ORIGINAL ARTICLE

Stamford Journal of Microbiology, 2023.
ISSN: 2074-5346 (Print); 2408-8846 (Online)

Vol. 13, Issue 1, p. 25-29
DOI: https://doi.org/10.3329/sjm.v13i1.70411

BIOREMEDIATION OF HEXAVALENT CHROMIUM BY BACTERIA ISOLATED FROM BURIGANGA RIVER, DHAKA CITY

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Received 10 October 2023/Accepted 27 November 2023

Environmental pollution due to hexavalent chromium (Cr^{6+}) is widespread because of the anthropogenic activities in various industrial processes, notably in leather tanning. Hexavalent chromium (Cr^{6+}) is considered as highly toxic, carcinogenic, and mutagenic due to its high solubility in water, interaction with cellular proteins, and biological membrane permeability. Trivalent chromium (Cr^{3+}), on the other hand, is less water-soluble, and relatively benign in nature. Thus, bioreduction of toxic Cr^{6+} to relatively non-toxic Cr^{3+} by microorganisms can be an inexpensive and eco-friendly option for chromium bioremediation. In this regard, the present study attempted to isolate chromium-reducing bacteria from Buriganga River in order to assess their capability for chromium bioremediation. Ten chromium-tolerating bacterial isolates were successfully identified. The results revealed that these isolates, particularly strains of *Bacillus subtilis*, exhibited a remarkable ability to remove up to 89% of hexavalent chromium from the contaminated medium within three days of incubation.

Keywords: Hexavalent chromium, trivalent chromium, environmental pollution, anthropogenic activities, bioremediation, diphenylcarbazide

INTRODUCTION

Hexavalent chromium (abbreviated as Cr (VI) or Cr^{6+}) is a toxic heavy metal that is classified as group 1 carcinogen by the International Agency for Research on Cancer (IARC) (1). Environmental pollution due to hexavalent chromium (Cr^{6+}) is widespread because of the anthropogenic activities in various industrial processes such as electroplating, leather tanning, steel production and pigment production. In these industries, chromium compounds are used in large quantities and are discharged into the environment. The discharge of untreated industrial effluents into rivers, lakes, and other water bodies can result in the contamination of the aquatic ecosystem and the surrounding soil (2, 3). This pollution of heavy metals can easily get access to the food web and contaminate food and water bodies. Ingestion of food and water contaminated with Cr^{6+} can cause a wide range of detrimental effects on animal and human health. Chromium exposure is associated with respiratory, hepatic, renal, and reproductive problems, as well as neurological disorders and cancers, e,g, lung cancer, stomach cancer (4). However, control of such pollution and removal of Cr^{6+} from industrial effluents and wastewater are critical environmental challenges.

Among heavy metals, chromium represents an intriguing case. In its stable oxidation states, III (+3) and VI (+6), it has remarkably different characteristics in terms of toxicity. Cr^{6+} is considered as highly toxic, carcinogenic, and mutagenic due to its high solubility in water, and high biological membrane permeability. Cr^{3+} (trivalent chromium), on the other hand, is less water-soluble, and relatively benign in nature. Cr^{3+}cations usually form complexes or chelates, and may enter minerals, where they substitute iron or aluminum (3). An effective way of immobilizing chromium is therefore to reduce $Cr^{6+}$to Cr^{3+}. While traditional remediation approaches for Cr^{6+} removal from water and wastewater are based on chemical and physical methods, bioremediation of Cr^{6+} by microorganisms can be an eco-friendly and cost-effective approach for chromium bioremediation.

Buriganga River, located in the capital city of Bangladesh, Dhaka, is one of the most polluted rivers in the world. The river is heavily contaminated with various pollutants, including Cr^{6+}, due to the discharge of untreated industrial effluents and domestic sewage (5, 6). Cr^{6+} pollution in the river is particularly due to release of tannery effluent from Hazaribagh tanneries (3, 6). A portion of the effluents from tanneries in Hazaribagh are conveyed to the lagoon while the other portion discharged into nearby low-lying areas through drain, which eventually falls into the Buriganga River (5). Considering the adverse effects of tannery waste on both human health and the environment in Hazaribagh, the government of Bangladesh has formulated a new plan "Hazaribagh Tannery Relocation Project (HTRP)" to shift Hazaribagh Tannery Complex to a new site in Savar (7). All tanneries were relocated to Savar by 2017; however, the contamination of the Buriganga River has persisted for such a long duration that a recent relocation is unlikely to be sufficient in significantly reversing the pollution. Some studies conducted post-relocation indicate that pollution levels in the Buriganga, including chromium levels, remain

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substantial (7, 8). In Cr^{6+}- laden environments such as Buriganga River, bacteria are likely to adapt specific resistance systems to survive by evading metal stress through efflux or minimizing uptake and reducing the Cr^{6+} to Cr^{3+}. Thus, bacteria that have the potential to bioremediate chromium can be found in Buriganga River. The aim of the research was to isolate Cr^{6+}-resistant bacteria from the Buriganga River, assessing their tolerance to high Cr^{6+} concentrations, identifying and characterizing the isolates, and evaluating their capacity for Cr^{6+} bioremediation.

MATERIALS AND METHODS

Water sample procurement: Water samples were collected aseptically from the Buriganga River and transported to the laboratory within 2 hours of collection (9). The sample was then subjected to further analysis as detailed below.

Primary screening of chromium-tolerant bacteria: The water sample obtained from the Buriganga River was serially diluted with normal saline to obtain five different dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} (9). 0.1 ml of each of these diluted samples were inoculated separately on freshly-prepared nutrient agar plates supplemented with 50 ppm potassium dichromate ($K_2Cr_2O_7$), which were then incubated overnight at 37°C. Ten distinct colonies with distinct morphologies were observed in the plate inoculated with the 10^{-3} diluted sample (10, 11). These Ten colonies were picked up and sub-cultured separately to obtain pure cultures which were named as BR 1, BR 2, BR 3, BR 4, BR 5, BR 6, BR 7, BR 8, BR 9 and BR 10.

Characterization and presumptive identification of the isolates: The isolates were identified based on the biochemical and morphological characteristics (12). The biochemical assays that were performed include Gram staining, Spore formation test, Catalase test, Mannitol Salt Agar (MSA) Test, Mannitol Egg Yolk Polymixin B (MYP) Agar Test, Citrate utilization test, Indole test, Methyl Red test, and Voges-Proskauer test.

Determination of MIC (Minimum Inhibitory Concentration): Nutrient agar plate containing different concentrations of Cr^{6+} (50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm) were inoculated aseptically from the cultures of each bacterial strain. These plates were incubated at 37°C for 48 hours. The MIC was considered to be the lowest concentration of Cr^{6+} at which no growth occurred (13, 14).

Chromium reduction assay: DPC (1,5-Diphenylcarbazide) assay was used to determine the chromium reduction ability of the five isolates. The DPC reagent was prepared by dissolving 250 mg 1,5 diphenylcarbazide in 50 ml acetone (15, 16). For the assay, the chromate solution was prepared in LB (Luria-Bertani, Himedia Ltd. India) broth at different concentrations of $K_2Cr_2O_7$, i.e., 50 ppm, 100 ppm, and 200 ppm. The pH of all these solutions was adjusted to 2 by the addition of 2M concentrated H_2SO_4 (sulphuric acid) followed by the addition of DPC. This immediately resulted in a colour change that showed different intensities of the colour at different concentrations of $K_2Cr_2O_7$ as confirmed by absorbance measurement at 540 nm (15, 16). In order to determine the chromium reduction by the isolates, the isolates were incubated in LB broths containing three different chromium concentrations (i.e., 50 ppm, 100 ppm and 200 ppm) at 37°C. Following 3 days of incubation, the isolates were centrifuged for 10 minutes at 4000 rpm. The supernatants containing the remaining concentration of Cr (VI) were collected and adjusted to a pH of 2 after which 2 drops of DPC were added. The colour change was observed and the absorbance at 540 nm was determined by spectrophotometry. The percentage reduction of Cr (VI) was calculated by using the following formula:

$$Cr^{6+} \text{ reduction } (\%) = \{(A-B) \div A\} \times 100$$

Where, A=Absorbance before incubation; B= Absorbance after incubation.

RESULTS

Determination of MIC (Minimum Inhibitory Concentration) of the isolates: The MIC of chromium varied between 300 ppm to 500 ppm across the strains. BR 4, BR 7 and BR 8 showed the highest resistance to chromium (500 ppm) (Figure 1).

Biochemical characterization and presumptive identification of the chromium-tolerant isolates: The bacterial isolates were biochemically characterized and presumptively identified by a set of biochemical assays. All isolates were found to be gram positive (Table 1).

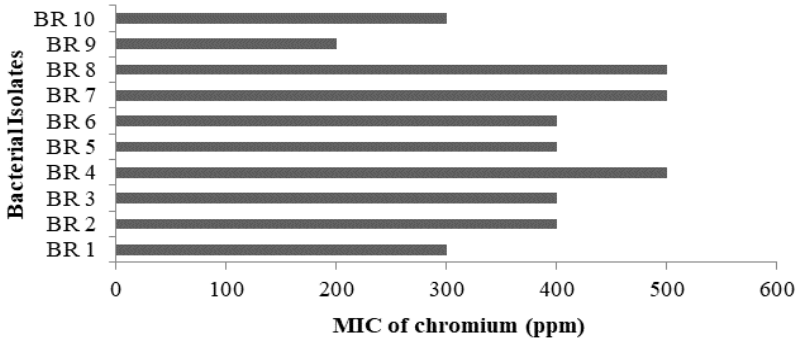

Figure 1: Determination of MIC of chromium for different isolates.

Table 1: Presumptive identification of the chromium tolerant isolates.

Isolates	Biochemical test								Presumptive identification
	Gram stain reaction	Spore formation test	Catalase test	Indole test	MR test	VP test	Citrate test	Mannitol fermentaion test	
BR 1	+	-	+	-	-	-	+	+	*Staphylococcus aureus*
BR 2	+	-	+	-	-	-	-	-	*S. epidermis*
BR 3	+	+	+	-	+	+	+	+	*Bacillus subtilis*
BR 4	+	+	+	-	+	+	-	+	*B. subtilis*
BR 5	+	+	+	-	-	+	-	-	*B. cereus*
BR 6	+	+	+	-	-	+	-	-	*B. cereus*
BR 7	+	+	+	-	+	+	-	+	*B. subtilis*
BR 8	+	-	+	-	-	-	+	+	*S. aureus*
BR 9	+	+	+	-	+	+	-	+	*B. subtilis*
BR 10	+	-	+	-	-	-	+	+	*S. aureus*

Reduction of 50 ppm hexavalent chromium by different bacterial isolates: The reduction percentages of the isolates at 50 ppm initial chromium concentration ranged from 15.07% to 89.19%, BR 4 being the highest reducer (Figure 2).

Reduction of 100 ppm hexavalent chromium by different bacterial isolates: The isolates showed reduction percentages ranging from 6.54% (BR 1) to 82.22% (BR 4) at 100ppm initial concentration of chromium (Figure 3).

Reduction of 200 ppm hexavalent chromium by different bacterial isolates: The isolates have been shown to reduce 4.44% (BR 1) to 31.41% (BR 4) chromium when incubated at 200 ppm initial Cr^{6+} concentration (Figure 4, 5).

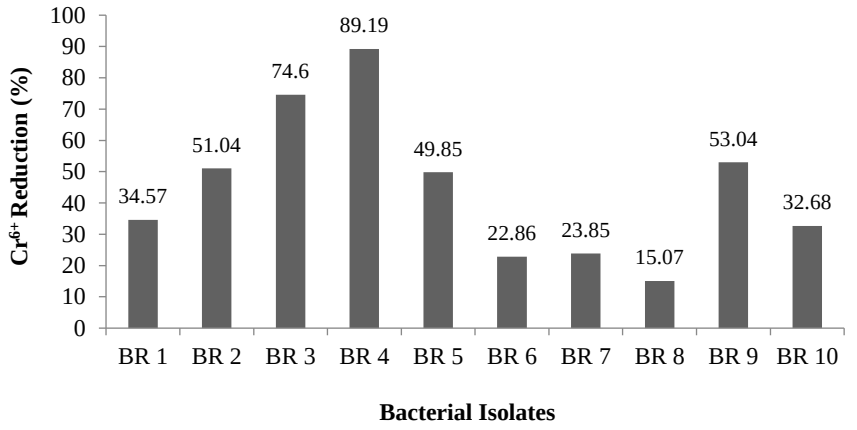

Figure 2: Reduction of 50 ppm hexavalent chromium by different bacterial isolates.

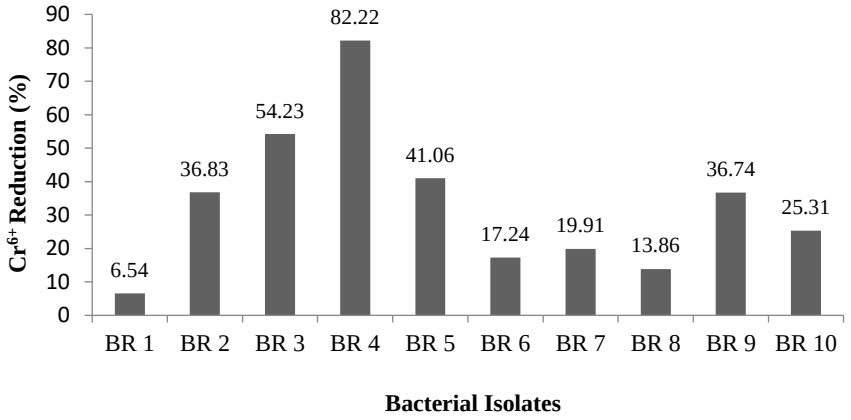

Figure 3: Reduction of 100 ppm hexavalent chromium by different bacterial isolates.

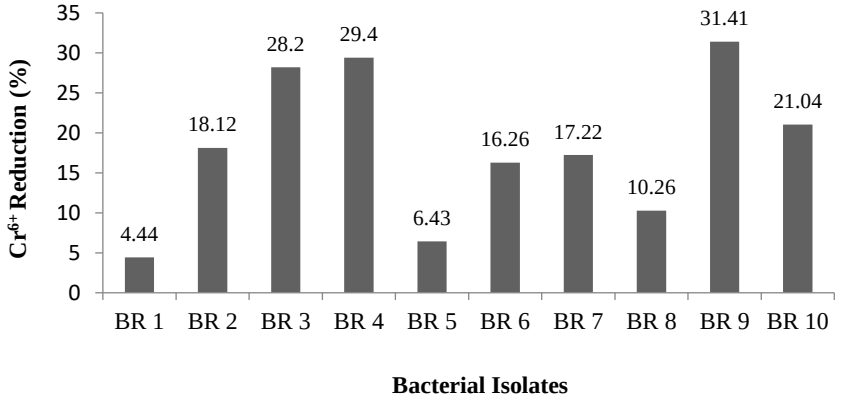

Figure 4**:** Reduction of 200 ppm hexavalent chromium by different bacterial isolates.

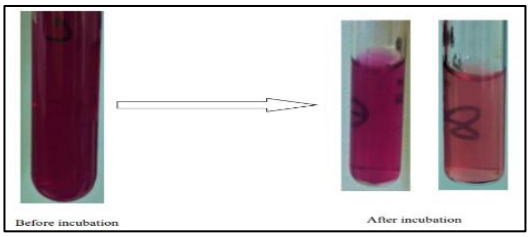

Figure 5: Reduction of 100 ppm hexavalent chromium by BR 3 and BR 4 following incubation as indicated by DPC (1,5-Diphenylcarbazide).

DISCUSSION

Water sample from Buriganga River was screened for chromium-tolerant bacteria. Ten morphologically distinct Cr^{6+} tolerant bacterial strains were screened for their Cr^{6+} tolerance limit. The MIC (Minimum Inhibitory Concentration) of chromium for the bacterial strains was ranged from 300 ppm to 500 ppm (Figure 1).

Buriganga River is severely contaminated with a range of harmful pollutants, including Cr^{6+}, as a consequence of unregulated discharge of industrial effluents such as tannery effluents (5). In such Cr^{6+}- laden environments, bacteria are likely to be evolved resistance systems to withstand heavy metal stress. According to ecological theory, a stressor such as chromium could lead to changes in the bacterial community structure by promoting the growth of metal-tolerant strains (13). Thus, it was not surprising to find highly chromium-tolerant bacteria from the river.

The chromium-tolerant isolates were further investigated for their chromium reduction capacity at different initial concentrations of potassium dichromate. The bacterial isolates were incubated in LB (Luria-Bertani) broth containing different concentration of potassium dichromate (i.e., 50 ppm, 100 ppm, and 200 ppm).

The reduction percentage of these bacterial isolates was found to be dependent on the initial concentration of chromium in the medium. At different initial concentrations (50 ppm, 100 ppm, and 200 ppm), the isolates displayed varied reduction capabilities, with a range from 15.07% to 89.19% at 50 ppm (Figure 2), 6.54% to 82.22% at 100 ppm (Figure 3), and 4.44% to 31.41% at 200 ppm (Figure 4).

The findings indicated that all bacterial strains were capable of reducing Cr^{6+}, but the reduction percentage tended to rise as the initial chromium concentration decreased. The maximum reduction was observed at the lowest concentration (50 ppm), while the least reduction occurred at the highest concentration (200 ppm). This trend aligns with similar concentration-dependent reduction patterns found in other studies (13, 17). This correlation could be attributed to the toxicity of Cr^{6+} at high concentrations, which might hinder the bacterial growth and activity.

The bacterial isolates BR 3 and BR 4 (presumptively identified as *Bacillus subtilis*), were able to tolerate and reduce Cr^{6+} in a range of concentrations, from 50 to 200 ppm, with the maximum reduction capacity of 74.6% and 89.19%, respectively, at 50ppm initial chromium concentration. This indicates their potential for use in the bioremediation of contaminated sites or industrial discharge site with high chromium levels.

Seven of the isolates belong to the genus of *Bacillus* spp., (BR 3, BR 4, BR5, BR 6, BR 7, BR 9, and BR 10) while three of the isolates belong to the genus *Staphylococcus* spp., (BR 1, BR 2 and BR 8) as presumptively identified by biochemical assay (Table 1). These findings are consistent with previous studies that have demonstrated the ability of *Bacillus* spp., (13, 18, 19) and *Staphylococcus* spp., (20, 21, 22) to reduce Cr^{6+}.

One notable limitation of this study is that it was conducted under laboratory conditions and thus, may not accurately reflect the ability of the isolates to bioremediate Cr^{6+} in complex environmental conditions present in contaminated sites or industrial discharges. Therefore, further studies are needed to evaluate the potential of these bacterial strains to bioremediate in field conditions. Moreover, the molecular identification of the isolates, as well as the molecular mechanism of chromium removal, and growth kinetics is also needed to be investigated.

CONCLUSION

The present study aimed to isolate and characterize bacteria from the Buriganga River capable of bioremediating hexavalent chromium, a highly toxic and carcinogenic pollutant. The study successfully identified ten bacterial isolates capable of reducing hexavalent chromium under laboratory conditions, and the results indicated that they were able to remove up to 89% of the hexavalent chromium from the contaminated medium within a period of three days. The bacterial isolates, particularly *B. subtilis* (BR 3 and BR 4) can be a potential candidate for chromium bioremediation in Cr^{6+} contaminated environment and Cr^{6+} containing industrial discharge. Further study is required to understand the growth kinetics and molecular mechanism of chromium reduction of the bacterial isolates, as well as the reduction capacity in field condition.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Department of Microbiology, Stamford University Bangladesh for their technical assistance throughout the study.

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