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Detoxification of Phenol by Haloalkaliphilic Bacteria Isolated From Lonar Lake

KEYWORDS: Lonar Lake, Phenol degradation, Prolinoborus fasciculus

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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. Phenol is the toxic pollutant and produces number of health hazardous therefore attempt was made to isolate phenol degrading bacteria from Lonar Lake. In these study sediment, matt and water samples were collected from alkaline Lonar Lake and inoculated in 100mL peptone water phenol medium. After enrichment, the isolated bacterium phenol degrader was identified as Prolinoborus fasciculus. This Prolinoborus fasciculus was further screened for its ability to utilize phenol by 4-Aminoantipyrine method. Results showed that the phenol-degrading isolate Prolinoborus fasciculus appears to have 72% phenol utilizer and might be greater potential for enhanced phenol removal through utilization of phenol as sole source of carbon and energy.*

INTRODUCTION

Lonar crater is a bowl-shaped, near-circular crater formed by meteor impact (Fredriksson *et al.*, 1973) around 52 000 years ago (Sengupta *et al.*, 1997) in the Deccan volcanic flood basalts in Maharashtra, India, harbors variety of halophillic micro-organisms having capacity to degrade or detoxify various pollutant such as phenol from environment. Phenol, one of the most common environmental pollutants, toxic even at low concentrations, poses significant risks to aquatic biota to fish at relatively low concentrations of (5-25 mg/L) and causes taste and odor problem in drinking water and its presence in natural waters can lead to form substituted compounds during disinfection and oxidation processes ((Ferhan *et al.*, 2002; Rittmann and McCarty, 2001; Chakraborty et al., 2010). Research on microbial degradation on phenol has intensified in recent years because it is the sustainable ways to clean-up contaminated environments (Tambekar *et al.*, 2012). Microbes will adapt quite rapidly and grow at extreme condition using hazardous compounds as carbon and energy sources, microbes can adapt rapidly to extreme conditions in waste streams. Important examples include phenol, chlorophenol, chlorobenzene, chloroalkanes, atrazine and nitroaromatics (Tambekar *et al.*, 2013).

The alkaline Lonar Lake harbours many industrially important microbes which degrade phenol like toxic industrial effluent, and such phenolic compounds possess various degrees of toxicity and their fate in the environment is therefore important (Nair *et al.*, 2008, Tambekar and Dhundale, 2012). The Phenol degrading bacteria present in the Lonar Lake has not been studied in detailed so far. Therefore attempt was made to isolate and apply culture dependent strategy to explore the diversity of phenol degrading bacteria from Lonar Lake and identification of this degrader based on cultural, morphological biochemical characters and 16S rRNA gene sequencing analysis.

MATERIALS AND METHODS

Collection of Samples: Total twelve sediment, matt and water samples were collected from Lonar, labeled and kept in sterile plastic bottle at 4^0C until analysis. For enrichment, collected samples were separately inoculated in 250 mL Erlenmeyer's flask containing 100 mL peptone water phenol medium and flask and incubated at 37°C on rotary shaker for 72 hrs. After every 72 h of interval, repeated subcultures were made for 5 times in same media (Kanekar *et al.*, 1999).

Isolation and biochemical characterization of Phenol-Degrading Bacteria: Isolation was performed on solid nutrient agar plate and isolated bacterium was characterized by standard biochemical test according to Bergey's manual of systematic bacteriology, Hi-Media Rapid Detection Kit KB003, and identified on the basis of 16S rRNA gene sequencing from the NCCS, Pune.

Determination of phenol degradation potential: Standard graph was prepared by preparing different concentrations of phenol (0.1 mg/mL- 0.5 mg/100mL) (Table. 1) and the degradation rate of phenol was estimated using 4-Aminoantipyrine method at each 24 h interval by taking absorbance at 510 nm wavelength on UV-VIS spectrophotometer (Mohammed *et al.*, 2003). From the absorbance recorded the percent utilization, rate of degradation and amount of phenol utilized/h was determined.

Table 1 Protocol for standard graph preparation

Reagent	Standard					
Concen. of phenol (mg/mL)	0.1	0.2	0.3	0.4	0.5	0.6
Phenol working solution	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Buffer solution	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
4-Aminoantipyrine solution (2%)	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Pot. Ferricyanide solution (8%)	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Distilled water	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
Absorbance was measured at 510 nm after 15 min.						

RESULTS AND DISCUSSION

One of the most alarming situations in today's world is the generation of a huge amount of waste water contaminated with the toxic organic substances like phenol from the industrial sector. Phenol is highly water soluble and its presence in the water imparts a carbolic odor to the receiving water bodies and can have baleful effects on aquatic as well as terrestrial flora and fauna including human beings (ATSDR, 2008). Hence removal of phenol from the discharged sewage and effluent is highly necessary.

Total twelve samples comprising of sediment, matt and water were collected from Lonar Lake, from which one bacterium was isolated in peptone water phenol medium having 5µg/100ml concentration of phenol, the isolate was characterized biochemically by Hi-media Rapid Detection Kit

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KB003. The isolate is gram negative sluggishly motile rod, ferment arabinose and trehalose with acid production (Table 2). The biochemically characterized isolate was identified by 16S rRNA sequencing from NCCS, Pune as *Prolinoborous fasciculus* (Table 3). Tambekar *et al.*, (2012, 2013) isolated the phenol degrading *Pseudomonas stutzeri, Staphylococcus arlettae* and *Staphylococcus* sp. from the samples collected from Lonar Lake, while Kanekar *et al.*, 1999 isolated alkaliphilic phenol degrading *Arthrobacter sp., Bacillus cereus, Citrobacter freundii, Micrococcus agilis* and *Pseudomonas putida* bacteria from the sediment of Lonar Lake. The phenol degrading *Staphylococcus aureus* strain was also isolated by Butani *et al.*, (2012) from the effluent sample of Amla Khadi, located in Unkaleshwar, India.

Table 2.Morphological and biochemical characteristics of bacteria isolated from Lonar Lake.

TEST	RESULT	TEST	RESULT	TEST	RESULT
Colony shape	Circular	Indole	-	Arginine	+
Colour of colony	White	Nitrate reduction	+	Sucrose	-
Gram staining	-	Citrate	-	Fructose	-
Shape	Rod	ONGP	-	Xylose	-
Arrangement	Clustered	Alkaline phosphatase	+	Mannitol	-
Motility	Sluggishly motile	Urease	+	Glucose	-
Catalase	+	Malonate	-	Arabinose	-
Oxidase	+	Voges Proskauer's	-	Trehalose	+

Note: + = Positive, – = Negative

The isolate *Prolinoborous fasciculus (JN 175353) utilized 72%* of phenol from the culture broth having 5µg/100ml concentration of phenol before incubation (Fig1). The rate of degradation of phenol was increasing for first 24h incubation as the bacterium was in log phase of division. The rate of degradation decreased slowly after the 24h incubation (Fig 2). The percent degradation was maximum at alkaline pH9 (68%) while rate of degradation was maximum in 72h (Fig. 3 and 4). At high temperature the percent utilization of phenol by this organism was maximum (Fig.5 and 6). The isolate *Prolinoborous fasciculus* removed phenol upto 800ppm from initial concentration 1000ppm Butani *et al.*, (2012).

Table 3: 16s rRNA sequencing, closed neighbour strain citation, accession no., and pair similarity of isolated phenol degrading organism from Lonar lake.

Closest Neighbour	Strain	Citation	Accession No	Pair wise Similarity (%),
Prolinoborous fasciculus	CIP 103579(T)	(Strength et al. 1976) Pot et al.1992	JN 175353	92.67

Sequence Text (in FASTA format): >A_MAR_14_015

GGCCTTAATACTATTCCCCTTCCTTGCTTTGCTAC-CTGGGTCGGCGATGACTAGGATTCCGACTTCATG-GAGTCGA

GTTGCAGACTCCAATCCGGACTACGATCGTTCTC-CTAAAAGAAGATTAGCATCCTCTCGCGAGGTAGCAAC-CCTTT

GTACCGACCATTGTAGCACGTGTGTAGCCCTGGTCG-TAAGGGCCATGATGACTTGACGTCGTCCCCGCCTTC-CTCC

ACCCTGTCACTGGCAGTATCCTTAAAGTTCCCG-GCTTAACCCGCTGGCAAATAAGGAAAAGGGTT-GCGCTCGTTG

CGGGACTTAACCCAACATCTCACGACACGAGCTGAC-GACAGCCATGCAGCACCTGTATGTAAGTTCCCGAAGGC

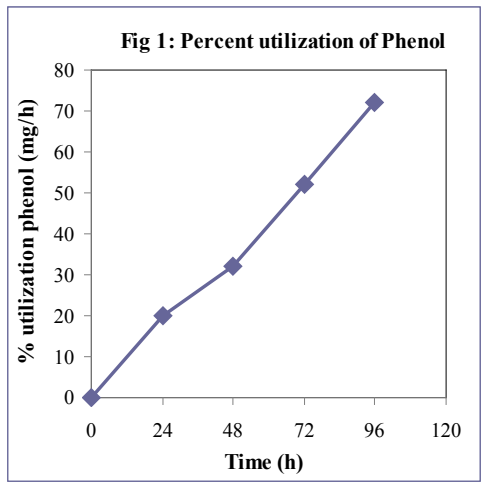
ACCAATCCATCTCTGGAAAGTTCTTACTATGTCAAGAC-CAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACA

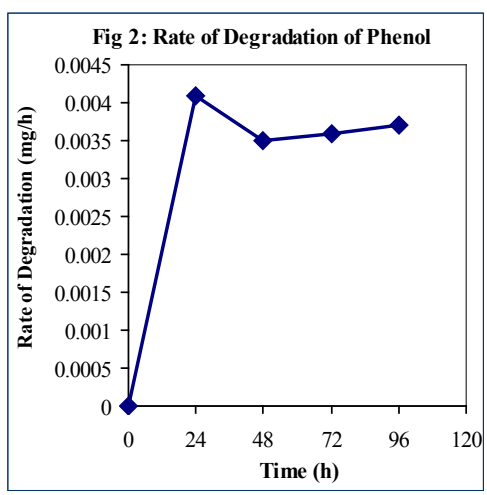
TGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTT-GAGTTTTAGTCTTGCGACCGTACTCCCCAGGCGGTCTAC

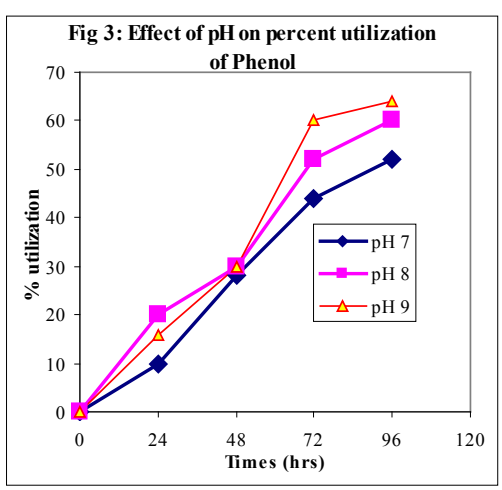
TTATCGCGTTAGCTGCGCCACTAAAGCCTCAAAGCCCC-CGCCCGTAGTAGACATCGTTACGGCATGGACTACCAG

GGAGTAAAACATTTT

Mrozik *et al.*, (2003), demonstrated that phenols and their compounds are the most recalcitrant and persistent organic chemicals in the environment. Vidyavathi *et al*, (2000) reported phenol degradation by *Nocardia* that resulted in complete degradation of phenol (100 ppm) within 96 hours. The isolates form the present study also reduces the phenol to a permissible limit and can be used in the bioremediation purpose. Chakraborty *et al.*, (2010) investigated the biodegradation of phenol by native bacterial strains isolated from coke oven processing waste water. The salinity and the alkalinity of the Lonar Lake is higher as compare to the industrial effluent, so the isolates from such environment sustain to the environment having high pH and salt concentration.







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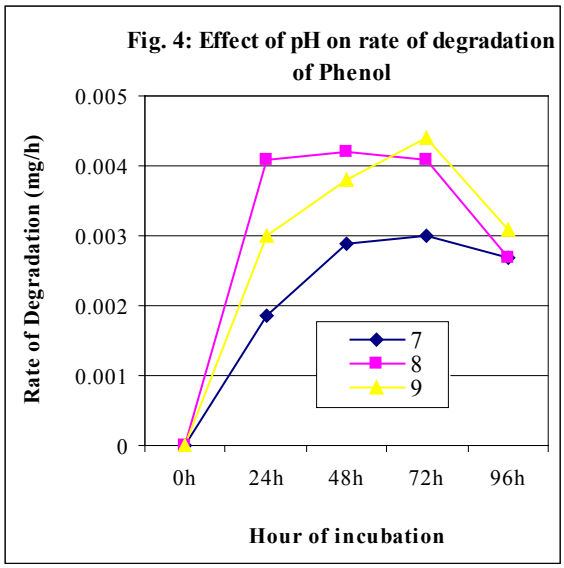

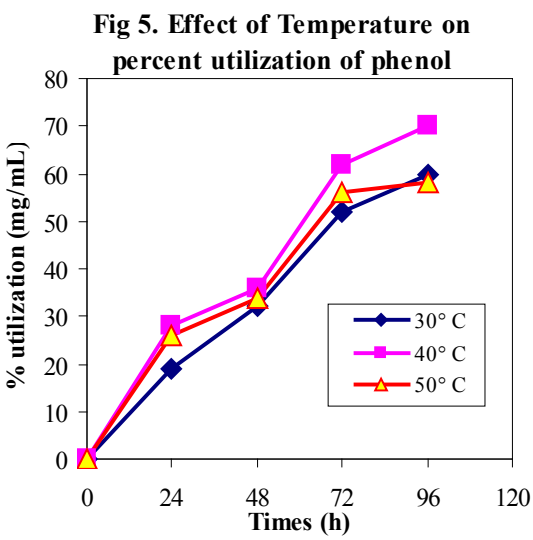

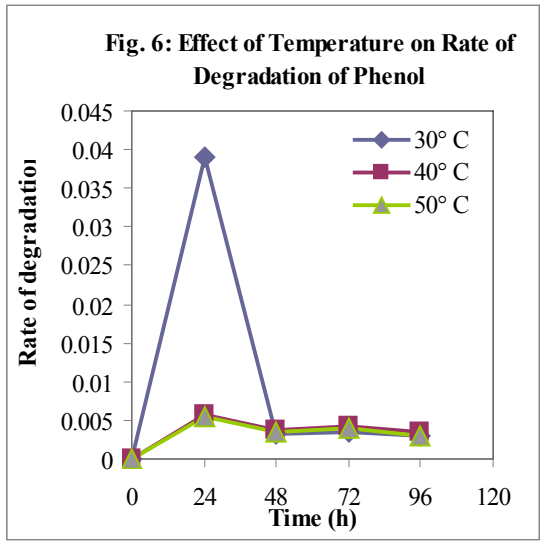

In present study the isolates *Prolinoborous fasciculus* showed phenol degradation at the rate of 72% at laboratory scale and optimum conditions were provided for the study. For field application of remediation of polluted sites the isolates may face the adverse condition with rapid change in the availability of nutrient and pollutants, so further processing and proper enrichment can help the isolates to improve the ability to remediate phenol. The study also showed that alkaline Lonar Lake harbors diverse microbial flora, which is endowed with the potential to degrade variety of chemical pollutants which helps to develop a new line of research in the field of bioremediation.

CONCLUSION

The aerobic phenol-degrading isolate *Prolinoborus fasciculus* appears to have greater potential for enhanced phenol removal through utilization of phenol as sole source of carbon and energy. The alkaliphilic bacteria, *Prolinoborus fasciculus* utilized up to 72% phenol, thus ensuring an acceptable phenol Lonar Lake bacterium, which could therefore be commercially exploit for bioremediation of phenol, which is major toxic pollutant in industrial waste effluents. It can also be concluded that isolated bacteria can be degrader at optimum pH of 9 and an optimum temperature of 40° C.

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