Studies on bioremediation of Chromium [VI] by bacteria isolated from alkaline Lonar Lake (MS) India

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ABSTRACT

Most of the industrial effluent contains chromium which is pulmonary carcinogen. Generally the industrial effluent has high pH, and bacteria isolated from normal environment cannot tolerate the alkaline condition in effluent. Therefore, aim of this study was to isolate bacteria alkaline environment having ability to survive and remediate the chromium from industrial effluent. The alkaline Lonar Lake is one of them, situated in the Maharashtra State (India). In present study, twelve water samples were collected from Lonar Lake and two bacterial strains were isolated on alkaline medium having pH 10 with 100µg/ml of chromium. The isolates were characterized biochemically and identified as *Pseudomonas* species by 16S rRNA sequencing.. The chromium remediation ability of the strains was estimated by the Spectrophotometric method of Di-phenyl carbazide and atomic absorption spectra (AAS) (Perkin Elmer) and the study revealed that the isolates SSG1 and SSG2 oxidized 65.38% and 64.88% of chromium after 96 hours of incubation respectively, showing the ability of organisms to tolerate the chromium concentration at alkaline pH and potential to remediate and detoxify Cr (VI) to Cr (III) as well.

Key words: Bioremediation of chromium, Lonar Lake, Di-phenyl-carbazide, *Pseudomonas*.

INTRODUCTION

Lonar is the third natural salt-water lake in the world. It is of immense interest as a natural phenomenon and the micro ecosystem that evolved within it. The uniqueness of this lake is its high salinity and alkalinity favouring the growth of alkaliphiles and haloalkaliphiles. The crater has international significance in terms of wet land, ecology, biology and limnology or hydrology. The extensive application of chromium in industries particularly leather tanning industries leads to the formation of chromium-contaminated soil and ground water which pose a serious threat to the living biota particularly to human health. Chromium is a potent pollutant which is mutagenic, carcinogenic and teratogenic in humans (Petrilli and Flora, 1977; Gale, 1978). Chromium is seventh most abundant element on earth and exists in several oxidation states from Cr^{2+} to Cr^{6+}. In nature it can be found either as Cr^{3+} or Cr^{6+}. Cr (III) is not toxic because, it easily gets adsorbed in soils and waters. But chromium VI is known to cause serious health hazard effects. It can cause allergic reactions, nose irritation and nose bleeds. Hexavalent chromium toxicity appears to be due to its rapid permeability through biological membranes and subsequent interaction of chromium with intracellular proteins and nucleic acids (Kotas and Stasicka, 2000).

At high levels, chromium damage cell membranes, alter enzyme specificity; disrupt cellular functions and damage structure of DNA (Bruins *et al.,* 2002). A conventional method used for the removal of hexavalent Cr is to use chemical procedures, which are expensive and lack specificity (Katiyar and Katiyar, 1997). As alternative, biological approaches utilizing microorganisms for highly selective removal of toxic metals coupled with considerable operational flexibility. Biological approaches may reduce Cr (VI) to Cr (III) intracellularly or by making extra cellular environment more reducing or lowering pH to favour Cr (VI) reduction. Immobilised cells as bio-films, beads or inert support have been found to be most effective in designing bioreactors for heavy metal degradation (James, 2002; Benazir *et al.*, 2009). Hence it will be more preferable to have a biological approach to remediate chromium.

The most efficient method for the detection of chromium is Di-phenyl carbazide method and atomic absorbance spectra. Benazir *et al*., (2009) studied the ability of consortia of three organisms to remediate the chromium from industrial effluent.

The remediation of chromium was detected by di-phenyl carbazide method and atomic absorbance spectra. Though there is no Cr source in aquatic ecosystem of Lonar Lake. But the uniqueness of Lake, make it centre of curiosity to study versatility of microbial flora present in Lonar Lake by all aspects. Present study also inspired in such aspect regarding the degradation of chromium by alkaliphilic bacteria isolated from Lonar Lake and study there potential by spectrophotometric method.

MATERIALS AND METHODS

Sampling site and Sample collection

Water and sediment samples were collected from four different sites of Lonar Lake during August 2012. Water samples were collected in sterile screw capped plastic bottles and sediment samples were collected in Zip lock bags. All collected samples were stored in a 4^0C to arrest any biological activity and transported to laboratory for experimental analysis.

Enrichment of samples

For isolation of Cr reducing bacteria, sediment (1 g) and water (10ml) samples from Lonar Lake were separately inoculated in 250 ml Erlenmeyer's flask containing 100 ml sterilised Nutrient broth medium having alkaline pH 10 and 1 ml of $K_2Cr_2O_7$ solution to make chromium concentration 100 µg/ml. the flasks were incubated at 37^0C for 24 h. after 24 h incubation 10 ml culture broth was transferred in freshly prepared nutrient medium having same composition. The same procedure was successively repeated 4 times for enrichment of bacterial culture.

Isolation and biochemical characterization

After enrichment, the isolation was made by inoculating the culture broth on solid nutrient agar plate with pH 10. The well isolated and morphologically distinct colonies from the plate were selected and stock cultures were prepared for further analysis. All these isolates were further characterized by standard biochemical test according to Bergey's manual of systematic bacteriology.

Identification of selected strain

Based upon morphological, biochemical characters identification of isolated bacterial cultures was carried out. Among the bacterial cultures one strain SSG1 was subjected for 16S rRNA sequencing based on unique biochemical traits and high 'Cr' remediation ability.

Cr (VI) analysis

Chromate reducing activity was estimated as the decrease in chromate concentration in supernatant with the time using the estimation method of Cr (VI) [1], by specific colorimetric reagent, 1, 5-diphenyl Carbazide (DPC) (250 mg in 50 ml Acetone; 10% H_2SO_4, to give pH 2 ± 0.5). Standard graph for estimation of chromium was prepared by using concentration of chromium 20µg/ml to 120µg/ml by using DPC. The rate of chromium degradation was estimated by taking the absorbance at 540 nm on UV-VIS spectrometer (make Systronics). The rate of degradation of chromium was estimated per hour of incubation.

Atomic absorbance spectra for chromium degradation

AAS is a quantitative technique used mostly for determining the concentration of a particular metal element within a sample. Atomic absorption method measures the amount of energy in the form of photons of light that are absorbed by the sample. The chromium concentration within the medium in presence of culture was determined by atomic absorbance spectroscopic technique (Perkin Elmer) available at University Central Instrumentation Cell (CIC), Amravati. The deionized chromium present in the medium was detected by detector at 357.4nm.

RESULTS AND DISCUSSION

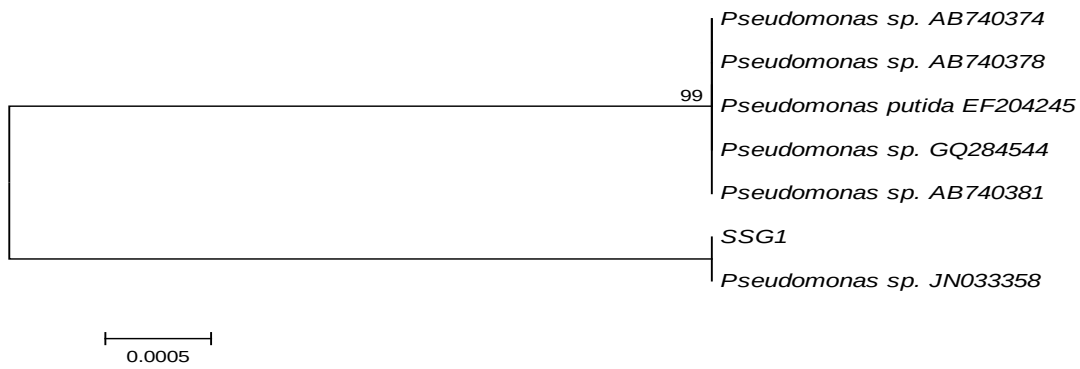
Two bacterial spp. were isolated from the twelve samples collected from the alkaline Lonar Lake on Nutrient broth having pH 10 and chromium 100 µg/ml concentrations. After enrichment for successive five times, isolation was made on alkaline nutrient agar plates. The isolates SSG1 and SSG2 were Gram negative short rod and Gram positive coccobacilli respectively. Both the isolates were different in there cultural and biochemical characteristics (Table 1). The biochemical characteristics of the isolate SSG2 were also done by the commercially available Hi-media rapid detection kit KB003. On the basis of morphological and biochemical characteristics and high 'Cr' remediation ability the isolate SSG1 was subjected to 16S rRNA sequencing by hiring services from Qube Biosciences, Hyderabad.

Table 1: Morphological and biochemical characteristics of the isolates

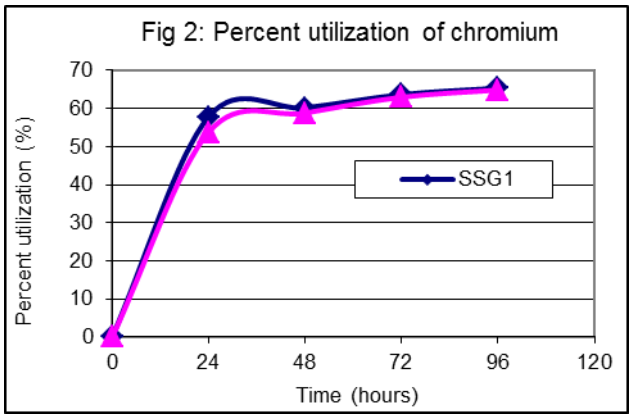
Test	**SSG1**	**SSG2**
Colour of colony	colourless	white
Colony shape	circular	circular
Size of colony	small	large
Opacity	translucent	opaque
Morphology of bacteria		
Gram staining	Gm -ve	Gm +ve
Shape	short rod	cocco-bacilli
Arrangement	single	single
Motility	+	-
Oxidase	+ve	+ve
Catalase	-ve	-ve
Indole	-ve	-ve
Methyl red	-ve	-ve
VP	-ve	-ve
Citrate utilization	+ve	-ve
Fermentation of sugars		
Lactose	-	-
Sucrose	-	-
Mannitol	A	-
Fructose	-	-
Dextrose	A	-
Trehalose	-	-
Galactose	A	-
Maltose	A	-

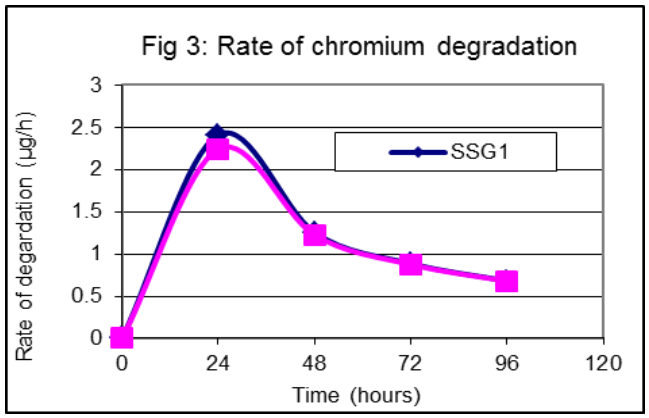
The result of the 16S rRNA sequencing showed that the isolate SSG1 belong to phylum Firmicutes and the genus was *Pseudomonas.* Bootstrap analysis was used to evaluate phylogenetic tree stability according to a consensus tree from the neighbor-joining method based on 1,000 replicates for each. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SSG1 was affiliated to phylum Firmicutes with genera *Pseudomonas* (Fig 1). The highest similarity values with the sequences of SSG1 relate with surfactant resistant *Pseudomonas* species JN033358 isolated from the estuarine surface micro layer (Louvado *et al.,* 2012). Wani *et al*., (2007) isolated the chromium [VI] degrading bacterium *Burkholderia cepacia* from this alkaline environment of Lonar Lake. The isolates were resistant to 1,000 ppm concentration of chromium. Farah *et al*., (2010) also isolated the three chromium degrading bacteria from waste water of industrial effluent, Lahore. The chromium degrading strains isolated on LB agar containing 100 µg/ml concentration of chromium and the isolates were identified as *B. pumilus*, *Alcaligenes faecalis* and *Staphylococcus* species. The biochemically characterized (both) isolates were further screened for there ability to degrade chromium from the experimental flask in laboratory scale bu UV-VIS spectrophotometer and atomic absorbance spectroscopy. Though the experimental flask contain the 100 µg/ml concentration of chromium, only 60 µg/ml of chromium was detected in the cases suggesting any conversion of chromium in deionized form. The percent utilization rate for chromium was found about 55% for both the isolates in first 24 h, while the rate gradually increases as period of incubation increases. In case of rate of degradation of chromium it was observed that for first 24 h of incubation the rate increased intensively as the bacterial cultures were in continuous phase of division. The rate of degradation decreased as incubation period increased, it means that the organisms were in phase of decline growth rate. The study showed that isolate SSG1 reduces 65.38% of chromium while isolate SSG2 degrades 64.88% of chromium after 96 h of incubation.

Fig 1: Phylogenetic Tree Based on a Comparison of the 16S Ribosomal DNA Sequences of the isolates.



The study of Farah *et al*., (2010) revealed that the isolates *B. pumilus, Staphylococcus* species and *Alcaligenes faecalis* reduces Cr^{6+} 95%, 91% and 97% within 24 h from the medium containing 100µg/ml chromium. In our study reduction rate for the isolates was studied in separate flask having same concentration of chromium.





Present study illustrate that the alkaliphilic bacterial isolates from Lonar lake SSG1 and SSG2 showed the excellent ability to reduce the hexavalent chromium to tri-valent chromium i.e. 65.38 % and 64.88% in 96 h. Proving the isolates ability to sustain at chromium containing waste as well as reduction potential at alkaline condition. The result also concludes that bacterial isolates can be exploited for bioremediation of toxic hexavalent chromium to tri-valent chromium from the industrial effluent and other polluted sites.

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