.9 |
| $Mn^{2+}$ | 67±1.6 |
| $Li^{+}$ | 45±1.6 |
| $Zn^{2+}$ | 35±2.1 |

**Table 2: Relative tolerance of bacterial isolate PM 08 to various concentration of Cr (VI)**

| $K_2Cr_2O_7$ Concentration (mg/l) | Growth (%) |
|---|---|
| Control | 100 |
| 100 | 95±2.0 |
| 200 | 85±2.3 |
| 300 | 72±3.2 |
| 400 | 60±2.5 |
| 500 | 30±1.8 |
| 600 | 11±1.6 |

## CONCLUSION

Leather industries and tanneries with conventional drainage systems emitting chromium enriched effluents pose a chronic chromium stress and can decrease microbial diversity, biomass and activity jeopardizing the ecological balance. Hence, successful microbial-based chromium remediation technologies are required to be implemented. It requires not only a suitable strain but also a better understanding of the microbial community response to these stress conditions [27].

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