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Detoxification of Methanol by Methylotrophic Bacteria Isolated from Hypersaline Environment

KEYWORDS: Methanol, Biodegradation, Lonar Lake, Methylotrophs

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ABSTRACT *Microorganisms have ability to metabolize a variety of chemical compound, other environmental pollutants and these capabilities make them useful for application as bioremediating agents. Methanol is the toxic and hazardous pollutant to health and environments. Lonar Lake harbors number of methylotrophic bacteria and found potential to detoxify methanol therefore the objective of the study was to isolate the methylotroph to detoxify it. Among the isolates, potential strains, Achromobacter ruhlandii and Pseudomonas aeruginosa were isolated and selected on its methanol utilization property and identified based on cultural, morphological, biochemical characterization and 16S rRNA gene analysis. These isolates were found to utilize methanol up to 77 – 78%. The study indicated that these potential bacterial isolates therefore can be employed effectively to detoxify methanol and other C1 compounds on polluted sites.*

INTRODUCTION

The pollution of aquatic and terrestrial environments with toxic chemicals is widespread and is created by industrial activities and produced major human health and environmental problems. Methanol is widely used in different industries and hazardous to human and environment health also plays an important role in global warming and its atmospheric concentration has been increasing over many decades (ATSDR, 2008; Antony *et al.*, 2010). Bioremediation is the breakdown of complex and possibly toxic organic contaminants to non-toxic by microbial activity and such contaminants can be considered as carbon source for microbial growth. Methylotrophs are the unique group of methylotrophic bacteria can be "obligate," capable of growth on one or more C1 compounds like methanol, methylamine, dimethylamine, dimethyldisulfide, dimethylsulfoxide, formaldehyde and formate (Trotsenko and Murrell, 2008; Chistoserdova *et al.,* 2009).

These microbes adapt quite rapidly and grow at extreme condition using hazardous compounds as carbon and energy sources such extreme environment was found in Lonar Lake situated at Buldhana district, Maharashtra (India). It is a unique saline Lake in Asia formed in basaltic rock on the earth (Jhingran and Rao, 1954; Nandy and Deo, 1961). It has also been known that there are "extreme" environments on earth, which harbors number of methylotrophic bacteria and found potential to detoxify methanol. In this habitat, environmental conditions, such as pH, temperature and salinity concentrations are extremely high. Lonar Lake water is green throughout the year because of dense cyanobacterial (*Spirulina*) blooms (Surakasi *et al.,* 2007) which decomposed cyanobacterial biomass in soda lakes and likely to produce high quantities of methane, methanol, methylamine and dimethylsulfide favouring the surveillance of methylotrophs in this lake (Antony *et al.,* 2013; Tambekar *et al.,* 2011). Therefore, attempt was made to isolate methylotrophic bacteria to detoxify methanol from Lonar Lake and its eco-friendly use by microbial technology which is cost effective, cheap and free from ill effect

MATERIALS AND METHODS

Sampling site and Sample collection: A total of twelve samples of water, matt and sediment were collected in sterile containers from four different sites of Lonar Lake during September 2015. They were labeled and transported to a laboratory for further analysis.

Enrichment, Isolation and Biochemical characterization of Methanol-Degrading Bacteria: The collected samples were inoculated in 100mL Minimal Salt medium with 2% methanol as a sole source of carbon (Haddad *et al.*, 2009) and incubated at 37˚C at 100 rpm on rotary shaker for 3 days and 5 times repeated subculturing was made in the same fresh medium. After enrichment the loopful of broth sub-cultured on nutrient agar and after incubation well isolated and morphologically distinct colonies were selected and stored as a stock culture. Isolate was further characterized by commercially available Hi-media Rapid Detection kit KB003 and KB009. The 16S rRNA sequencing identification was performed at Agharkar Research Institute, Pune and NCCS, Pune (Maharashtra).

Methanol Degradation Studies: For determination of methanol utilization, the broth cultures of isolates were inoculated in 100 mL minimal salt medium containing 5mg/mL methanol as sole source of carbon and energy. The methanol utilization was determined by analyzing residual methanol after 24, 48, 72 and 96h by Sodium nitroprusside (SNP) method using UV-Visible spectrophotometer at 481 nm (Zhan *et al.,* 2010). The effect of environmental effect such as pH, temperature and salt concentration on methanol utilization was also determined.

RESULTS AND DISCUSSION

One of the most alarming situations in today's world is the generation of a huge amount of waste water with the toxic chemicals from the industrial sector. Microorganisms have an ability to grow in polluted environment and are generally assumed to be tolerant to pollutant (Van *et al.,* 2004). Methanol is widely used in a number of industries and its presence in the water imparts can have harmful effects on

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aquatic as well as terrestrial flora and fauna including human beings (ATSDR, 2008).

As per the objectives, present work started for isolation of methanol utilizing bacteria; water, sediment and matt samples were collected from alkaline Lonar Lake; eight methanol utilizing bacterial strains were successfully isolated by enrichment culture technique. Out of eight bacterial Methylotrophs, two isolates (DHT 7 and DHT 15) were prominent methanol utilizer, selected for detail study. Cultural, morphological and biochemical finding of these two isolates were done (Table 1). Isolates was further subjected for 16S rRNA gene analysis and shows a sequence homology of 97.09% with *Achromobacter ruhlandii* (DHT 7) and 100% with *Pseudomonas aeruginosa* (DHT 15) respectively and also phylogeny was constructed (Table 2 and 3). Tambekar and Pawar, (2013) isolated six *Pseudomonas* strains from Lonar Lake having good potential to degrade methanol. Tambekar *et al.,* (2012) isolated *Achromobacter xylosoxidans* from the alkaline Lonar Lake for remediation purpose. Four methylotrophic strains including *Acinetobacter baumani, Achromobactrum xylosoxidans, Ochromobactrum tritici* and *Pseudomonas aeruginosa* in the sediments of Lonar Lake were isolated by Tambekar *et al.,* (2011). Gainutdinova *et al.,* (2005), isolated methanotrophs from the surface layers of all the sediment samples and sometimes from dipper horizons.

The effect of environmental parameters such as pH, temperature and salt concentration on methanol utilization efficiency was studied. *A. ruhlandii* utilized 77% and *P. aeruginosa* 78% (rate of degradation 0.040 mg/mL and 0.041 mg/mL) methanol in 96 h respectively (fig. 1 and 2). The optimum methanol utilization was 78% at pH 8 for *A. ruhlandii* and 86% at pH 9 for *P. aeruginosa* (fig. 3 and 4). The optimum utilization was 73 % for *A. ruhlandii* at 30˚C and 76 % respectively at 40˚C for *P. aeruginosa* respectively (fig. 5 and 6). The effect of salt concentration (8% - 12%) on methanol utilization, *A. ruhlandii* utilized 72% in 8%, 69% in 10% and 60% in 12% and *P. aeruginosa* utilized 73% in 8%, 69% in 10% and 62% in12% (fig. 7 and 8).

Tambekar and Rajgire, (2015) isolated methanol bioremediating *P. aeruginosa* and *E. cloacae* from Lonar Lake and showed prominent methanol utilization by these methylotrophs. Tambekar *et al.*, (2013) reported 70% degradation of methanol within 72 h by *Ps. aeruginosa*. Some bacteria are known for their bioremediation potential, including members of *Pseudomonas* sp., *Enterobacter-clostridium* species sated by Van *et al.,* (2004). Tambekar *et al.,* (2015) isolated strains *Pseudomonas hibiscicola* and *Pseudomonas aeruginosa* and showed methanol utilization up to 79% to 82 %. Tambekar *et al.,* (2014) isolated *Ochrobactrum oryzae* from Lonar Lake and observed that *O. oryzae* utilized methanol optimally up to 78% at pH 7 and 40˚C. Tambekar *et al.,* (2011) reported that *Pseudomonas aeruginosa* (DQ989018) was new species and not previously recorded bacterial species from Lonar Lake to utilize methanol as carbon source. Trotsenko and Khmelenina, (2002), first isolated *M. kenii* methylotrophs from Soda Lake. These results are concurrence with present study.

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

TEST	*A. ruhlandii* (DHT 7)	*Ps. aeruginosa* (DHT 15)	TEST	*A. ruhlandii* (DHT 7)	*Ps. aeruginosa* (DHT 15)	TEST	*A. ruhlandii* (DHT 7)	*Ps. aeruginosa* (DHT 15)
Shape	R	R	Catalase	+	+	Lactose	+	-
Color of colony	Cream	Green	Oxidase	+	+	Arginine	-	-
Gram staining	-ve	-ve	MR	-	-	Sucrose	-	-
Texture	Sm	Sm	VP	-	-	Maltose	-	-
Arrangement	S	S	Citrate	+	+	Fructose	-	-
Motility	+	+	Xylose	-	+	Dextrose	-	+
Growth at different temperature			Lysine Utilization	-	-	Nitrate reduction	+	-
30 c	++	++	Arabinose	-	-	Mannose	-	-
40 c	++	++	Glucose	-	+	Melibiose	-	-
50 c	+	+	Galactose	-	+	Glycerol	-	-
Growth at different pH			Raffinose	-	-	Salicin	-	-
pH 7	+	+	Trehalose	-	-	Dulcitol	-	-
pH 8	+	+	Mannitol	-	-	Inocitol	-	-
pH 9	+	+	Adonitol	-	-	Sorbitol	-	-
pH 10	+	+	Saccharose	-	-	Erythritol	-	-
pH 11	+	+	Esculin hydrolysis	-	+	Melezitose	-	-
Growth at different salt conc.			- Methyl-D-Glucoside	-	-	Ornithine	-	+
1%	+	+	Rhamnose	-	-	Xylitol	-	+
2%	+	+	Cellibiose	-	-	Sorbose	-	-
3%	+	+	ONPG	-	-	L-Arabinose	-	-
4%	+	+	Esculin	-	+	Inulin	-	-
5%	+	+	Malonate	-	+	Sodium Gluconate	-	-

Note:- + = Positive; - = Negative; R= Rod; S= Single; Sm=Smooth;

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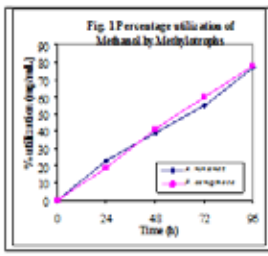

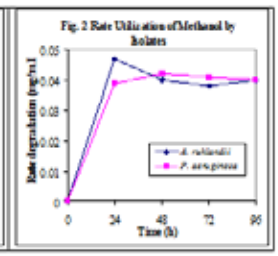

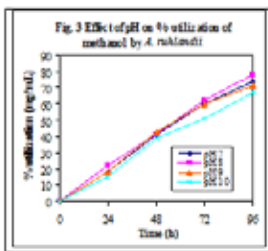

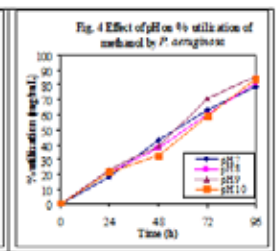

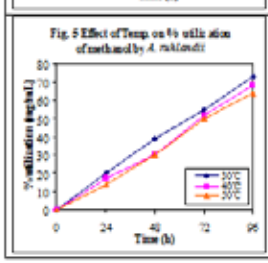

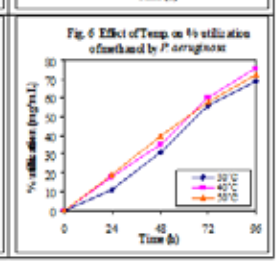

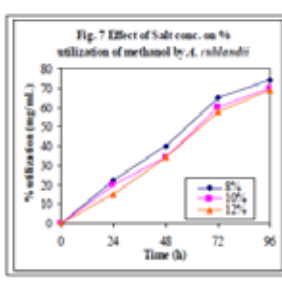

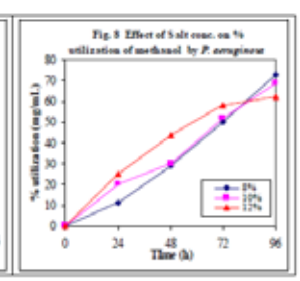

Table 2: Molecular detection and Closest phylogenetic affiliation and pair similarity of *A. ruhlandii*

Strain Designation	Closest phylogenetic affiliation	Max ident
DHT 7	*Achromobacter ruhlandii* (T) 16S ribosomal RNA gene partial sequence (AB010840)	97.09 %

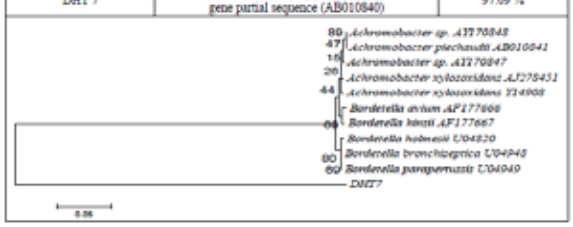

Table 3: Molecular detection and Closest phylogenetic affiliation and pair similarity of *P. aeruginosa*

Strain Designation	Closest phylogenetic affiliation	Max ident
DHT 15	*Pseudomonas aeruginosa* (T) 16S ribosomal RNA gene partial sequence (KF851246)	100.0%

found potential to detoxify methanol and the solution of pollution problems. These Methylotrophs were found to utilize methanol up to 77 – 78%. Thus study indicated that these potential bacterial isolates therefore can be employed effectively to detoxify methanol and other C_1 compounds on polluted sites.

CONCLUSION

Lonar Lake harbors number of methylotrophic bacteria and

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