

Bacterial Tolerance and Reduction of Chromium (VI) by *Bacillus cereus* Isolate PGBw4

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Abstract: This study aimed to determine the bacterial tolerance to chromium (Cr^{6+}) in three growth media, such as nutrient broth, Luria Bertani (LB) broth and mineral salt media in terms of Minimum Inhibitory Concentration (MICs). Among the seven metal resistant soil bacteria, *Bacillus cereus* isolate PGBw4 and *Bacillus cereus* strain ES-4a1 showed highest tolerance against Cr^{6+} in all three media. *Bacillus cereus* isolate PGBw4 was used as an effective and environment friendly agent for detoxifying Cr(VI) and reduction study in this research. The bacterial isolate mitigated toxic effects of Cr(VI) more efficiently from 100mg/L to 500mg/L within 24 and 48 hours respectively. The maximum amount of reduction of Chromium (VI) was 70.67 percent at 100 of Cr(VI) mg/L concentration after 48 hours of incubation and the lowest was 42 percent at 500mg/L Chromium concentration after 24 hours of incubation.

Keywords: Chromium (VI), *Bacillus cereus* Isolate PGBw4, Tolerance, Reduction

1. Introduction

Heavy metals contamination is a global environmental concern because it is difficult to remove these contaminants from the environment unlike many other pollutants [1]. These are recognized to be powerful inhibitors of biodegradation activities [2]. The metal elements such as lead, nickel, copper, cadmium, zinc, chromium and mercury are accumulated in different environmental segments and can make toxic effects to the living organisms.

Chromium salts are widely used by tannery industries of Bangladesh and led to large quantities of this element being released to the surrounding environment. Only hexavalent chromium and trivalent chromium are ecologically important because these are the more stable oxidation states. But the chromium (VI) is one of the most dangerous environmental pollutants due to its ability to cause mutations and cancer in biological system. Being mutagenic, carcinogenic and teratogenic, Cr(VI) is approximately 100-fold more toxic than Cr(III) [3]. But recent years this heavy metal is used with large industrial application in textile dyeing, chemicals

and pigments production, wood preservation, tanning activity and electroplating for surface treatment [4].

Serious concern about the toxicity of Cr(VI) compounds necessitates the recovery and reuse of chromium from industrial wastes and it is essential to convert the unrecovered Cr(VI) to a less toxic form. Several bacteria have been described as being able to reduce Cr(VI) to Cr(III) under aerobic and anaerobic conditions and the biological transformation of the very toxic Cr(VI) to the less toxic and less mobilized Cr(III) has been reported as an alternative to the physical and chemical approaches [5, 6, 7]. Accordingly, the decontamination of hexavalent chromium is of great importance. The conventional methods for heavy metals removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation, reverse osmosis [8]; evaporation, solvent extraction and membrane separation [4]. These processes are expensive and present some technological problems, mainly when applied to diluted metal solution. Bio-sorption is a process in which certain types of biomasses, viable or dead, may bind and concentrate

heavy metals from aqueous solutions [9]. Microorganisms have a high surface area-to-volume ratio because of their small size and therefore, they can provide a large contact interface, which would interact with metals from the surrounding environment. Chromium (VI) reduction by different microorganisms has been well documented in different studies [10].

The objective of this study was to evaluate the Cr(VI) resistance and Cr(VI)-reducing ability of metal-stressed bacteria.

2. Materials and Methods

2.1. Chromium Tolerant Bacteria

All the bacteria used in this study were earlier reported [11]. A highly chromate-resistant bacterium (*Bacillus Cereus* isolate PGBw4) was also earlier reported [11] and used in this study isolated from Agricultural field of Dhamrai, Bangladesh.

2.2. Method of Bacterial Tolerance Determinations and MICs

For evaluation of the sensitivity as MIC [12] to chromium (Cr^{6+}) of seven bacterial isolates, three media such as nutrient broth, LB broth, and mineral salt media with different concentrations of Cr^{6+} (ranging from 250 to 1500 $\mu\text{g/mL}$ using $\text{K}_2\text{Cr}_2\text{O}_4$) were prepared and then the bacteria were cultured readily in screw cap tubes. After 48 hours of incubation the growth of bacteria was confirmed by

spreading over 0.1mL of liquid culture of isolates from each tube on nutrient agar plate to test the appearance of growth. Growth of the bacterial culture was determined visually as positive or negative. The absence of bacterial growth indicated its sensitivity, while the presence of bacterial growth in corresponding metal concentrations indicated that bacteria were resistant at those concentrations. All of the experiments were replicated twice.

2.3. Chromium (VI) Reduction Activity

Chromate reductive activity was determined by measuring the decrease of hexavalent chromium. Chromium (VI) was analyzed by the S-diphenylcarbazide method [13], [14]. The 100mL of LB broth containing varying concentrations (100 to 500mg/L) of Cr(VI) as $\text{K}_2\text{Cr}_2\text{O}_7$ were inoculated with 1mL of enriched cells suspension and incubated under 30°C for 24 hours. Un-inoculated medium containing Cr(VI) served as controls. Samples were collected at different time intervals up to 48 hours, and centrifuged at 10,000 rpm for 10 min. The supernatant obtained after centrifugation was used to measure Cr(VI) concentration. The Cr(VI) content in each supernatant was determined by measuring absorbance of the purple complex of Cr(VI) with 1, 5-diphenylcarbazide at 540nm using spectrophotometer. The efficiency of chromate reduction was determined in terms of % Cr(VI) reduction by measuring the difference between Cr(VI) concentration at 24 and 48 hours. The rate of reduction of chromium was determined by the following equation.

$$\text{Percent chromium reduction} = \frac{\text{Un-inoculated initial concentration of Cr(VI)} - \text{inoculated final concentration of Cr(VI)}}{\text{Un-inoculated initial concentration of Cr(VI)}} \times 100$$

3. Result and Discussion

3.1. Determination of MICs and Metal Resistance of Bacteria

Seven bacterial strains were separated based on high degree of chromium resistance and were used for evaluation

of bacterial sensitivity to chromium. Minimum Inhibitory Concentrations (MICs) of all the bacterial strains against Cr^{6+} in three media viz. in nutrient broth, LB broth and mineral salt media have shown that the strains were sensitive to high concentration of Cr^{6+} ions and were incapable to grow at that concentrations. The MICs of Cr^{6+} ions of each bacterium in three media are given in Table 1.

Table 1. MICs against chromium (Cr^{6+}) of each bacterium cultured in Nutrient broth, LB broth and mineral salt media.

Identified bacterial strain	MIC against Cr^{6+} ($\mu\text{g/mL}$) in nutrient broth	MIC against Cr^{6+} ($\mu\text{g/mL}$) in LB broth	MIC against Cr^{6+} ($\mu\text{g/mL}$) in mineral salt media
<i>Bacillus amyloliquefaciens</i> strain SCSAAB0007	1050	1200	700
<i>Bacillus cereus</i> isolate PGBw4	1300	1450	1050
<i>Bacillus cereus</i> strain ES-4a1	1300	1450	1050
<i>Bacillus subtilis</i> strain 1320	1000	1200	1000
<i>Micrococcus luteus</i> strain P4_3	650	850	300
<i>Bacillus pocheonensis</i> strain TR2-6	300	400	200
<i>Bacillus megaterium</i> strain H2	850	1000	550

In this study, the results of bacterial sensitivity showed that the MIC of identified strains against Cr^{6+} ranged from 300 to 1300 $\mu\text{g/mL}$ in nutrient broth, 400 to 1450 $\mu\text{g/mL}$ in LB broth and 200 to 1050 $\mu\text{g/mL}$ in mineral salt medium. The bacterial metabolism depends on the composition of media where bacteria are cultured and the composition of growth medium influences the adaptation mechanisms. In the present study

the MIC values were found higher in LB broth than that in nutrient broth and mineral salt media. Reference [15] reported that the metal-microbe interactions viz. metal uptake mechanisms and precipitation are largely influenced by the composition of growth media. Reference [16] reported the results of MIC for chromium was 150 to 500 $\mu\text{g/mL}$ and for copper 200 to 300 $\mu\text{g/mL}$ for different bacteria. The orders of

bacterial sensitivity to Cr^{6+} in this study are *B. pocheonensis* > *Micrococcus luteus* > *B. megaterium* > *B. amyloliquefaciens* > *B. subtilis* > *B. cereus*.

3.2. Chromium Reduction Measurement

The ability of *Bacillus cereus* isolate PGBw4 of reduction of Cr(VI) at varying concentrations are shown in the Figures 1 and 2. Chromium (VI) reduction from the medium after 24 hours was started with the concentration of 100mg/L, 200mg/L, 300mg/L, 400mg/L, and 500mg/L respectively. Reduction of chromium (VI) was more or less similar to the observation made by reference [17] who reported 100% removal of chromium (VI) by *Pseudomonas putida* and *Serratia proteamaculans* starting with an initial concentration of 2mg/L. Both the strains were isolated from tannery waste [17]. But this study for chromium removal indicated that the isolate could tolerate chromium (VI) and further studies were done with the isolate for reduction of chromium (VI).

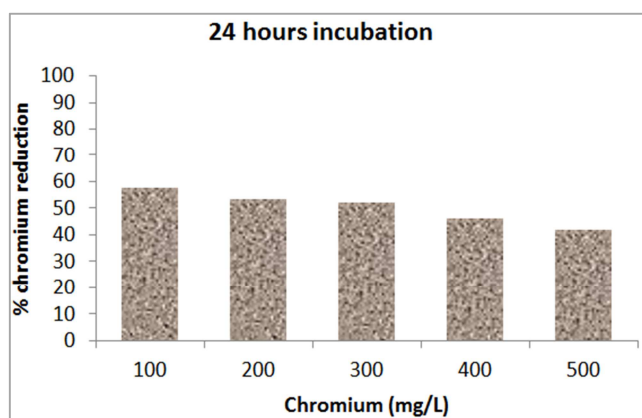


Figure 1. Reduction of chromium (VI) at different concentrations after 24 hours of incubation.

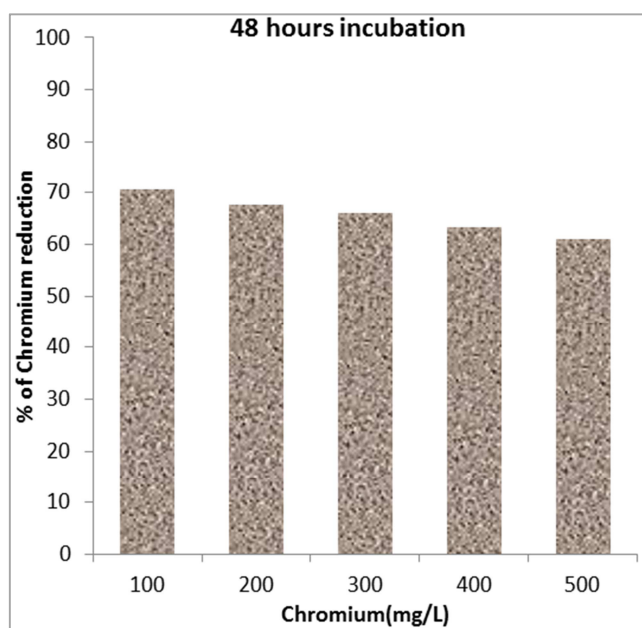


Figure 2. Reduction of chromium (VI) at different concentrations after 48 hours of incubation.

Chromium (VI) reduction by *Bacillus cereus* isolate PGBw4 was found to depend on the time or days of incubation (Figure 1 and 2). The maximum average percentage of chromium (VI) reduction was 57.66 and 70.67 at 100mg/L concentration after 24 and 48 hours of incubation, respectively (Figure 1 and 2).

Percent reduction of chromium (VI) was decreased with increasing chromium concentrations (Figure 1 and 2). This is due to the fact that as the volume of inoculums was constant relatively fewer amounts of bacterial cells were available for chromium (VI) reduction from the media, when concentrations were increasing. The lowest amount of reduction (42%) was occurred at 500mg/L of chromium concentration after 24 hours of incubation (Figure 1) but this reduction was increased to 61% after 48 hours period (Figure 2). Reference [18] similarly with *Pseudomonad* strain CRB5 reported that the reduction rate decreased during the first 24 hours at Cr(VI) concentrations of 30 and 40μg/mL. Reference [19] also reported 99.7% reduction of 112.5μg/mL Cr(VI) by *Pseudomonas fluorescence* LB300 within a period of 289 hours. The *Pseudomonad* strain CRB5, however, showed complete reduction of 20μg/mL of chromate after 120 hours [20]. Reference [21] however showed that the rate of Cr(VI) reduction by *Pseudomonas putida* strain SDCr-5 decrease with time irrespective to initial Cr(VI) concentration used.

4. Conclusion

The results of this study have confirmed that *Bacillus cereus* isolate PGBw4 is one of the most promising bacterial isolate that could tolerate and reduce chromium (VI). The rate of reduction has an increasing trend with increasing the incubation time and concentration upto 500mg/L within 48 hours. The highest percentage of reduction was found at low concentration of chromium. In this study, it is also confirmed that the sensitivity of bacterial isolates showed that the MIC of bacterial strains used in this research against Cr^{6+} was highest in LB broth medium. Information obtained through this study revealed that among various types of chromium resistant bacterial strain of *Bacillus* and *Pseudomonas*, *Bacillus cereus* isolate PGBw4 can be used with other bacteria found by other scientists to mitigate the toxicity effect of chromium in the environmental samples.

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