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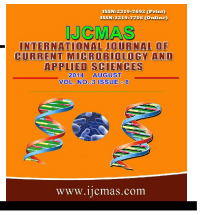


**Original Research Article**

# Bioremediation of Chromium by *Bacillus subtilis* and *Pseudomonas aeruginosa*

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**A B S T R A C T**

The polluted environment with toxic heavy metals is found throughout the world along with industrial progress. Industrial wastes containing toxic metals can arise from a wide variety of industrial processes. Today indiscriminate and uncontrolled discharge of metal contaminated industrial effluents in the environment has become an issue of major concern. Heavy metals are difficult to be removed from the environment and usually accumulate in animal and plant tissues. Chromium tolerant bacterial strains namely, *Bacillus sp.* and *Pseudomonas sp.* were isolated from the chromium contaminated soil. The two isolates tolerated high concentrations of up to 50 ppm/L chromium in nutrient broth. The growth responses of the bacterial isolates to different concentration of chromium were carried out in this study. The responses of the bacteria were dependent on time of incubation and chromium concentration. The analysis of the results showed that there was significant difference in the growth of the isolates at different concentration of chromium. As the concentration of chromium increased, the growth of the bacterial isolates decreased. The growth of the isolates were slightly inhibited at chromium concentration of 25 ppm/L and highly inhibited at 50 ppm/L when compared to other lower concentrations such as 5 and 10 ppm/L. The present study revealed the capacity of the bacterial isolates to grow in Cr, and the isolates can be used to remove Cr from the environment.

## Introduction

Pollution due to chemicals including heavy metals is a problem that may have negative consequences on the biosphere. The most abundant pollutants in the wastewater and in sewage are heavy metals (Hong *et al.*, 1996). Human activities such as mining operations and the discharge of industrial wastes have resulted in the accumulation of metals in the environment and eventually are accumulated through the food chain, leading to serious ecological and health problem.

Heavy metals have vast industrial applications due to their technological importance. Chromium is the seventh most abundant element on earth and exists in several oxidation states. The most prevalent forms of chromium in the natural environment are hexavalent and trivalent (Chung *et al.*, 2006). Chromium is a strong oxidant, Crystalline; steel-gray, lustrous, hard metallic, is an odourless. Found in rocks, animals, plants, and soil. Chromium

maintaining efficient glucose, lipid, carbohydrate and protein metabolism. It released by a large number of industrial operations such as electroplating, chromate manufacturing, leather tanning industry, dyes and pigment fabrication and wood preservation. Chromium is toxic to the reproductive system and the unborn child. Hexavalent chromium is a widespread industrial waste. Environmentally friendly processes need to be developed to clean-up and protect the environment by bioremediation technique (Stratten, 1987).

Microorganisms can sense variety of chemical signals in there surrounding. Depending on kind of signal is there, they shows a different action for example can move toward higher concentration of chemical as well as could repel itself in anti direction of toxic chemical. Soil bacteria are also attracted to various aromatic pollutants, such as heavy metals, benzene, naphthalene and chlorinated herbicides, leads to degradation of these compounds (Handelsman and Lawrence, 2002).

Bioremediation defined as any process that uses micro-organisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation may be employed to attack specific soil contaminants, such as degradation of metal contamination by bacteria.

## Materials and Methods

The soil samples were collected from the higher concentration of chromium contaminated pot. They were brought to the laboratory immediately and analysed.

The samples were serially diluted with sterile distilled water and plated with nutrient agar medium. The plates were incubated at 37°C for 48 hours. Among the bacterial colonies grown, two dominant colonies were selected and cultivated in nutrient broth for 48 hours. This two isolated organisms were subjected to biochemical tests for tentative identification following Bergy's manual of Systematic Bacteriology (Krieg and Holt, 1984).

After testing the resistance of the chosen bacterial strains to different concentrations of chromium prepared by dissolving required amount of potassium chromate ($K_2Cr_2O_7$), control, 5, 10, 25 and 50 ppm concentrations were selected for the experiment. Standard nutrient broth was prepared and autoclaved at 121°C for 15 minutes and was cooled in a water bath. In 250 ml Erlenmeyer flasks, 50 ml nutrient broth was taken along with the above mentioned concentration of chromium. Under aseptic conditions, the two chosen organisms were inoculated individually into these flasks with 0.1ml cells. The flasks were incubated at shaking incubator 140 rpm, $37^0$C temperatures. Uninoculated control flasks were also maintained in the same manner. After 48 hours, samples were taken from each flask and centrifuged at 8,000 rpm for fifteen minutes.

The supernatants were analysed with AAS for chromium concentration adopting standard methods (APHA, 1995). Percentage reduction in chromium concentration was calculated for each chromium concentration based on the initial and final readings.

## Results and Discussion

In this study, *Pseudomonas aeruginosa* was a gram negative rods, whereas *and Bacillus subtilis* was gram positive rods, observed based on the biochemical analysis (Table 1).

**Table.1** Biochemical characteristics of *Bacillus subtilis* and *Pseudomonas aeruginosa* from the higher concentration of chromium contaminated pot culture soil

| S.No | Biochemical test | *Bacillus subtilis* | *Pseudomonas aeruginosa* |
|---|---|---|---|
| 1 | Methyl red | _ | _ |
| 2 | Indole | _ | + |
| 3 | Catalase | + | + |
| 4 | Oxidase | _ | + |
| 5 | Urease | _ | + |
| 6 | Glucose | _ | + |
| 7 | Citrate | + | + |
| 8 | Lactase | _ | _ |
| 9 | $H_2S$ | _ | _ |

**Notes:** + Possitive ; - Negative

**Fig.1** Reduction initial and final concentration in chromium concentration in media by the isolates after 48 hours

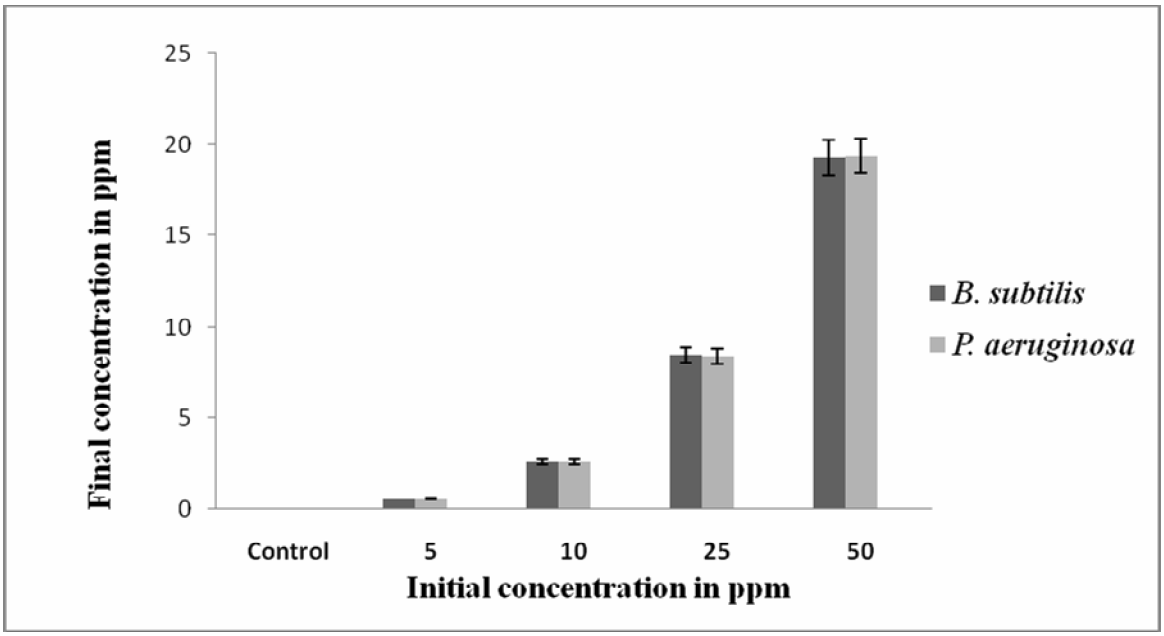
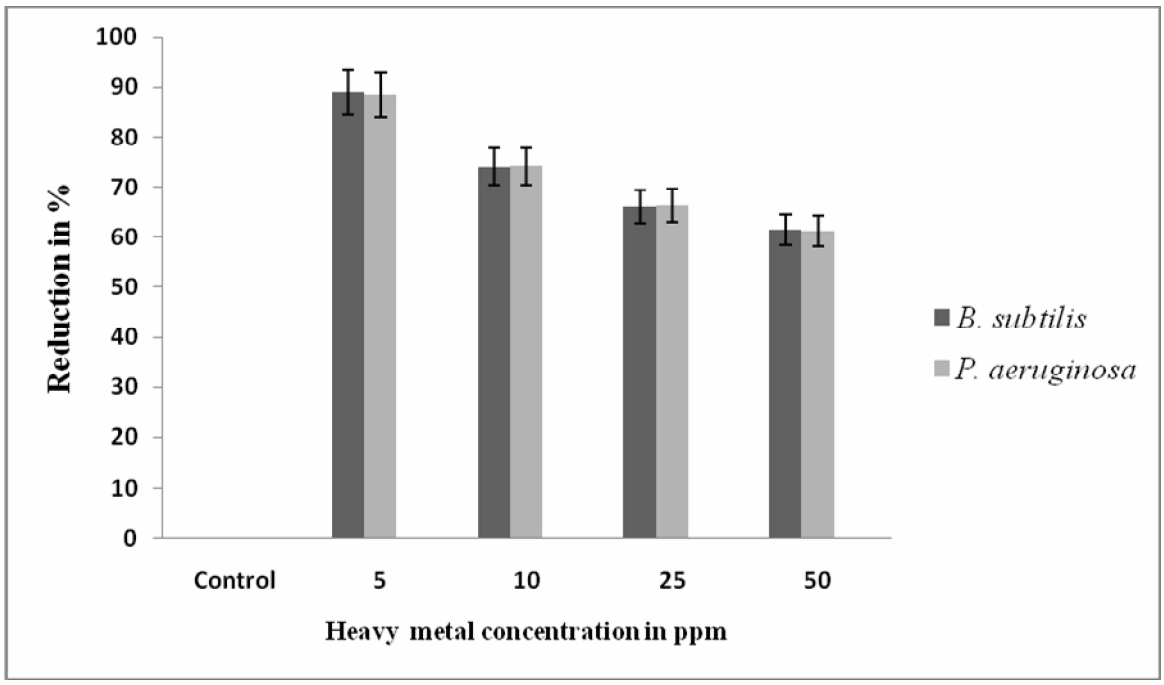


**Fig.2** Reduction percentage in chromium concentration in media by the isolates after 48 hours ($30^0$C, pH 7.0, Volume of media 50ml)




*B. subtilis* and *P. aeruginosa* were inoculate into Nutrient broth containing chromium at varying concentrations (control, 5, 10, 25 and 50 ppm). A control tube was also inoculated which lacked chromium.

The bacterial strain removed 87%, 73%, 65% and 60% chromium from medium in 48 hours starting with the initial concentration of 5 ppm/L, 10 ppm/L, 25 ppm/L and 50 ppm/L respectively (Fig. 1). Percentage removal of chromium was similar observation made by Basu *et al* (2014) who reported 97% removal of chromium *Basillus subtilis* starting with an initial concentration of 2.5 mg/L. The strain was isolated from wetlands Basu *et al.* (2014). Results of the present study for chromium removal indicate that the isolates could tolerate to chromium.

Percentage reduction in chromium concentration was calculated for each chromium concentration based on the initial and final readings (Fig. 2). Percentage removal was decreased with increasing chromium concentration (Fig. 1). This is due to the fact that as the volume of inoculum was constant relatively less biomass was available for chromium removal from the media, in case of higher concentrations. This result is similar to the observation of Basu *et al.* (2014) who reported lower chromium degradation with higher initial concentrations by a *Bacillus subtilis*. Raghuraman *et al.* (2013) has also reported that the higher reduction of chromium for lower initial concentrations by *P. aeruginosa* and *P. fluorescens.*

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