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Microbial Reduction, Detoxification and Possible Bioremediation of Hexavalent Chromium By *Lysinibacillus Mangiferihumi*

KEYWORDS: Lonar Lake, Chromium, L. mangiferihumi and Di-phenyl carbazide).

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ABSTRACT *Hexavalent chromium (VI) is particularly worrying because of it's widespread contamination of soil, water and produce toxic influence on human health. The main objective of the study is to reduce toxic chromium (VI) to nontoxic chromium (III) by bacteria from halophilic alkaline Lonar Lake, situated in Buldhana District of Maharashtra State, India. In these studies, water and sediment samples were collected from Lonar Lake and inoculated in Nutrient broth containing K2Cr2O7 (100µg/mL). The potential chromium reducing bacterial strain (DHT14) was isolated from Lonar Lake and characterized through cultural, morphological, biochemical characterization and identified by 16S rRNA gene analysis as Lysinibacillus mangiferihumi. The chromium reducing efficiency of the L. mangiferihumi was estimated to be 84% in 96 hrs. Results of this study showed that, the L. mangiferihumi found to be highly efficient chromium reducer and could use for detoxification on polluted sites.*

INTRODUCTION

Heavy metals such as lead, mercury, chromium, and nickel are highly toxic even at very low concentrations. Among the various heavy metals, hexavalent chromium (VI) has been found in harmful in surface waters due to contamination introduced from industrial pollution. 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 (Cervantes *et al,* 2001) and also cause dermatitis, damage to liver, kidney circulation, nerve tissue damage and death in large doses (Das and Mishra, 2008, Malik, 2004). The trivalent chromium (Cr^{+3}) is not major problem since its solubility in water is low and can be easily separated from water and it is 100 times less toxic than hexavalent chromium (Tambekar, 2014).

Microbes can adopt quite extreme condition rapidly and grow using hazardous compounds as energy sources from waste and polluted streams. The Lonar lake is natural alkaline environment, contains high amount of sodium carbonate, which is a major cause of alkalinity and harbors diverse microbial flora of alkaliphilic microbes growing at pH 10 and at high salt concentrations (Joshi *et al,* 2007, Tambekar et al, 2010). Hence attempt was made to isolate and study chromium reducing bacteria from alkaline environment of Lonar Lake and its potential to reduced chromium (VI) to chromium (III).

MATERIALS AND METHODS

Collection of Samples:.

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 4^0C until analysis (Thakker and Ranade, 2002).

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 4 sub-cultured in freshly prepared nutrient medium was made with same composition for enrichment of bacterial culture.

Isolation and biochemical characterization:

After enrichment, the isolation of bacterium was made and it was identified by cultural, morphological and biochemical test by commercially available Hi-media rapid detection kit K3003 and KB009. The bacterial strain was also identified by 16S rRNA gene sequence analysis from Agharkar Research Institute, Pune.

Di-phenyl carbazide assay for Cr (VI) reduction:

For the determination of chromium, diphenyl carbazide (DPC) method of Cr estimation was adopted. Chromium (VI) reacts with DPC and 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. Cr estimated by taking the absorbance at 540nm on UV-VIS spectrometer (APHA, 2012).

RESULTS AND DISCUSSION

The bacterial species are able to grow in the toxic conditions and are generally assumed to be tolerant first hexavalent chromium (Viti and Giovannetti, 2001). Tolerance is defined as "the ability of a microorganism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity (Gadd, 1992). In the present investigation, attempt was made to isolate chromium reducing 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 isolation of chromium reducing bacterium for reducing toxicity of chromium.

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A total of twelve water and sediment samples were collected from the alkaline Lonar Lake in the season 2014 and a morphologically distinct colony from sediment sample was selected. The colony and bacterial morphology of isolated and selected bacterium was studied. The colony was circular, cream in colour and bacterium was Gram positive, short rod and motile. The biochemical characteristics of the isolate DHT 14 was done by the commercially available Hi-media rapid detection kit KB003 and KB009 (Table 1).

The strain DHT 14 isolated from the sediment of Lonar Lake was also identified by 16S rRNA gene sequencing from the Agharkar Research Institute, Pune. The result of 16S rRNA gene sequencing showed that the organism was *Lysinibacillus mangiferihumi* (Table 2 and figure1).

Table 1. Cultural, morphological and biochemical characteristics of bacteria 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	δ	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	δ
Fructose	+	L-Arabinose	+	Glycerol	+
Dextrose	+	Mannose	-	Salicin	+
Galactose	+	Insulin	+	Dulsitol	-
Inositol	-	Mannitol	+	Arabitol	-

Note: + = Positive, – = Negative

Table 2: The 16S rRNA gene sequencing Closest phylogenetic affiliation and pair similarity of isolated chromium bioremediating organism from Lonar lake

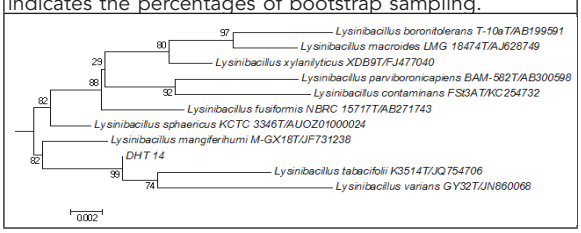
Strain	Closest phylogenetic affiliation	Max indent
DHT 14	*Lysinibacillus mangiferihumi* M-GX18(T)16S ribosomal RNA gene partial sequence (JF731238)	99.73%

Sequence Text (in FASTA format) :>

TGGGAGACTT-
GAGTGCAGAAGAGGATAGTGGAATTCCAAGTGTAGCG-
GTGAAATGCGTAGAGATTTGGAGGAACACCAGTG-
GCGAAGGCGACTATNTGGTCTGTAACTGACANTGAG-
GCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC-
CTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGT-
TAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCAT-
TAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACT-
GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG-
GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC-
CTTACCAGGTCTTGACATCCCGTTGACCACTGTAGAGA-
TATGGTTTTCCCTTCGGGGACAACGGTGACAGGTGGT-
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT-
TAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTT-
GCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCG-
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT-
CATCATGCCCCTTATGACCTGGGCTACACACGTGCTA-
CAATGGACGATACAAACGGTTGCCAACTCGCGAGAGG-
GAGCTAATCCGATAAAGTCGTTCTCAGTTCGGATTGTAG-
GCTGCAACTCGCCTACATGAAGCCGGAATCGCTAG-
TAATCGCGGATCAGCATGCCGCGGGAATACTTC

In the present study, the isolate *Lysinibacillus mangiferihumi* (DHT14) was also studied to determine its ability to utilization and reduction in hexavalent of chromium in to trivalent chromium. Determination of chromium was performed by using diphenyl carbazide (DPC) method. *Lysinibacillus mangiferihumi* reduced 84% chromium in 96 h and the rate of degradation was 0.875 µg/mL (fig. 2 and 3). The effect of environmental conditions such as pH and temperature on chromium reduction, detoxification and possible remediation was also studied. In various pH condition, (pH 7 to pH 11), the *Lysinibacillus mangiferihumi* utilized 80% at pH 7, at pH 8 utilized 89%, pH 9 utilized 94%, pH 10 utilized 78%, and on pH 11 utilized 74% after 96 h. From these data it was concluded that pH 9 was optimum for chromium reduction, detoxification and possible remediation (fig. 4 and 5). The *L. mangiferihumi* optimally reduced and detoxify chromium at 37^{0}C to 84% (rate degradation 0.875 µg/mL). At various temperatures, at 30^{0}C, 40^{0}C and at 50^{0}C utilized 80%, 72% and 70% and rate degradation was 0.833 µg/mL 0.750 µg/mL and 0.729 µg/mL respectively (fig. 6 and 7).

Fig 1: Phylogenetic tree based on a comparison of the 16S r RNA sequences of DHT 14 and some of their closest phylogenetic relatives. The tree was created by the neighbor joining method. The numbers on the tree indicates the percentages of bootstrap sampling.



Farah *et al.* (2010) 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 (2013) isolated *Pseudomonas* species from Lonar Lake and revealed that isolates reduces chromium 65.38% to 64.88% in 96 hrs. Wani *et al*, (2007), isolated the chromium [VI] degrading bacterium *Burkholderia cepacia* from alkaline environment of Lonar Lake which was resistant to 1,000 ppm of chromium. Tambekar *et al.*, (2014) isolated *Proteus mirabilis* from alkaline Lonar Lake having potential to reduced and detoxify chromium efficiently. Poornima *et al.*, (2010), isolates two chromium degrading bacterial strains SP8- *Pseudomonas putida* and SP2- *Pseudomonas plecoglossicida* from Rhizosphere soils from Amrithi forest. Also Muhammad (2013), isolated two different bacterial strains; *Pseudomonas* sp. and *Bacillus* sp. from waste water, they found that *Bacillus* sp. was found to give higher chromium utilizer in compared to *pseudomonas* sp.

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 *Lysinibacillus mangiferihumi* 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 *Lysinibacillus mangiferihumi* can be exploited for bioremediation of toxic hexavalent chromium to trivalent chromium from the industrial effluent and other

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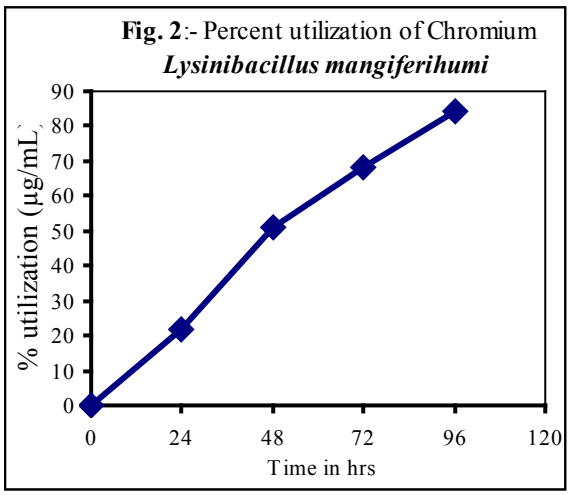
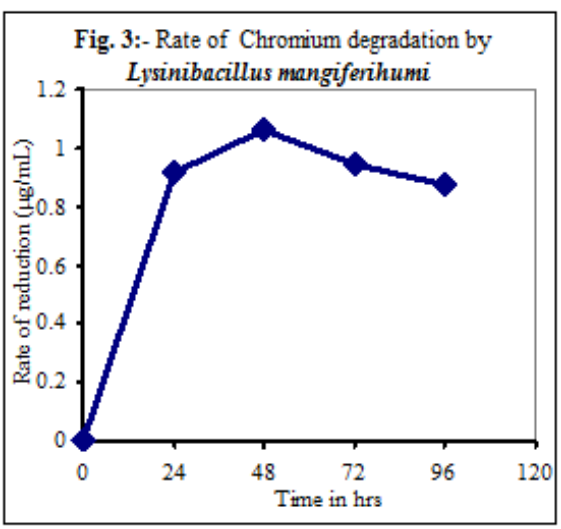
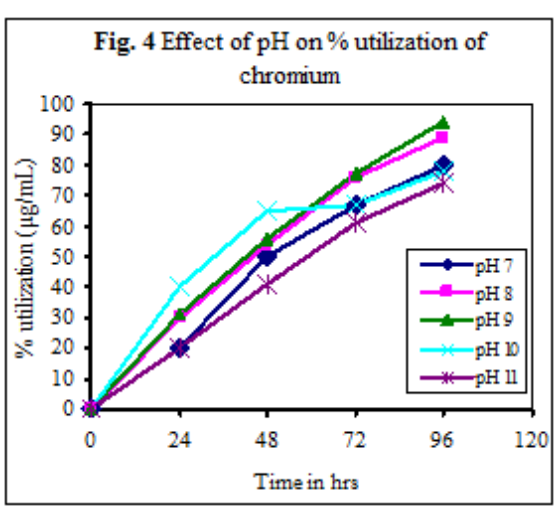
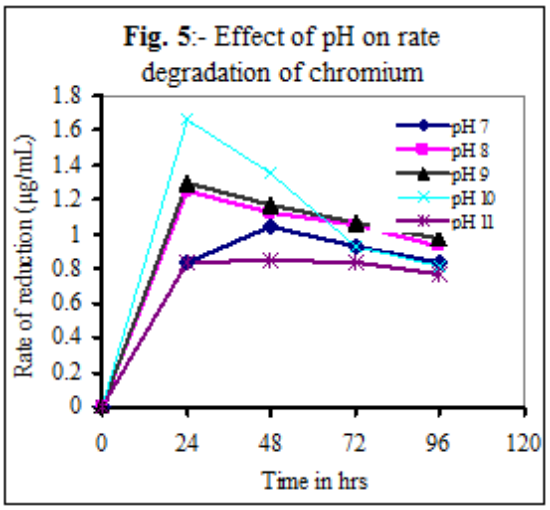
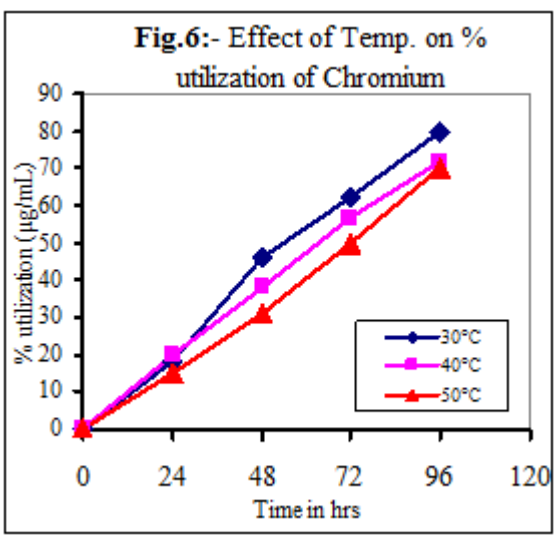
polluted sites

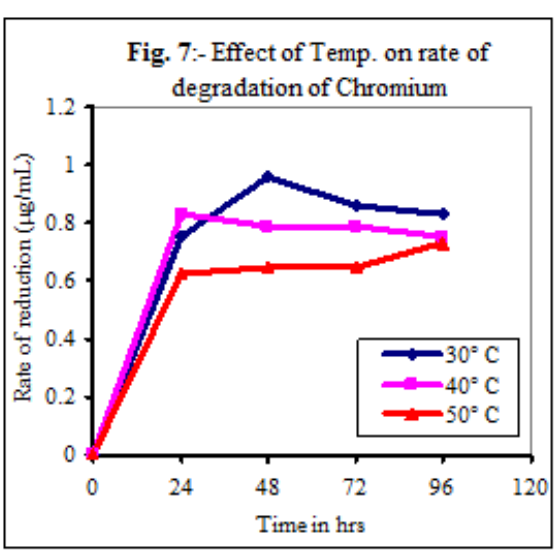












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REFERENCE

1. APHA -AWWA-WPCF, (2012). Standard methods for the examination of water and wastewater. American Public Health Association (APHA), 22nd edition. Washington. DC. 201-204. | 2. Cervantes C, Campos GJ, Devars S, Gutierrez CF, Loza TH, Torres GJC and Moreno SR, (2001). Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev. 25: 335–347. | 3. Das A and Mishra S, (2008). Hexavalent chromium (VI): Health hazards and Environment pollutant. J Env Res Dev. 2(3). | 4. Farah R, Shakoori S, Tabassum A, Rehman A and Shakoori R, (2010). Isolation and Characterization of Cr (VI) Reducing Bacteria and Their Potential use in Bioremediation of Chromium Containing Wastewater. Pak J Zoology., 42(6): 651-658. | 5. Gadd GM, (1992). Metals and microorganisms: a problem of definition, FEMS Microbiol. Lett. 100 (1992) 197–204. | 6. Joshi, AA, Kanekar PP, Kelkar AS, Shouche YS, Wani AA, Bogave SB and Sarnaik SS, (2007). Cultivable bacterial diversity of an alkaline Lonar Lake, India. Microb Ecol. 55:163–172. | 7. Malik A, (2004). Metal bioremediation through growing cells. Env Int. 30: 261–278. | 8. Muhammad Q, (2013). Biodegradation of hexavalent chromium (Cr+6) in wastewater using Pseudomonas sp. and Bacillus sp. bacterial strains. INT J ENE ENV. Vol 4(4) pp.653-662. | 9. Poornima K, karthik L, Swadhini SP, Mythili S and A.Sathiavelu, (2010). Degradation of Chromium by Using a Novel Strains of Pseudomonas Species. J Microbial Biochem Technol. 2(4): 095-099. | 10. Tambekar DH and Gayakwad SS, (2013). Studies on bioremediation of Chromium [VI] by bacteria isolated from alkaline Lonar Lake (MS) India. Sci. Res. Rept., 3(1): 87-90. | 11. Tambekar DH, Pawar AL and Dudhane MN, (2010). Lonar Lake water: Past and present. Nat Env Poll Tech. 9(2): 17-221. | 12. Tambekar DH, Waradkar PA and Tambekar SD, (2014). Bioremediation of chromium (VI) by haloalkaliphilic bacteria isolated from alkaline Lonar Lake. Int J Pharm, Chem Biol Sci, 4(3), 601-605. | 13. Viti C and Giovannetti L (2001). The impact of chromium contamination on soil heterotrophic and photosynthetic microorganisms, Ann. Microbiol. 51, 201–213. | 14. Wani R, Kodam KM and Gawai KR, 2007. Chromate reduction by Burkholderia cepacia MCMB-821, isolated from the pristine habitat of alkaline Crater Lake. Appl. Microbiol. Biotechnol. 75:627-632.