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Research Article

# BIOREMEDIATION OF CHROMIUM (VI) BY HALOALKALIPHILIC BACTERIA ISOLATED FROM ALKALINE LONAR LAKE

**Tambekar DH[1]*, Waradkar PA[1] and Tambekar SD[2]**

[1]PG Department of Microbiology, SGB Amravati University, Amravati, India.
[2]Department of Microbiology, D.B. Science College, Gondia -441614, India.

**ABSTRACT**

Alkaline Lonar Lake in India having a unique ecosystem, formed by meteorite impact on basaltic rock, situated in Buldhana District of Maharashtra State, India. The hexavalent form of chromium is the most toxic and carcinogenic and produces health hazardous effect. Hence attempt was made to isolate chromium remediating microorganisms from halophilic environment such as Lonar Lake. In these studies, water and sediment 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, biochemically and 16S rRNA gene sequencing identified the organism as Proteus *mirabilis.* The chromium remediation ability of the *Proteus mirabilis* was estimated by the Spectrophotometric method of Di-phenyl carbazide. *Proteus mirabilis* oxidized 68% of chromium after 96 hrs of incubation respectively. Results of this study showed that, the *Proteus mirabilis* found to be highly efficient chromium utilizer and could use for bioremediation on polluted sites.

**Keywords:** Lonar Lake, Chromium *Proteus mirabilis* and Di-phenyl carbazide.

## 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). The lake has circular periphery and is 0.14km hollow below the ground level with amphitheatre of practically vertical cliffs[1]. In natural alkaline environment, contains high amount of sodium carbonate, which is a major cause of alkalinity and it is closed system without outlets and regular influents are responsible for its existence[2, 3]. The Lonar Lake harbors diverse microbial flora of alkaliphilic microbes growing at pH 10 and or at high salt concentrations; haloalkaliphilies requiring up to 33% NaCl along with $Na_2CO_3$[4].

Chromium is the most abundant of the Group VIA family of metallic elements. It is one of the world's most strategic, critical and highly soluble metal pollutants having wide range of uses in the metals and chemical industries[5,6]. Chromium exists in the environment in several diverse forms such as trivalent Cr (III) and hexavalent Cr (VI), of which hexavalent chromium Cr (VI) is carcinogen and a potential soil, surface water and ground water contaminant[7,8] and also long term exposure at level above maximum contaminant can cause dermatitis, damage to liver, Kidney circulation nerve tissue damage and death in large doses[9] and is associated with decreased plant growth and mutagenicity[10,11] whereas it's reduced trivalent form, (Cr3+) is less toxic, insoluble and a vital nutrient for humans. These toxic metals ions not only cause potential human health hazards but also affect other life forms.

Microbes will adapt quite rapidly and grow at extreme condition using hazardous compounds as energy sources in extreme conditions in waste streams. Hence attempt was made to isolate chromium bioremediating halophilic micro-

organism from alkaline environment of Lonar Lake. In the present investigation, an indigenous bioremediation of Cr (VI) bacterial strain was isolated and characterized it by 16S rRNA gene sequencing and detoxification potential were studied.

## MATERIALS AND METHODS

**Collection of Samples:** The Lonar Lake, the third largest in the world, is the only crater in basaltic rock and the lake water is alkaline with pH[2]. Total twelve sediment and water samples were collected from four different location of alkaline Lonar Lake during monsoon season 2013 using sterilized spatula. All samples were labeled and kept in sterile plastic bottle (water sample) and zip lock bag (sediment and matt sample) at $4^0$C until analysis.

**Enrichment of samples:** All twelve water and sediment samples were mixed immediately in separate sterile containers for isolation of Cr reducing bacteria inoculated in 250mL Erlenmeyer's flask containing sterilized Nutrient broth medium (pH 10) containing 10 mL of $K_2Cr_2O_7$ (100µg/mL). After 72h of incubation 10mL culture broth was repeated subcultured in freshly prepared nutrient medium having same composition for 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 by streak plate method. The well isolated and morphologically distinct colonies from the plate of water and sediment sample were selected and stock cultures were prepared for further analysis. Isolated bacterial strain was identified by cultural, morphological and biochemical was identified by commercially available Hi-media rapid detection kit K3003. The bacterial strain was also identified by 16S rRNA gene sequence analysis from NCCS, Pune.

**Di-phenyl carbazide assay for Cr (VI) reduction**: For the determination of chromium, diphenyl carbazide (DPC) method of Cr estimation was adopted with the help of Spectrophotometer. Chromium (VI) reacts with DPC to form reddish violet complex. The reaction is selective for chromium and very sensitive. Standard graph for estimation of chromium was first prepared by using different concentration of chromium 20µg/mL to 120µg/mL by using DPC. The estimation method of Cr (VI)[12] 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).Volume was made up to 100 mL in borosil volumetric flasks. Cr estimated by taking the absorbance at 540nm on UV-VIS spectrometer (make Systronics).

## RESULTS AND DISCUSSION

In the present investigation, attempt was made to isolate chromium degrading microorganisms from halophilic environment such as Lonar Lake. There are certain microorganisms which have been reported by various researchers but detail studies on the biodegradation of chromium from Lonar Lake were yet not to be done. Hence this study focuses on the study of isolation of chromium degrading organism for biodegradation of chromium. A total of twelve water and sediment samples were collected from the alkaline Lonar Lake in the season 2013 and well isolated and morphologically distinct colonies from the plate was selected and Gram negative short rod and motile bacteria was isolated and identified. The biochemical characteristics of the isolate PAW1 was done by the commercially available Hi-media rapid detection kit KB003 (Table 1).

**Table 1: Morphological and biochemical characteristics of bacteria isolated from Lonar Lake**

| TEST | RESULT | TEST | RESULT | TEST | RESULT |
|---|---|---|---|---|---|
| Colony shape | Circular | ONPG | + | Rhamnose | + |
| Colour of colony | Colourless | Esculin hydrolysis | + | Glucose | + |
| Gram staining | Gm-ve rod | Adonitol | - | Lactose | + |
| Arrangement | Single | Meliboise | - | Arabinose | - |
| Motility | Motile | Reffinose | - | Trehalose | + |
| Catalase | + | Phynyl alanine deamination | + | Ornithine utilization | + |
| Oxidase | + | Malonate | - | $H_2S$ production | + |
| Nitrate reduction | + | Voges Proskauer's | - | Xylose | - |
| Citrate | + | Methyl red | + | Cellobiose | - |
| Lysine utilization | - | Indole | - | Saccharsoe | - |
| Note: + = Positive, – = Negative | | | | | |

The isolate PAW1 was also identified by 16S rRNA gene sequencing from the NCCS, Pune. The result of 16S rRNA gene sequencing showed that the organism was *Proteus mirabilis* and accession No ACLE01000013 (Table 2).

In the present study, the isolate when studied for chromium estimation then the rate of degradation and percent utilization of chromium was found to be 0.71 and 68% µg/mL after 96 hrs (fig. 1 and 2). In case of pH the chromium degraded on 10 pH was maximum (fig. 3 and 4). The effect of various environmental parameters on chromium utilization efficiency was also studied and it was found that the optimum temperature for organism is 37$^0$C for utilization of chromium, while on 20$^0$C and 40$^0$C, the percent utilization and rate of degradation slows down In case of pH the chromium degraded on 10 pH was maximum (fig. 5 and 6). Farah *et al*., [13] revealed that the isolates *B. pumilus, Staphylococcus* species and *Alcaligenes faecalis* reduces Cr6+ 95%, 91% and 97% within 24 h from the medium containing 100µg/ml chromium. Tambekar and Gayakwad[14] isolated *Pseudomonas* species from Lonar Lake and revealed that isolates oxidized 65.38% and 64.88% of chromium after 96 hrs of incubation. Wani *et al*. [15], 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.

**Table 2: 16S rRNA sequencing closed neighbour strain citation, accession no., and pair similarity of isolated chromium bioremediating organism from Lonar lake**

| Closest Neighbour | Strain | Citation | Accession No | Pair wise Similarity (%), |
|---|---|---|---|---|
| *Proteus mirabilis* | ATCC 29906 (T) | Hauser 1885 | ACLE01000013 | 97.85 |

*Sequence Text (in FASTA format):*>MAR_14_014

CTAACACATCCAAGTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGATGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCCCGATAGAG
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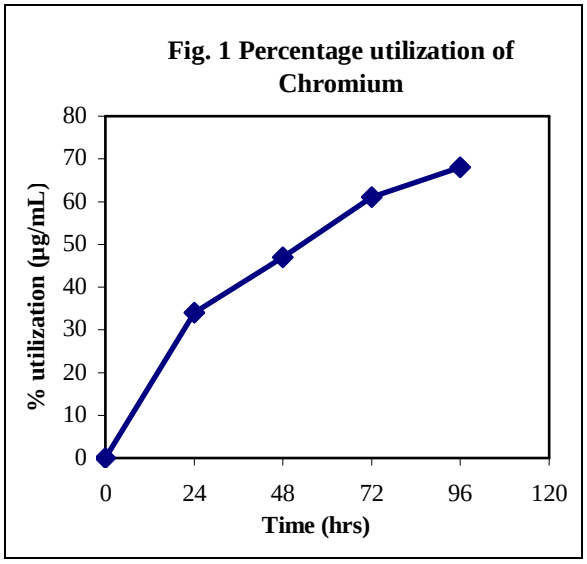


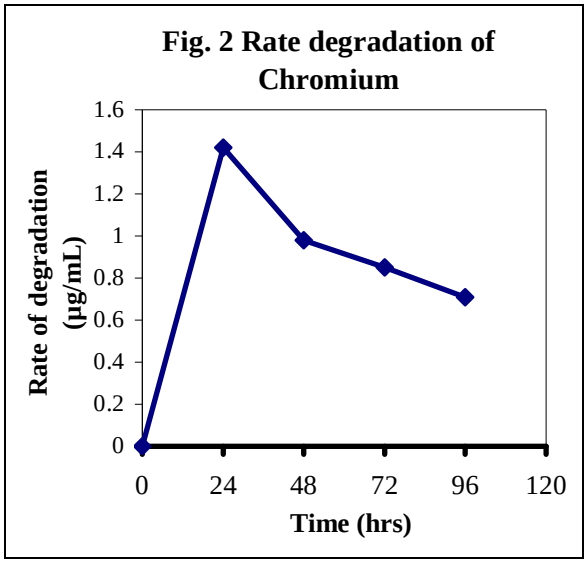

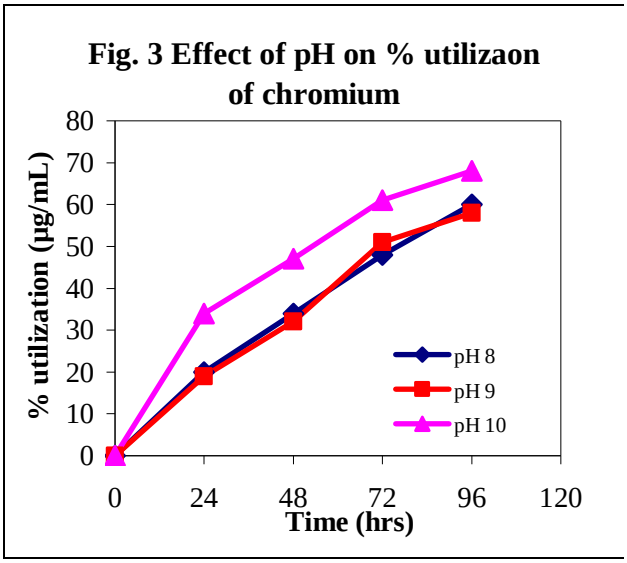


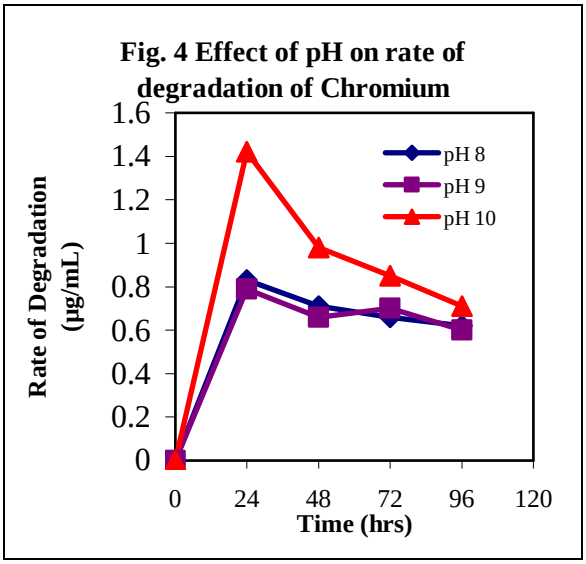


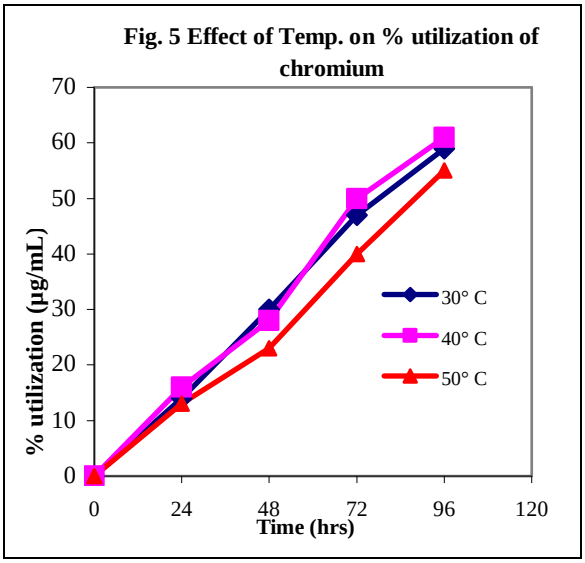


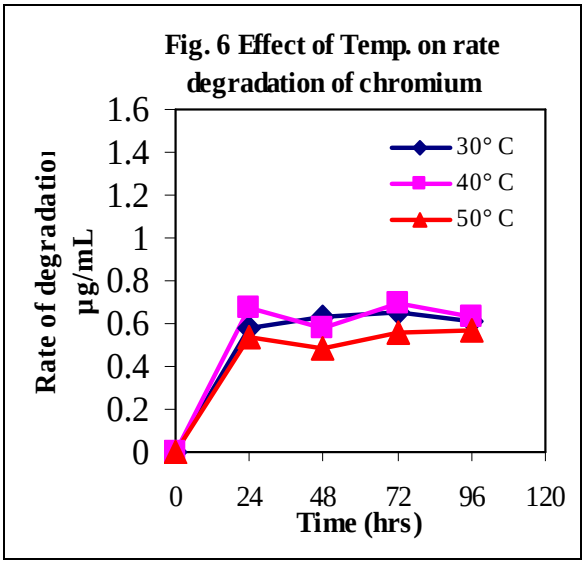


## CONCLUSION

The strain isolated from Lonar Lake was identified as *Proteus mirabilis* and showed the ability to utilize chromium. These isolated *Proteus mirabilis* having potential to detoxify and degrade chromium efficiently, ecofriendly by which it reduces the pollution from water. The results also concluded that *Proteus mirabilis* can be exploited for bioremediation of toxic hexavalent chromium to trivalent chromium from the industrial effluent and other polluted sites.

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