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Research Article

Studies on detoxification of Chromium (VI) by *Pseudomonas aeruginosa* from alkaline Lonar Lake

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ABSTRACT

Alkaline Lonar Lake (Maharashtra state, India) is unique ecosystem and harbors diversified groups of bacteria having potential to degrade various pollutants in the environment such as chromium. Hence attempt was made to isolate chromium detoxifying bacteria from halophilic environment of Lonar Lake. In these studies, water, sediment and matt samples were collected from alkaline Lonar Lake and inoculated in Nutrient broth containing $K_2Cr_2O_7$ (100µg/mL). Isolate was characterised by cultural, morphological and biochemically and 16S rRNA gene sequencing and identified as *Pseudomonas aeruginosa*. The chromium reduction ability of the *Ps. aeruginosa* was estimated by Di-phenyl carbazide, a Spectrophotometric method. *Ps. aeruginosa* reduced 82% of chromium after 96 h of incubation. Results of this study showed that, the *Ps. aeruginosa* was found to be highly efficient chromium reducer and could use for detoxification on polluted sites.

Keywords: Lonar Lake, Hexavalent Chromium, *Pseudomonas aeruginosa* and Di-phenyl carbazide

INTRODUCTION

We live in a toxic world. Every day we are exposed to hundreds of toxic metals and chemicals. Chromium is a toxic heavy metal, which primarily exists in two inorganic forms, Cr (VI) and Cr (III). Chromium, especially Cr(VI) is of particular environmental concern owing to its high solubility, bioavailability and toxicity. The reduction of Cr(VI) to innocuous Cr(III) is an important step in the remediation of Cr(VI)-contaminated environments. Highly soluble hexavalent chromium is carcinogenic due to its oxidizing nature[1]. Chromium is one of the most toxic chemical compounds because of its increased level in the environment as a result of metallurgies refractory, chemical and tannery industries as well as by agricultural practices. It has become one of the most abundant pollutants in aquatic and terrestrial ecosystems[2,3]. Cr (III), which is less toxic and less soluble than Cr (VI), is readily being converted into Cr (VI) under natural conditions through various oxidation processes and this oxidized Cr (VI) reacts with nucleic acids and other cell components to produce mutagenic and carcinogenic effects on biological systems [4-7].

Because of the wide range of exposure of chromium in the environment there is the need of identifying potential microbes having potential to immobilize Cr (VI). Few microorganisms in the environment have been identified as potential chromium immobilizer[8-13]. These microbes are adapting rapidly and grow at extreme condition using hazardous compounds as energy sources in waste streams. Such extreme conditions such as high pH (pH 10.5), high alkalinity, high salt etc found in Lonar Lake, which situated in Buldhana district of Maharashtra state, India, ranks third in the world based on diameter attracted to isolate extremophilic chromium detoxifying bacteria from its water[12].

MATERIALS AND METHODS

Collection and Enrichment of samples: Total twelve sediment, matt and water samples were collected from four different location of alkaline Lonar Lake during monsoon season. In the laboratory these were mixed immediately in sterile containers for isolation of Cr reducing bacteria in Nutrient broth medium (pH10) containing $K_2Cr_2O_7$ (100µg/mL).

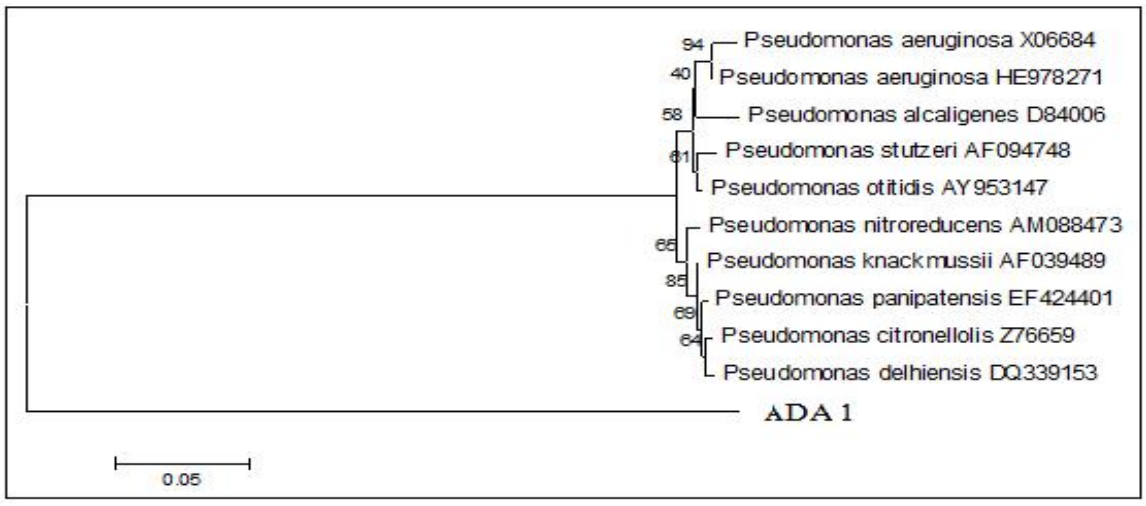
Table 1
Cultural, morphological and biochemical characteristics of chromium reducing bacteria isolated from alkaline Lonar Lake

TEST	RESULT	TEST	RESULT	TEST	RESULT
Shape	Rod	Catalase	+	Cellibiose	-
Colour of colony	Green	Oxidase	+	ONPG	+
Gram staining	-ve	MR	+	Esculin	+
Texture	Sm	VP	-	Nitrate reduction	-
Arrangement	S	Citrate	+	Lactose	-
Motility	+ve	Xylose	+	Arginine	-
Growth at different temperature		Ornithine	-	Sucrose	-
30ºC	+	Xylitol	-	Maltose	-
40ºC	+	Sorbose	-	Fructose	-
50ºC	+	Lysine	+	Dextrose	+
Growth at different pH		D-Arabinose	-	Mannose	+
pH 7	+	Glucose	+	Melibiose	-
pH 8	+	Galactose	+	Glycerol	-
pH 9	+	Raffinose	-	Salicin	-
pH 10	+	Trehalose	-	Dulcitol	-
pH 11	+	Mannitol	-	Inocitol	-
Growth at different salt conc.		Adonitol	-	Sorbitol	-
9%	+	Saccharose	-	L-Arabinose	+
10%	+	Esculin	Hydrolyzed	Rhamnose	-
11%	+	Erythritol	-	Sodium Gluconate	-

Note:- + = Positive; - = Negative; R= Rod; S= Single; Sm=Smooth

Table 2
The 16S rRNA gene sequencing closest phylogenetic affiliation, pair similarity and ribosomal database project report of isolated chromium bioremediating organism ADA 1 from Lonar lake

Strain Designation	Closest phylogenetic affiliation	Maximum Identification
ADA 1	***Pseudomonas aeruginosa***	**100%**



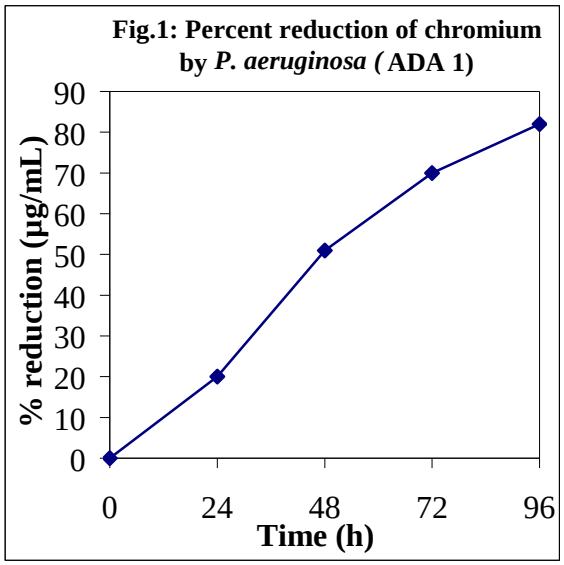


Fig.1: Percent reduction of chromium by *P. aeruginosa* (ADA 1)

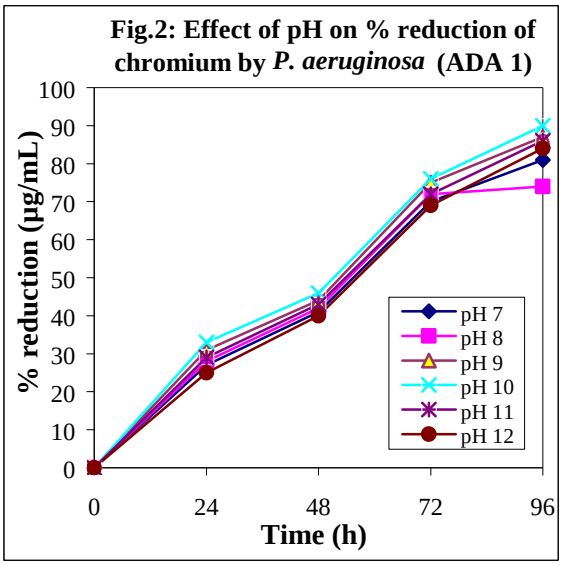


Fig.2: Effect of pH on % reduction of chromium by *P. aeruginosa* (ADA 1)

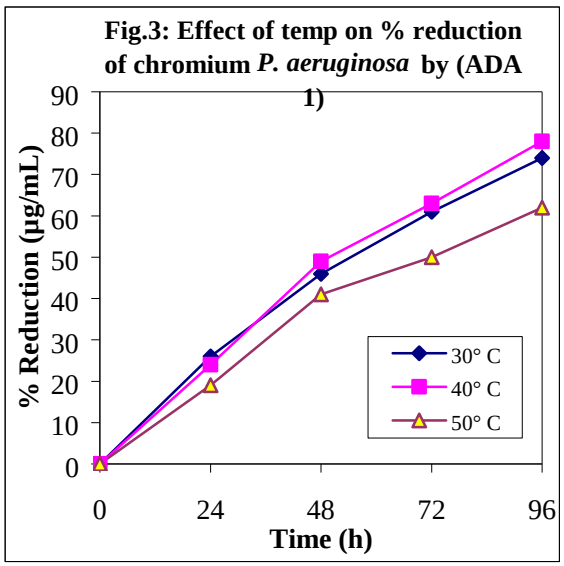


Fig.3: Effect of temp on % reduction of chromium *P. aeruginosa* by (ADA 1)

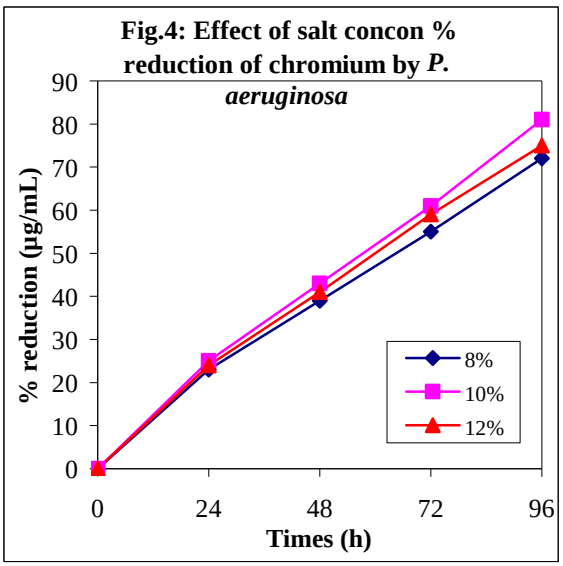


Fig.4: Effect of salt concon % reduction of chromium by *P. aeruginosa*

After every 72 h of incubation, 10mL culture broth was repeated subcultured in freshly prepared nutrient medium having same composition for five times for enrichment.

Characterization of isolate: After enrichment, the isolation was made by inoculating the culture broth on solid nutrient agar plate with pH 10 by streak plate method. The well isolated and morphologically distinct colonies were identified by cultural, morphological and biochemically by Hi-media rapid detection kit K3003. The 16S rRNA gene sequence analysis was performed at Agharkar research institute, Pune.

Di-phenyl carbazide assay for Cr (VI) reduction: The chromium reduction was determined by diphenyl carbazide (DPC) Spectrophotometeric method[14,15]. Standard graph for chromium was prepared by using 20µg/mL to 120µg/mL of chromium. Cr estimated by measuring the absorbance at 540nm on UV-VIS spectrometer (make Systronics).

RESULTS AND DISCUSSION

In the present investigation, attempt was made to isolate chromium reducing microorganisms from Alkalophilic environment such as Lonar Lake. There are certain chromium detoxifying microorganisms which have been reported by various researchers but detail studies were yet not to be done. Hence this study focuses on the study of isolation of chromium reducing bacteria for biodegradation of chromium. A total of twelve water, matt and sediment samples were collected from the alkaline Lonar Lake in the season mansoon and well isolated and morphologically distinct colonies from the plate was selected for further analysis. The isolate was Gram negative, motile, short rod identified biochemically by the commercially available Hi-media rapid detection kit KB003 (Table 1). It is also identified as *Pseudomonas aeruginosa* on the basis of 16S rRNA gene sequencing at Agharkar Research Institute, Pune (Table 2).

A wide variety of mechanisms exist for the removal of heavy metal from aqueous solution by bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants. The cellular response to the presence of metalsincludes various processes such as biosorption by cell biomass, active cell transport, binding by cytosolic molecules, entrapment into cellular capsules, precipitation and oxidation-reduction reactions. Chromium resistant bacteria have been isolated from tannery effluents by several groups. During the present investigation *Pseudomonas aeruginosa* could tolerate Cr6+ up to 2 mg/ml.

In the present study, the isolate reduces chromium in the tune of 0.854 and 70-82% µg/mL within 96 h of incubation (Fig.1). The effect of various environmental parameters on chromium reduction efficiency was also studied and pH 10 was found to be optimum for reduction of chromium to 90% µg/mL and rate of reduction was 0.937 µg/mL (Fig.2). The optimum temperature for chromium reduction was recorded as 40^0C (Fig.3). The optimum salt concentration for chromium reduction was 10% (81% µg/mL and 0.843 µg/mL) (Fig.4). Farah *et al.*,[16] revealed that the isolates *B. pumilus*, *Staphylococcus* species and *Alcaligenes faecalis* reduces Cr6+ to 95%, 91% and 97% within 24 h. Tambekar and Gayakwad,[17] isolated *Pseudomonas fulva* from alkaline environment. Wani *et al*, [18] isolated the chromium (VI) degrading bacterium *Burkholderia cepacia* from alkaline environment of Lonar Lake and the isolate was resistant to 1,000 ppm concentration of chromium. Similar result was found by Tambekar *et al.*,[19] for *Lysinibacillus mangiferihumi* from Lonar Lake.

CONCLUSION

Reduction, detoxification and possible remediation chromium by using various microorganisms has been the topic of scientific interest for a number of decades. A large number of natural and synthetic organic compounds are biodegradable by microorganisms as part of their normal metabolism for energy and growth. From the data, the strain *Pseudomonas aeruginosa* was isolated from Lonar Lake showed the potential to reduce, detoxify and possibly remediate chromium effectively and eco-friendly by which it reduces the pollution from water. From the study, it can be concluded that *Pseudomonas aeruginosa* can be exploited for detoxification of toxic hexavalent chromium to trivalent chromium from the industrial effluent and other.

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