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BIODEGRADATION OF PHENOL BY NATIVE MICROORGANISMS ISOLATED FROM LONAR LAKE IN MAHARASHTRA STATE (INDIA)

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ABSTRACT

The present study was aimed to isolate phenol degrader from 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). From 4 sediments and water samples of Lonar Lake, a bacterium *Pseudomonas stutzeri* was isolated and identified based on morphological, cultural, biochemical properties and 16S rRNA gene sequencing. Our experiment showed that the isolate removes almost 87% phenol in the peptone water-phenol medium at laboratory level. Thus, the alkaliphilic bacterium, *Pseudomonas stutzeri,* ensuring an acceptable Lonar lake bacterium, which could therefore be commercially exploit for bioremediation of phenol, a major toxic pollutant in industrial waste effluents.

Key Words: Lonar Lake, Phenol degrader, bioremediation, *Pseudomonas stutzeri,* alkaliphilic bacterium

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. 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 (Thakker and Ranade, 2002). The lake harbors diverse microbial flora of alkaliphilic microbes growing at pH 8 to 10 and or at high salt concentrations; haloalkaliphilies requiring up to 33% NaCl along with Na_2CO_3. The alkaliphilic bacteria isolated from this lake include the genera of *Bacillus sp, Flavobacterium sp, Aeromonas sp, Micrococossus sp, Archaeobacteria sp, Natronobacterium* and *Natronococcus,* are responsible for production of enzymes like amylases, proteases, lipases, cellulases, cyclodextrin glycosyl transferase, pectinases, phosphatases etc., which have industrial applications (Joshi *et al*, 2007, Tambekar and Dhundale, 2012).

Phenol is known to be toxic to both aquatic and terrestrial life including human beings and necessary to remove from industrial effluents before discharging them in to the environment. Biological methods for the removal of phenol are possible because some micro-organisms are endowed with the property of degrading phenol but meagre data are available on the use of these microorganisms for treatment of phenol containing industrial waste effluents (Kanekar *et al*, 1999, Catia *et al*, 2010). Since some of the phenol-bearing industrial waste waters are alkaline in nature, it was thought worthwhile to explore the use of alkaliphilic bacteria for the removal of phenol. The alkaline Lonar Lake harbours many industrially important microbes which can degrade phenol like toxic industrial effluent. These phenolic compounds possess various degrees of toxicity and their fate in the

environment is therefore important (Nair *et al*, 2008). Therefore bioremediation of phenol from industrial effluents is of great importance (Alva and Peyton 2003; Chakraborty *et al*, 2009). Kanekar *et al,* (1999) isolated alkaliphilic bacteria from the sediment of alkaline Lonar Lake, which has the capacity to degrade phenol. They were identified as *Arthrobacter sp, Bacillus cereus, Citrobacter freundii, Micrococcus agilis* and *Pseudomonas putida*. Due to widespread distribution of phenol in the environment, some microorganisms adapted to use the compound both as carbon and energy source. These microorganisms use both aerobic and anaerobic pathway for phenol degradation and aerobic biodegradation has been studied (Abdullah *et al*, 2010).

The Phenol degrading bacteria present in the Lonar Lake has not been studied in detailed. Therefore attempt was made to apply culture dependent strategy to explore the diversity of phenol degrading bacteria from Lonar Lake and identification of this degrader based on morphological, biochemical, cultural characters and 16S rRNA analysis and evaluation of phenol degradation potential in different phenol concentrations.

MATERIALS AND METHODS

Collection of Sample: Total four sediment and water samples were collected from four different location of alkaline Lonar Lake during monsoon season 2011 using sterilized spatula. All samples were labeled and kept in sterile plastic bottle at 4^0C until analysis.

Isolation of Phenol degrading bacteria: To 90 ml of peptone water-phenol medium (in 250 ml conical flask) 10 ml of water sample or 1 gm sediment sample was added separately. These flasks were shaken and incubated on rotary shaker for seven days at 120 rpm at 37^0C. After seven days of incubation, 10 ml of cultured broth was sub-cultured in fresh 250 ml media and allowed to incubate in shaker for next seven days. Same procedure was repeated for five times (Kanekar *et al*, 1999).

Identification of isolates: Isolated bacteria were identified based on standard morphological, cultural, biochemical tests and 16s rRNA sequence analysis which is carried at NCCS Pune.

Determination of phenol degradation potential: The isolates showing growth on peptone phenol agar slants were used for further studies on bioremediation of phenol. Hundred ml peptone phenol broths were inoculated with a 24-h-old culture of the isolates, grown on a respective agar medium and the flasks were incubated under shake culture condition on a rotary shaker for 48h at an ambient temperature. After an appropriate incubation period, the cells were removed by centrifugation and the cell-free supernatants were used for estimation of residual phenol (Table 2). The residual phenol was estimated by 4-Aminoantipyrine method (Mohamed *et al,* 2003).

RESULTS AND DISCUSSIONS

Mrozik *et al*, (2003), demonstrated that phenols and their compounds are the most recalcitrant and persistent organic chemicals in the environment. Abdulla *et al*, (2010), Vidyavathi *et al*, (2000) reported phenol degradation by *Nocardia* that resulted in complete degradation of phenol (100 ppm) within 96 hours. The bioremediation potential of an indigenous *Pseudomonas fluorescence* was studied in batch culture using synthetic phenol in water in concentration range of (100-500) mg/L as model limiting substrate. Viraraghavan and Rao (2002), used the cells of *Aspergillus niger* to treat the effluent of many waste water treatment plants to remove the phenol from aqueous solution. Catia *et al*, (2010) studied the comparison of the biodegradation performance of phenol by using free and encapsulated cells of a new *Aspergillus* sp. isolated from a crude oil contaminated soil. Chakraborty *et al*, (2010) investigated the biodegradation of phenol by native bacteria strains isolated from coke oven processing waste water. Ahamad and Kunhi (1996), demonstrated that generally *Pseudomonas* degrade phenol through the *meta*-pathway, but *Pseudomonas stutzeri* strain SPC2 isolated by flask enrichment of municipal sewage degraded phenol through the *ortho*-pathway.

Two sediment and two water samples were collected from different sites of Lonar Lake during year 2011. Four bacterial cultures were

isolated, out of which one with high potential to degrade phenol was selected and identified on the basis of the biochemical test, 16S rRNA sequencing. The result of biochemical and 16S rRNA sequencing showed that the organism is *Pseudomonas stutzeri* and phenol degrading capacity was determined.

Table 1 : Morphology and biochemical characteristic of bacteria isolated from Lonar lake

Test	Result	Test	Result	Test	Result
Colony shape	Circular	**Urease**	Negative	**Mannitol**	Negative
Colour of colony	Pale Yellow	**Starch hydrolysis**	Negative	**Xylose**	Negative
Gram staining	Gram negative	**Trehalose**	Positive	**Raffinose**	Negative
Shape	Short rod	**Salicin**	Negative	**Celloboise**	Negative
Arrangement	Single	**Sucrose**	Negative	**Sorbitol**	Negative
Motility	Actively motile	**Fructose**	Negative	**Indole**	Negative
Catalase	Positive	**Maltose**	Negative	**MR**	Negative
Oxidase	Positive	**Lactose**	Negative	**VP**	Negative
Nitrate reductase	Positive	**Dextrose**	Acid	**Citrate**	Positive
Bacteria on the basis of 16S rRNA: *Pseudomonas stutzeri* ATCC 17588					

Table 2: Protocol for phenol estimation

Reagent	Standard						Experimental				
							0h	24h	48h	72h	96h
Phenol working solution (ml)	0.5	1	2	3	4	5	3	3	3	3	3
Buffer solution (ml)	2	2	2	2	2	2	2	2	2	2	2
4-Aminoantipyrine solution (ml)	2	2	2	2	2	2	2	2	2	2	2
Potassium ferricyanide (ml)	2	2	2	2	2	2	2	2	2	2	2
Absorbance at 420 nm	0.001	0.002	0.004	0.006	0.008	0.01	0.001	0.006	0.005	0.004	0.003
Conc. of phenol	0.05	0.1	0.2	0.3	0.4	0.5	0.05	0.3	0.25	0.2	0.15

Fig, 1: Rate of degradation of phenol

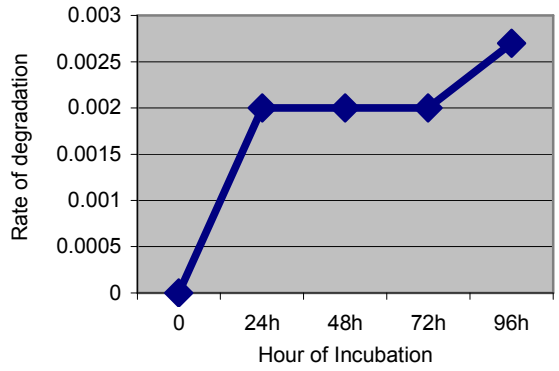
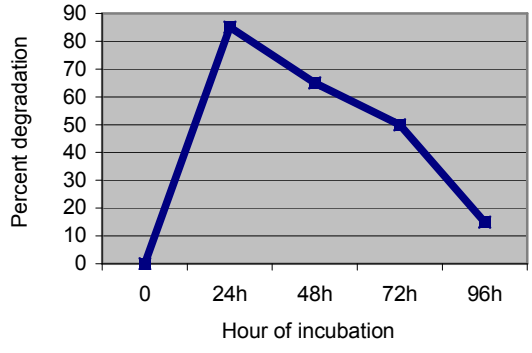


Fig. 2: Percent degradation of phenol



In the present study, on basic of morphological, cultural characteristics biochemical properties (Table 1) and based on 16S rRNA gene sequencing; the isolates should be classified as a species belonging to the, *Pseudomonas* species i.e. *Pseudomonas stutzeri* (Table 1). Our experiment also showed that the isolate removes phenol up to 87% in the peptone water-phenol medium at laboratory level. Although not exposed to any industrial waste effluents in nature, the microbial species isolated from the Lonar Lake were able to utilize phenol in the laboratory by a simple adaptation technique. Kanekar *et al*, (1999) isolated 14 microbial species from Lonar Lake which can degrade phenol and the overall extent of phenol removal by the isolates ranged between 34% and 100% from the industrial effluents but our isolate from Lonar lake, *Pseudomonas stutzeri,* removed almost more than 87% within 24 h of incubation (Fig. 1 and 2)

The alkaliphilic bacteria, *Pseudomonas stutzeri,* removed phenol almost completely, thus ensuring an acceptable Lonar lake bacterium, which could therefore be commercially exploit for bioremediation of phenol, a major toxic pollutant in industrial waste effluents.

RIBOSOMAL DATABASE PROJECT

AATGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGGGAGACGTTTACGGCGTGGACTACCAGGGTATCTAA
TCCTGTTTGCTCACCACGCTTTCGCACCTCAGTGTCAGTATTAGCCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATAT
CTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCATACTCTAGCTCGCCAGTTTTGGATGCAGTTCCCAGGTT
GAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTT
CGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTTACTGC
TTCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTTCCAGTGTGACTGATCATCCTCTCAGACCAGT
TACGGATCGTCGCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAAGA

Results for Query Sequence: seqmatch_seq, 642 unique oligos norank

Root (20) (match sequences)
domain Bacteria (20)
phylum "Proteobacteria" (20)
class Gammaproteobacteria (20)
order Pseudomonadales (20)
family Pseudomonadaceae (20)
genus Pseudomonas (20)

S000022295	-	not calculated	0.955	1275	Pseudomonas stutzeri; = ATCC 17588;
S000127562	-	not calculated	0.955	1292	Pseudomonas stutzeri; DSM 5190T; AJ288151
S000372382	-	not calculated	0.955	1355	mucus bacterium 10; AY654828
S000427765	-	not calculated	0.955	1389	Pseudomonas sp. BRW1; AF025349
S000427767	-	not calculated	0.955	1389	Pseudomonas sp. BRW3; AF025351
S000428789	-	not calculated	0.955	1368	Pseudomonas stutzeri (17588; AF094748
S000434631	-	not calculated	0.955	1376	Pseudomonas sp. JPL-1; AY030314
S000437364	-	not calculated	0.955	1366	Pseudomonas stutzeri; ATCC 17589; U25432
S000437391	-	not calculated	0.955	1366	Pseudomonas stutzeri; CCUG 11256; U26262

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