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Bioremediation of chromium (VI) by Haloalkaliphilic Bacteria Isolate from alkaline environment

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ABSTRACT

Most of the industrial effluent contains various chemical compounds such as chromium, which is pulmonary carcinogen and health hazardous to living system. Generally, the industrial effluent is alkaline, hence, attempt was made to isolate the bacteria from alkaline environment, having ability to survive and remediate the chromium. In the present study, twelve sample of water, matt and sediment were collected from alkaline Lonar Lake and bacterial strain were isolated in nutrient broth (pH 10) containing 100µg/mL of chromium. The bacterial isolate was characterized morphologically and biochemically and identified as *Lysinibacillus mangiferihumi* by 16S rRNA gene sequencing. The chromium remediation ability of the strain was estimated by the Spectrophotometric method of Di-phenyl carbazide and it showed that 89% of chromium reduction in 96 h of incubation. Thus, it indicated that the isolated *Lysinibacillus mangiferihumi* bacillus could remediate and detoxify hexavalent Chromium (VI) to trivalent Chromium (III) in alkaline environment.

Keyword: Bioremediation, chromium, *Lysinibacillus mangiferihumi*, haloalkaliphiles Lonar lake

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INTRODUCTION

The alkaline Lonar Lake, situated in Buldhana district of Maharashtra state, India, ranks third in the world based on diameter and its high alkalinity (pH 10.5). In natural alkaline environment, contains high amount of sodium carbonate, which is a major cause of alkalinity and it is a closed system without outlets and regular influents are responsible for its existence[1,2]. The uniqueness of this lake is its high salinity and alkalinity favoring the growth of alkaliphiles and haloalkaliphiles. The Lonar Lake harbors diverse microbial flora of alkaliphilic microbes growing at pH 10 and or at high salt concentrations; haloalkaliphiles requiring up to 33% NaCl along with Na_2CO_3[3]. The extensive application of chromium in industries particularly leather tanning industries, dye and pigment manufacturing, wood preservation, textile dyeing, steel and alloy industries to the formation of chromium-contaminated soil and ground water which poses a serious threat to the biological living system particularly to human health. Hexavalent Cr (VI) is a carcinogen and a potential source of soil, surface water and ground water contaminant[4,5] and also long term exposure to chromium can cause dermatitis, damage to liver, kidney and nerve tissues[6] and is also associated with decreased plant growth[7,8]. Microbes have ability to reduce Cr (VI) in to less soluble and non-toxic Cr (III). Hence, attempt was made to isolate a bacterium from Lonar Lake, having ability to survive, remediate, detoxify and reduce the chromium from industrial polluted sites.

MATERIALS AND METHOD

Sample collection, enrichment and characterization of culture:

Twelve samples of water, matt and sediment were collected from four different sites of Lonar Lake during September 2015. Water samples were collected in sterilized pack capped plastic bottles, matt and sediment samples were collected in zip lock bags. All collected samples were stored in a 4^0C and transported to a laboratory for further experimental analysis. These water, matt and sediment samples were inoculated in 250mL flask containing sterilized nutrient broth medium (pH 10) containing 100µg/mL $K_2Cr_2O_7$ and incubated for 72h. Four times repeated sub culturing was made in freshly prepared nutrient medium for enrichment of bacterial culture. The well-isolated and morphologically distinct colonies from the plate of water, matt and sediment samples were selected and stock cultures were prepared for further analysis. The isolated bacterial strain was identified by cultural, morphological and biochemical with the help of commercially available Hi-media rapid detection kit K3003. The isolated bacterial strain was also identified by 16S rRNA gene sequence analysis from Agharkar research institute, Pune.

Chromium (Cr (VI)) assay by diphenyl carbazide (DPC) method:

Reduction of chromium was determined by diphenyl carbazide (DPC) method using DPC. Standard graph was prepared using different concentration of chromium (20µg/mL to 120µg/mL). Chromium estimated by taking the absorbance at 540nm on spectrophotometer[9].

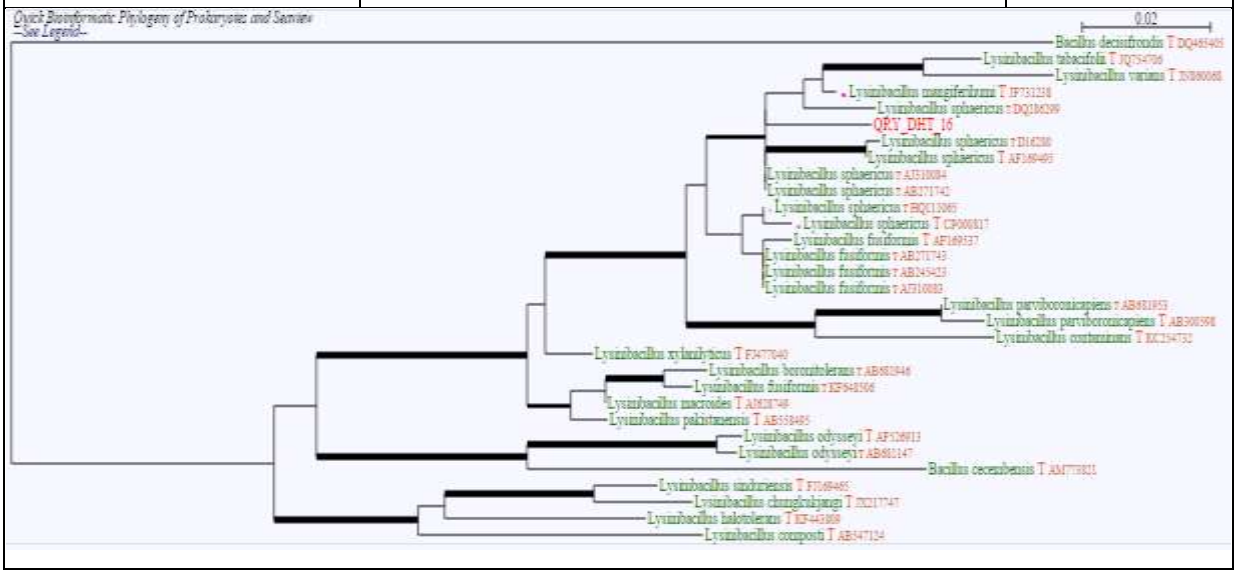
Table 1: Cultural, morphological and biochemical characteristics of chromium bio-remediating *Lysinibacillus mangiferihumi* (DHT-16) isolated from Lonar Lake

Test	Result	Test	Result	Test	Result
Colony shape	Circular	ONPG	+	Rhamnose	-
Colour of colony	Cream	Esculin hydrolysis	-	Glucose	+
Gram staining	Gm+ve rod	Adonitol	-	Lactose	-
Arrangement	Single	Meliboise	+	Arabinose	+
Motility	Motile	Reffinose	-	Trehalose	+
Catalase	+	α-Methyl-D-glucoside	-	α -Methyl-D-mannoside	-
Oxidase	+	Malonate	-	Melezitose	-
Nitrate reduction	+	Voges Proskauer's	-	Xylose	-
Citrate	-	Arginine	+	Cellobiose	+
Sorbitol	-	Cellobiose	+	Erythritol	-
Maltose	+	Sucrose	+	Sodium Gluconate	-
Fructose	+	L-Arabinose	+	Glycerol	+
Dextrose	+	Mannose	-	Salicin	+
Galactose	+	Insulin	+	Dulsitol	-
Inositol	-	Mannitol	+	Arabitol	-

Note: + = Positive, – = Negative

Table: 2. The 16S rRNA gene sequencing phylogeny affiliation and pair similarity of chromium bio-remediating organism from Lonar lake

Strain Designation	Closest phylogenetic affiliation	Max ident
DHT 16	***Lysinibacillus mangiferihumi*** (T) 16S ribosomal RNA gene partial sequence (JF731238)	100.0%



RESULTS AND DISCUSSION

A total of twelve water, sediment and matt samples were collected from alkaline Lonar Lake during September 2015 and enrichment was done for the isolation of chromium reducing bacteria. After enrichment well isolated and morphologically distinct colony (DHT 16) was selected and characterized for cultural, morphological and biochemical. The isolate (DHT 16) was found as Gram positive, short rod and motile and also biochemically characterized by commercially available Hi-media rapid detection kit K3003 (Table 1) and also identified as *Lysinibacillus mangiferihumi* by 16r RNA gene sequencing at Agharkar Research Institute, Pune (Table 2). In the present study, *Lysinibacillus mangiferihumi* was studied for chromium estimation and showed percent reduction (89%) and rate of reduction (0.92µg/mL) after 96h at 37^0C (Figure 1). The effect of environmental parameters such as temperature, pH and salt concentration on chromium reduction and rate reduction was also studied and *Lysinibacillus mangiferihumi* showed that percent reduction 78% and rate of reduction 0.812µg/mL at optimum temperature 40^0C (Figure 2). The different pH ranges (pH 7 to pH 12) were studied and was found that percent reduction (88%) and rate of reduction (0.916µg/mL) after 96h at optimum pH 10 (Figure 3). The different salt concentration (8%, 10%, 12%) were studied and was found that percent reduction (74%) and rate of reduction (0.770µg/mL) after 96h at optimum salt concentration 10% (Figure 4). Tambekar and Gayakwad[10], isolated *Pseudomonas* species from Lonar Lake and revealed that isolates oxidized 65.38% and 64.88% of chromium after 96h of incubation. Farah *et al.*[11], revealed that the isolates *B. pumilus*, *Staphylococcus* species and *Alcaligenes faecalis* reduces Cr (VI) 95%, 91% and 97% within 24h from the medium containing 100µg/ml chromium. Wani *et al.*[12], isolated the chromium (VI) degrading bacterium *Burkholderia cepacia* from an alkaline environment of Lonar Lake and the isolates was resistant to 1,000 ppm concentration of chromium. Tambekar *et al.*[13], isolated *Proteus mirabilis* as a chromium reducing bacteria from hypersaline environment and found to be highly chromium reducer.

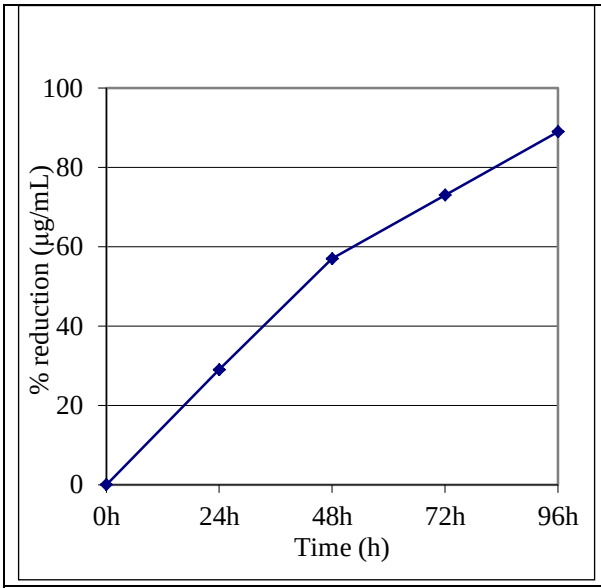


Figure 1: Percent reduction of chromium by *Lysinibacillus mangiferihumi.*

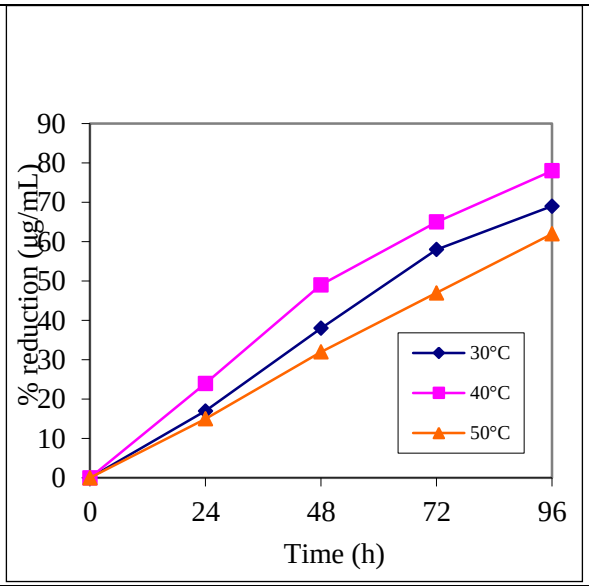


Figure 2: Effect of temperature on % reduction of chromium by *Lysinibacillus mangiferihumi*

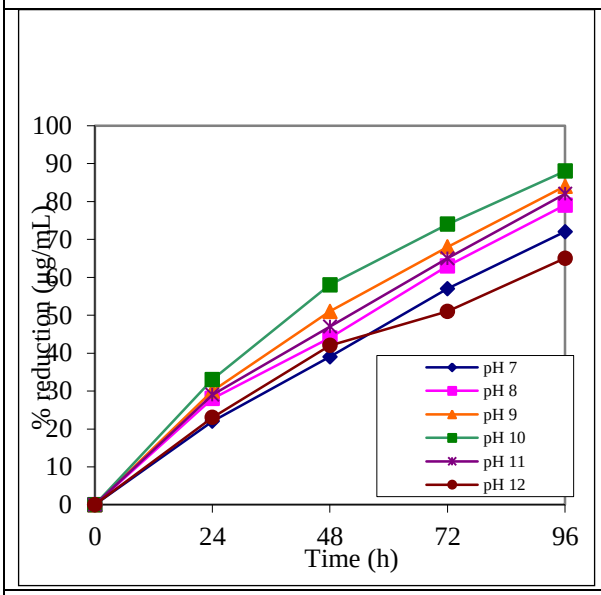


Figure 3: Effect of pH on % reduction of chromium by *Lysinibacillus mangiferihumi*

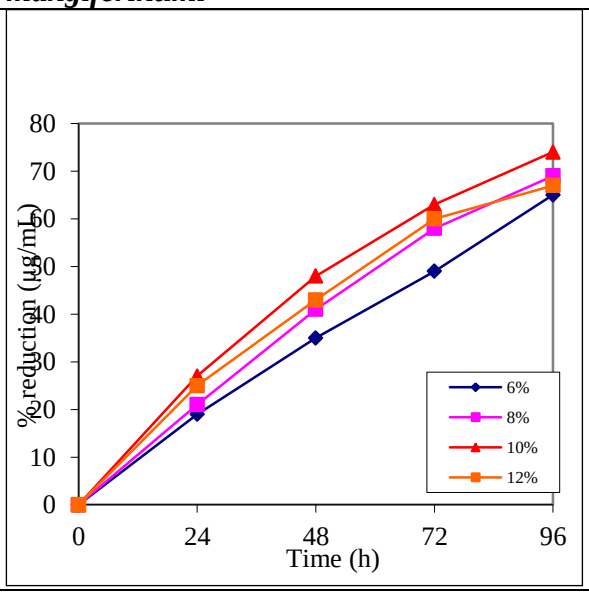


Figure 4: Effect of salt concentration % reduction of chromium by *Lysinibacillus mangiferihumi*

CONCLUSION

Bioremediation is the efficient method in which microorganism is used for the removal of toxic hexavalent Chromium from contaminated water. Because the industrial effluent contains high pH, the bacteria were isolated from Lonar Lake. The isolate was identified by 16S rRNA gene sequencing, which shows the highest similarity 100% with was *Lysinibacillus mangiferihumi*. Reduction rate of *Lysinibacillus mangiferihumi* was identified by Spectrophotometric method, which showed that the percent reduction of isolate was 89% after 96h. Various environmental

factors such as pH, salt concentration and temperature shows an optimal reduction of pH 10, salt concentration 10% and the temperature was 40^0C. These isolated *Lysinibacillus mangiferihumi* having potential to detoxify and reduction of chromium efficiently, ecofriendly by which it reduces the pollution of water. The results also concluded that *Lysinibacillus mangiferihumi* could be exploited for bioremediation of toxic hexavalent chromium to trivalent chromium from the industrial effluent and other polluted sites.

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