Spatial and Temporal Distribution of Methanotrophs in Lonar Lake Environment

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Lonar Lake is an alkaline ecosystem formed entirely on basalt rock. It is a completely closed system. The Methanotrophs play a major role for conserving environment by utilizing toxic C1 carbon compound and reduces the pollution produced by methanol, green houses gases etc. In present study, methanotrophic bacterial isolates were isolated from water and sediment of Lonar Lake using minimal salt medium containing 2% (v/v) methanol as sole carbon and energy source.. These strains were further screened for its ability to utilize methanol by Spectrophotometric method. The results indicated that there was no remarkable change in the distribution methanotrophs on spatial scale, with the methanotrophs showing presence in all the areas of the Lonar Lake and no remarkable change in the distribution methanotrophs on spatial scale, with the methanotrophs showing presence in all the areas of the Lonar Lake

Keywords: Lonar Lake, Methanotrophs, Bioremediation, Methanol

INTRODUCTION

The Lonar Lake is a saline and hyperalkaline ecosystem formed entirely on basalt rock by meteor impact. The crater is located in India ~ 550 km east of Mumbai and Arabian Sea is 150 m deep and 1830 m across (Fredrikson, 1973) with raised rim up to the 100m in width and 20m to 30 m high and alkalinity of the Lake water is attributed to the high content of sodium carbonate (Thakker and Ranade, 2002). The Lonar Lake is unique in the world for its alkalinity (pH 10) and salinity (NaCl 0.9%) of the water but it was seen that chlorides and salinity of the Lake water is decreasing day by day (Tambekar *et al*., 2010).Microbiological studies using culture-dependent and culture-independent strategies have identified and characterized both bacterial (Kanekar *et al*.,1999, 2002; Nilegaonkar *et al*., 2002; Wani *et al*., 2006; Surakasi *et al*., 2007) and archaeal (Thakker and Ranade, 2002; Surakasi, 2007; Surakasi *et al*., 2007) communities in the Lonar Lake water and sediment.

Methanotrophs are a unique group of methylotrophic bacteria, which utilize one-carbon (C1) compounds such as methane, methanol and methylamine which constitute an important component of microbe-driven food web chains in many ecosystems. Methylotrophic bacteria, are phylogenetically distributed across diverse phyla such as Gammaproteobacteria (type I methanotrophs), Alphaproteobacteria (type II methanotrophs) (Trotsenko and Murrell,2008), filamentous methane oxidizers (Stoecker *et al*., 2006; Vigliotta *et al*., 2007) and Verrucomicrobia (Dunfield *et al*., 2007; Pol *et al*., 2007; Islam *et al*., 2008) and contribute significantly in

biogeochemical cycling of carbon by facilitating the incorporation of C1 compound-derived carbon into biomass (Anthony, 1992; Chistoserdova *et al*., 2009). The global cycling of methane and related C1 compounds further affects the important environmental phenomena related to climate change.

MATERIALS AND METHODS

Isolation of Bacterial Strain by Enrichment Method The sediment and water samples were collected from different sites of Lonar Lake (Buldhana District in Maharashtra, India). The medium containing ingredient (g/l): NaNO3 2.5g, KCl 0.1g, KH2PO4 3g, K2HPO4 7g, CaCl2 0.01g, MgSO4 0.5g, FeSO4 0.116g, H3BO3 0.232g, CuSO4 0.41g, MnSO40.008g, (NH4)6 Mo7O24, 0.008g, and ZnSO4 0.174g, 20 ml methanol, is used for isolation of methytrophic bacteria (Haddad *et al*., 2009) The medium was then incubated at 37^0C on a rotary shaking incubator at 100 rpm for 3 days. The subculturing was made for 5 times for enrichment and isolation of pure culture was done on solid nutrient agar plate. Well isolated colonies were selected and stored at 4^0C as stock culture (Pawar , 2018).The methanol utilization was determined by analyzing residual methanol at 480 nm in the medium after the each interval 24 h up from 0 h to 96 h by using UV- visible spectrophotometer (Zhan *et al*., 2010).

RESULTS AND DISCUSSION

Lonar Lake represents an extreme environment with high pH and moderate salinity. It is the only known depression in the region and hence may serve as a drain for excess runoff from anthropogenically influenced surrounding areas. However, the contribution of such natural or anthropogenic factors towards elevated phosphate and nitrate levels in the lake sediments warrants further investigation. Lonar Lake water is green throughout the year because of dense cyanobacterial bloom dominated by *Arthrospira* (Surakasi *et al*., 2007). Sediment and water samples were chosen as the source of bacterial isolation and these were enriched in minimal medium using 2% (v/v) methanol as sole source of carbon and energy for one month by repeated subculture after every 96 h.Those bacterial isolates able to grow on medium containing 2% (v/v) methanol as carbon source were identified as methanotrophs and subsequently isolated in pure culture.

In this investigation, a new method for the direct determination of methanol using sodium nitroprusside (SNP) is used (Zhan *et al*., 2010). It has been reported that SNP can react with nucleophilic agent such as primary and secondary aliphatic amine however; no studies in the literature to date have been reported on the reaction of SNP and alcohol. This experiment results showed that SNP can react with methanol to form colored product. Absorbance of product is linear with certain extent of the concentration of methanol.These morpho-biochemically characterized bacteria were identified by 16S rRNA sequencing and phylogenetic tree was constructed. The result of the phylogeny showed that methylotrophic strains isolated from Lonar Lake were related to phylum Proteobacteria. Sediment is the potential source of methanotrophs from Lonar Lake.

Spatial Distribution of Methanotrophs

Table 1: Presence of Methanotrophs in the different types of samples

SN	Nature of Sample	Abundance of Methanotrophs
1	Sediment	2.8 X 10^3
2	Water	3.9 X 10^2
3	Mat	Nil

Table 1 provides information pertaining to the presence of methanotrophs in different type of samples. It was evident from the data that 2.8 X 10^3 methanotrophs bacteria were present in the sediment of Lonar Lake, 3.9 X 10^2 methanotrophs bacteria were present in water, whereas no methanotrophs were present in mat of Lonar Lake.The methanotrophs were relatively more in sediments than other samples i.e. water and mats.

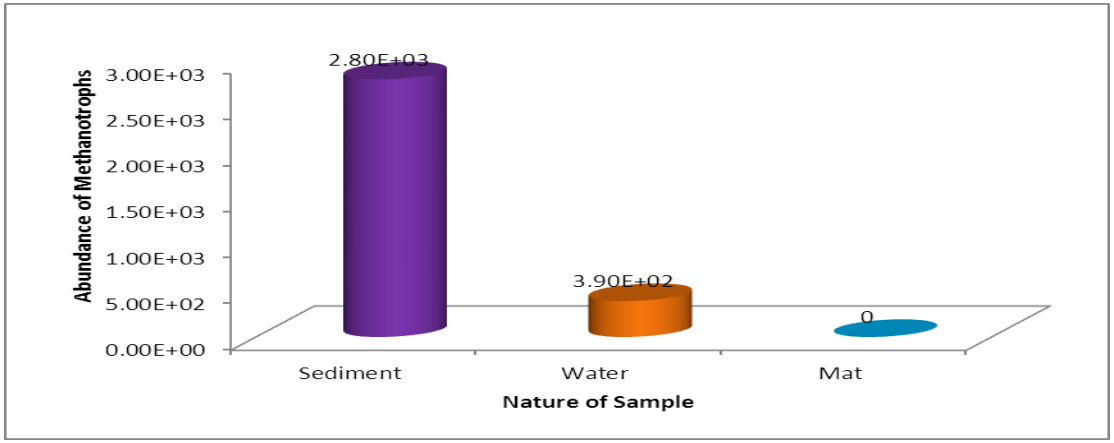

Fig. 1: Presence of Methanotrophs in the different types of samples

Table 2: Presence of Methanotrophs in the different layers of sediment samples

SN	Sampling Site	Abundance of Methanotrophs
1	Top most layer of sediment	1.7 X 10^3
2	2 inches deep in sediment	1.4 X 10^2
3	Below 2 inches (depth sediment)	0.2 X 10^2

Above **Table 2** provides information regarding presence of methanotrophs in different layers of sediment samples collected from Lonar Lake. It was observed that 1.7 X10^3 methanotrophs were present in top most layer of sediment, 1.4 X 10^2 methanotrophs present in 2 inches deep sediment, whereas 0.2 X 10^2 methanotrophs were present below 2 inches depth of the sediment in Lonar Lake. Methanotrophs population fluctuations occurred primarily within the top 0-2 cm of sediment, where methanotrophic cells increased by a factor of 3-5 over the depth sediments.

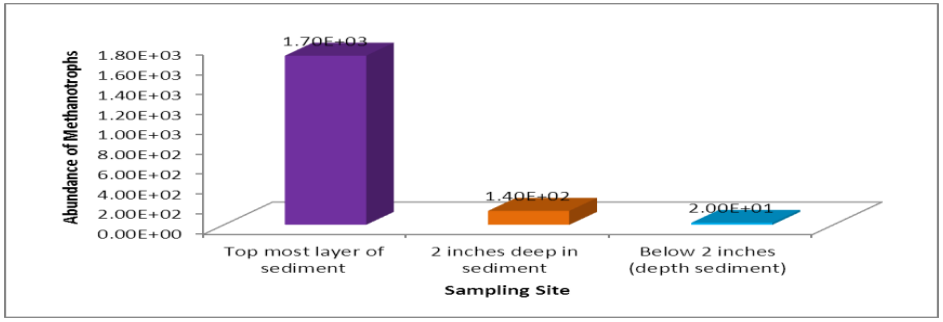

Fig. 2: Presence of Methanotrophs in the different layers of sediment samples

Table 3: Presence of Methanotrophs in the top 0-2 cm layer sediment samples

SN	Sampling Location	Abundance of Methanotrophs
1	East shore of Lonar Lake	2.1 X 10^2
2	West shore of Lonar Lake	1.7 X 10^3
3	North shore of Lonar Lake	4.2 X 10^2
4	South shore of Lonar Lake	3.7 X 10^3

Table 3 shows information pertaining presence of methanotrophs in top (0-2 cm) layer sediments at different sampling location of Lonar Lake. It was apparent from the information that 2.1 X 102 methanotrophs were present in the top layer sediments of east Shore of Lonar Lake, 1.7 X 103 methanotrophs present in the top layer sediment of west Shore of Lonar Lake, 4.2 X 102 methanotrophs were present in the top layer sediment of north shore of Lonar lake, whereas 3.7 X 103 methanotrophs were present in the top layer sediment of south shore of Lonar lake. The results indicated that there was no remarkable change in the distribution methanotrophs on spatial scale, with the methanotrophs showing presence in all the areas of the Lonar Lake.

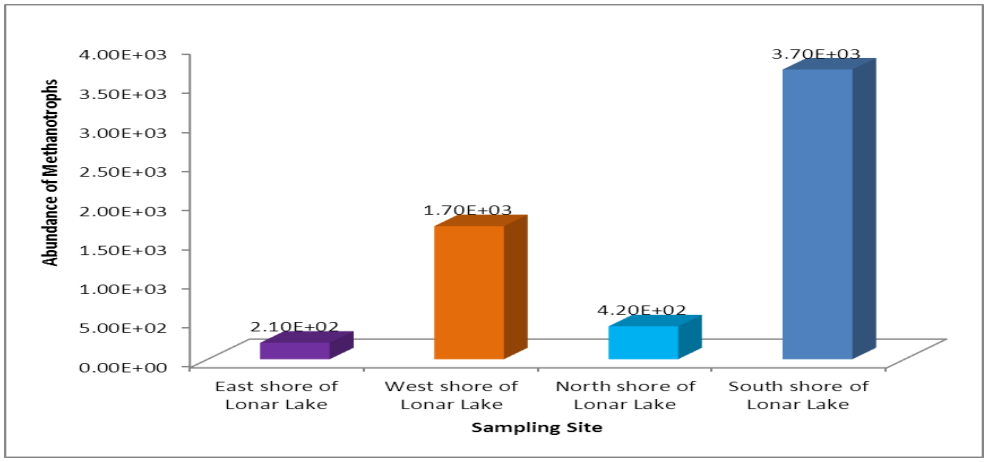

Fig. 3: Presence of Methanotrophs in the top 0-2 cm layer sediment samples

Seasonal variation in the presence of Methanotrophs in the top 0-2 cm layer sediment samples

Table 4: Information regarding seasonal variation in presence of methanotrophs in top (0-2cm) layer sediment sample of Lonar Lake

SN	Sampling Location	Abundance of Methanotrophs
1	Summer	0.6 X 10^2
2	Pre Monsoon	2.4 X 10^2
3	Post Monsoon	3.5 X 10^3
4	Winter	2.1 X 10^2

Table 4 illustrates information regarding seasonal variation in presence of methanotrophs in top (0-2cm) layer sediment sample of Lonar Lake. It was observed that the abundance of methanotrophs was 3.5 X 10^3 during post-monsoon season. During pre-monsoon and winter season the abundance of methanotrophs was 2.4 X 10^2 and 2.1 X 10^2 respectively. However; during summer season the abundance of methanotrophs was 0.6 X 10^2. It was apparent from the information that the abundance of methanotrophs was more during post-monsoon season whereas it is low during summer season. It was apparent from the study results that on temporal scale, methanotrophic abundance showed significant ($P < 0.05$) difference, with highest recorded during the post monsoon season at majority of sampling sites.

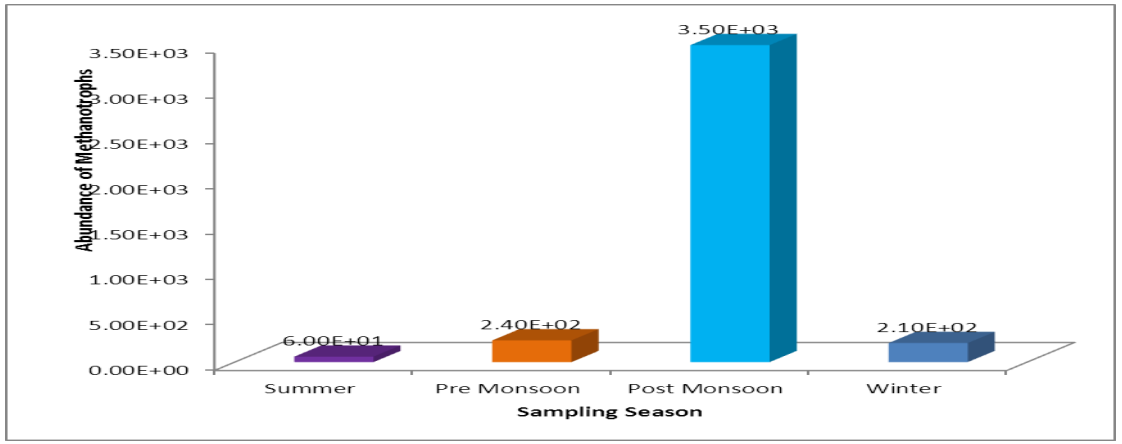

Fig. 4: Seasonal variation in presence of methanotrophs in top (0-2cm) layer sediment sample of Lonar Lake

The findings of this study provide a window into the diversity of bacterial community members which are methane degrading from the Lonar Lake. These isolated bacterial species may be used to combat industrial pollution of methanol or to control global warming which may found better choice for studies like methane, methanol or toxic chemical degradation to combat Global warming. Till date several works are in progress to isolates efficient microbial strain that have ability to utilize methanol. We report methanotrophs were relatively more in sediments than other and population fluctuations occurred primarily within the top 0-2 cm of sediment, where methanotrophic cells increased by a factor of 3-5 over the depth sediments. The results indicated that there was no remarkable change in the distribution methanotrophs on spatial scale, with the methanotrophs showing presence in all the areas of the Lonar Lake.

CONCLUSION The unexplored site of alkaline Lonar Lake contains many methanogenic and methanotrophic genera which might be helpful for the remediation of pollution environment. In present study results that on temporal scale, methanotrophic abundance showed significant ($P < 0.05$) difference, with highest recorded during the post monsoon season at majority of sampling sites and provides a new unexplored site for researcher.

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