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Molecular Characterization and Detoxification of Methanol by Haloalkaliphilic *Pseudomonas* Spp

***Tambekar DH Rajgire AV, SG Tembhare**

PG Dept. of Microbiology SGB Amravati University, Amravati, India
**diliptambekar@rediffmail.com*

Abstract: *Methylotrophs play a major role for saving our environments by utilizing toxic methanol, which are hazardous to environment as well as to human being hence attempt was made to degrade it by eco-friendly microbial techniques which is cost effective, cheap and free from ill effect. In present study, two bacterial strains Ps. hibiscicola (DHT 11) and Ps. aeruginosa (DHT 12) were isolated from sediment samples by using enrichment technique, in which minimal salt medium containing 2% methanol used as sole source of carbon and energy. The bacterial strains were characterised by cultural, morphological, biochemically and 16S rRNA gene sequencing. The selected microbial strain was able to utilized methanol up to 82% by Ps. hibiscicola and 79% by Ps. aeruginosa. Therefore these two bacterial cultures can be employed effectively to bioremediation of methanol and other C_1 compounds on polluted sites. Outcome of this study may offer useful information in evaluating potential bacterial methanol degraders from the environment.*

Keywords: *Lonar Lake, Methylotrophs, Ps. hibiscicola and Ps. aeruginosa*

1. INTRODUCTION

Methanotrophs are a unique group of microbes utilize methanol as sole source of carbon and energy and play a major role for saving our environments by utilizing toxic methanol, which are hazardous to environment as well as to human being. Currently, biodegradation of toxic chemicals and compounds received a great attention by many people from industries and researchers due to its toxicity. In future, bioremediation by microbial systems might be the potential tools to deal with the environmental pollutants. Methanol is one of the popular organic solvents and finds extensive applications in industries and household uses. It is rapidly and well absorbed by inhalation and by oral and topical exposure [1, 2, 3, 4]. Methylotrophic bacteria grow on a number of different one carbon compounds including methane, methanol, methylated amines and methylated compounds containing sulphur and utilized as source of food [5, 6].

Microbes rapidly adapt and grow at extreme condition using hazardous compounds as energy sources in waste materials. Lonar Lake, which is situated in Buldhana district of Maharashtra state, India have a unique ecosystem and harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment [7, 8]. Lonar Lake water is green throughout the year due to dense cyanobacterial blooms. Decomposition of cyanobacterial biomass in soda lakes is likely to produce high quantities of methane, methanol, methylamine and dimethylsulfide favouring the surveillance of methylotrophs in this lake [9]. Methanol is the toxic to health therefore attempt was made to degrade it by eco-friendly microbial techniques which is cost effective, cheap and free from ill effect by Lonar lake bacteria to control industrial methanol and C1 compound pollution.

2. MATERIALS AND METHODS

2.1. Collection, Enrichment, Isolation and Identification of Microbes

A total of 12 (water, sediment and matt) samples were collected from 4 different sites of Lonar lake in September, 2014. The samples were inoculated in minimal salt media containing 2% methanol as carbon source. All flasks were incubated at 37^0C in rotary shaker (100 rpm) for three days and five times repeated sub culturing was made in same medium. After enrichment the broth were subcultured 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 and BLAST identification was performed at Agharkar Research Institute, Pune (Maharashtra).

2.2. 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 [10]. The effect of environmental effect such as pH, temperature and salt concentration on methanol utilization was also determined

3. RESULTS AND DISCUSSION

The bacterial species are able to grow in the toxic conditions and are generally assumed to be tolerant to toxic chemical. Tolerance is defined as "the ability of a microorganism to survive chemical toxicity by means of intrinsic properties and or environmental modification of toxicity [11]. In the present investigation, attempt was made to isolate methanol degrading microorganisms from halophilic environment such as Lonar Lake, having a unique ecosystem and harbors various unidentified, unique haloalkaliphilic bacterial species which have potential to degrade or remove chemical toxic pollutant from environment and certain microorganisms which have been reported by various researchers but detail studies on the biodegradation of methanol from Lonar Lake were yet not to be done.

Table1. *Morphological and biochemical characteristics of bacteria isolated from Lonar Lake*

TEST	***Ps. hibiscicola* (DHT 11)**	***Ps. aeruginosa* (DHT 12)**	**TEST**	***Ps. hibiscicola* (DHT 11)**	***Ps. aeruginosa* (DHT 12)**	**TEST**	***Ps. hibiscicola* (DHT 11)**	***Ps. aeruginosa* (DHT 12)**
Shape	R	R	Catalase	+	+	Lactose	-	-
Color of colony	Green	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.			α-Methyl-D-Glucoside	-	-	Ornithine	+	+
1%	+	+	Rhamnose	-	-	Xylitol	-	+
2%	+	+	Cellibiose	-	-	Sorbose	-	-
3%	+	+	ONPG	+	-	L-Arabinose	-	-
4%	+	+	Esculin	+	+	Inulin	-	-
5%	+	+	Malonate	+	+	Sodium Gluconate	-	-

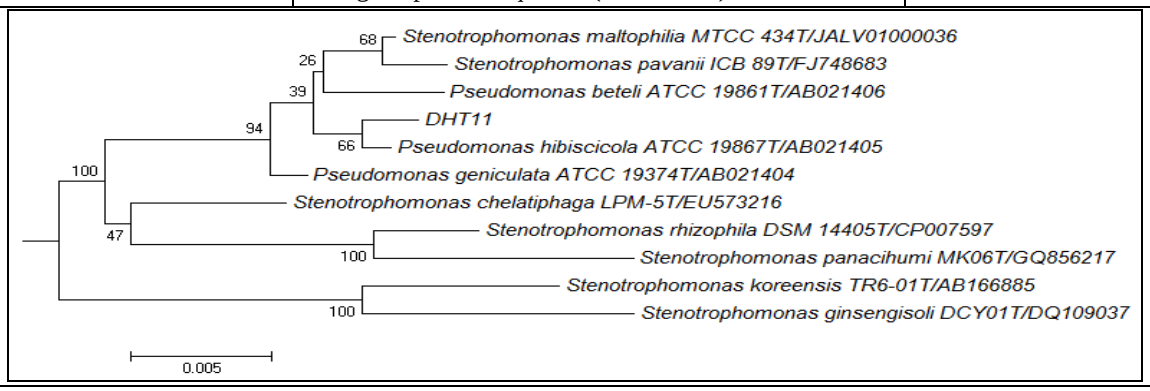
Note: *+ = Positive; - = Negative; R= Rod; S= Single; Sm=Smooth;*

In the present study, a total of 10 different bacterial species were isolated from water, sediment and matt samples of Lonar Lake. Out of 10 bacterial Methylotrophs, two isolates (DHT 11 and DHT 12) were prominent methanol utilizer, selected for detail study. Morphological, cultural and biochemical

finding of these two isolates were given in table 1. These DHT11 and DHT12 bacterial isolates were analyzed for the 16S rRNA gene sequencing and results suggested that it belongs to genus *Pseudomonas* of Pseudomonadaceae family having the nearest neighbor as *Pseudomonas hibiscicola* with 99.70% similarity (DHT 11) and *Pseudomonas aeruginosa* with 100% similarity (DHT 12). The phylogenetic tree was performed by neighbor joining tool, which shows the relation between the isolates and their respective neighbor type strains along with their respective distances (Table 2 and 3). 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.*, [12, 13]. Thakkar and Ranade, [14] isolated alkaliphillic *Methanosarcina* from Lonar Lake. Tambekar and Pawar, [15] isolated six *Pseudomonas* strains from Lonar Lake having good potential to degrade methanol.

Table2. *Molecular detection and closest phylogenetic affiliation and pair similarity of Ps. hibiscicola*

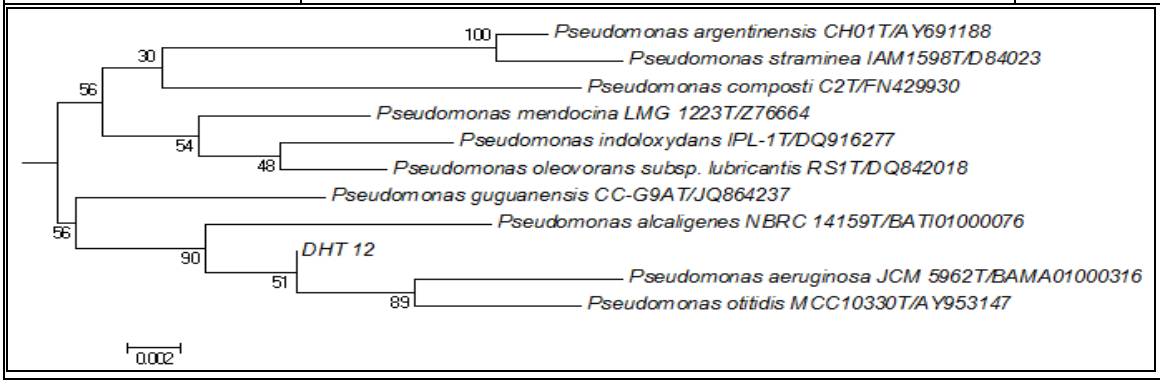
DHT 11	*Pseudomonas hibiscicola* ATCC 19867(T)16S ribosomal RNA gene partial sequence (AB021405)	99.70%

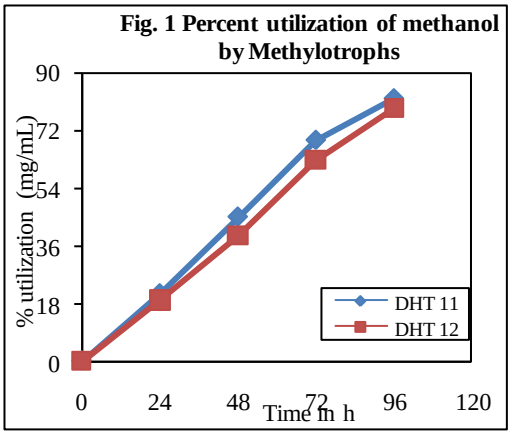


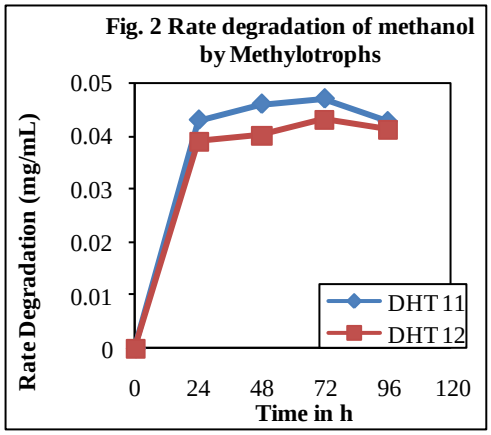
In the present study, the isolates *Ps. hibiscicola* (DHT 11) and *Ps. aeruginosa* (DHT 12) were studied to check its ability to utilize or degrade methanol by analyzing residual methanol after each interval of 24h upto 96 h by using UV- visible spectrophotometer at 481 nm. The effect of environmental parameters such as pH, temperature and salt concentration on methanol utilization efficiency was also studied. *Ps. hibiscicola* utilized 82% and *Ps. aeruginosa* 79% (rate of degradation 0.0427 mg/ml and 0.041 mg/ml) methanol in 96 h respectively (fig. 1 and 2). The optimum methanol utilization was 88% and 83% respectively recorded for both *Ps. hibiscicola* and for *Ps. aeruginosa* at pH 10 (fig. 3 and 4). The optimum utilization was 80 % and 77 % respectively at 40˚C for *Ps. hibiscicola* and for *Ps. aeruginosa* respectively (fig. 5 and 6). The effect of salt concentration (8% - 12%) on methanol utilization, *Ps. hibiscicola* utilized 74% in 8%, 78% in 10% and 70% in 12% and *Ps. aeruginosa* utilized 73% in 8%, 69% in 10% and 63% in12% (fig. 7 and 8).

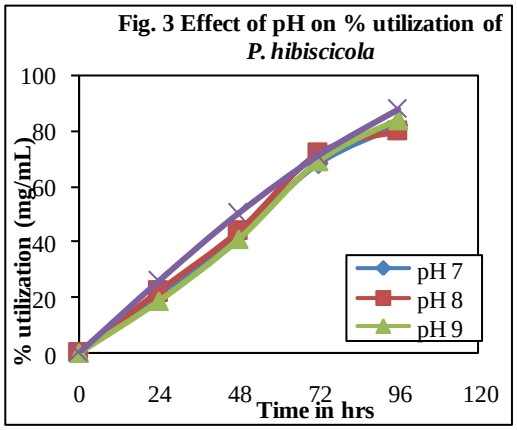
Table3. *Molecular detection and closest phylogenetic affiliation and pair similarity of Ps. Aeruginosa*

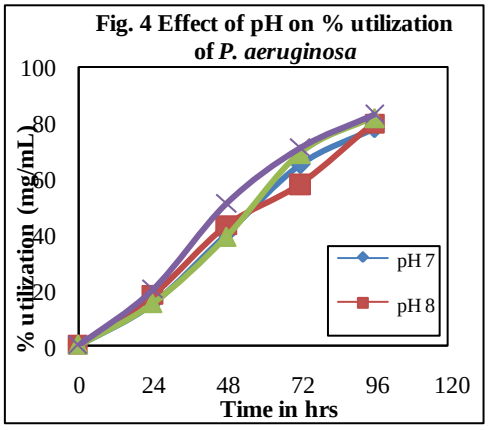
DHT 12	*Pseudomonas aeruginosa* JCM 5962(T)16S ribosomal RNA gene partial sequence (BAMA01000316)	100%

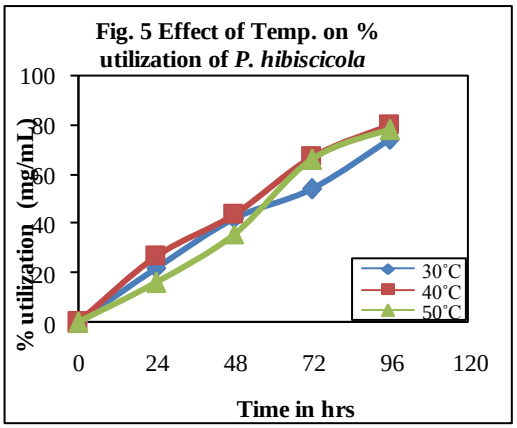


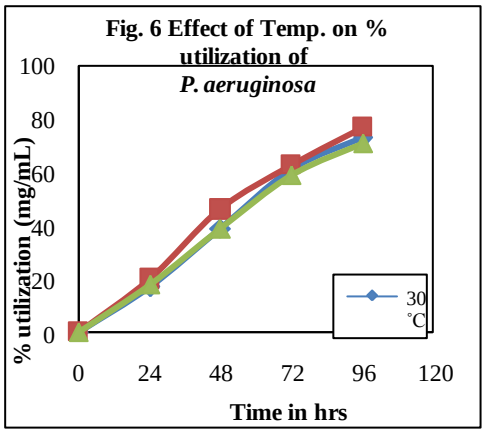


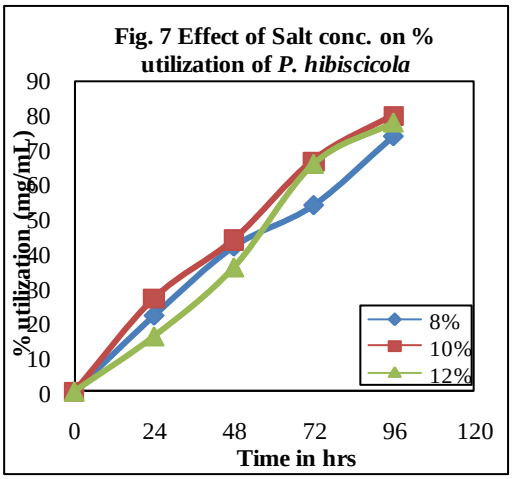


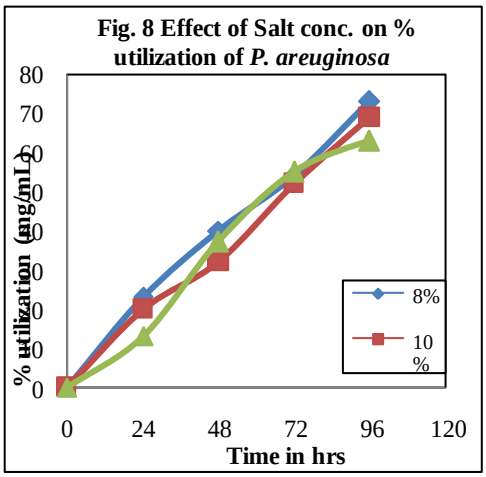












Tambekar *et al.*, [13] studied percent utilization and rate of degradation of methanol and reported 70% and 0.036mg/mL from *Ps. aeruginosa*. Some bacteria are known for their bioremediation potential, including members of *Pseudomonas* sp., *Enterobacter-clostridium* species [11]. Tambekar *et al.*, [8]

isolated *Ochrobactrum oryzae* from Lonar Lake and observed that *O. oryzae* utilization 78% methanol, and pH 7 and temperature 40˚C was optimum for methanol utilization. These results are in concurrence with present study. Tambekar *et al.*, [12] reported that *Pseudomonas aeruginosa* is new species and not previously recorded bacterial species from Lonar Lake to utilize methanol as carbon source.

4. CONCLUSION

Till date several works are in progress to isolate new and efficient microbial strain that have ability to degrade methanol. The presently isolated strains of *Pseudomonas* spp have potential to utilize and clean the environment polluted with methanol or related C1 chemicals. This work may be provided a useful guideline in evaluating potential methanol biodegrades isolated from environment.

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AUTHOR'S BIOGRAPHY

Prof.Dr. Dilip H. Tambekar, MSc, PhD (microbiology), is presently Prof and Head, P.G. Department of Microbiology. SSG Amravati University, Amaravati (Maharashtra State) having 33 years of teaching experience. He has produced 24 PhD students and published more than 185 research papers in National and international journals of reputes. He is specialized in water hygiene and remediation of environmental pollutions.