Bacterial tag encoded FLX titanium amplicon pyrosequencing (bTEFAP) based assessment of prokaryotic diversity in metagenome of Lonar soda lake, India

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Bacterial diversity and archaeal diversity in metagenome of the Lonar soda lake sediment were assessed by bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). Metagenome comprised 5093 sequences with 2,531,282 bp and 53 ± 2% G + C content. Metagenome sequence data are available at NCBI under the Bioproject database with accession no. PRJNA218849. Metagenome sequence represented the presence of 83.1% bacterial and 10.5% archaeal origin. A total of 14 different bacteria demonstrating 57 species were recorded with dominating species like Coeliella burnetii (17%), Fibrobacter intestinalis (12%) and Candidatus Cloacamonas acidaminovorans (11%). Occurrence of two archaeal phyla representing 24 species, among them Methanosaeta harundinaceae (35%), Methanoculleus chikugoensis (12%) and Methanolinea tardo (11%) were dominating species. Significience presence of 11% sequences as an unclassified indicated the possibilities for unknown novel prokaryotes from the metagenome.

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Soda lakes are unique to all other aquatic ecosystems due to their high productivity rates, high pH and salinity, but information on the prokaryotic diversity in such environments is sparse. Microbes in the soda lake have attracted significant attention as a source of biotechnological valuable biomolecules. Lonar Lake is a soda lake, being formed by meteorite impact on Deccan basalts in India, approximately 570,000 years ago [1]. Lonar lake is the third largest terrestrial crater in the world filled with breccia and unconsolidated sediment [2]. The lake sediments are rich in iron, magnesium, phosphate, nitrate, organic carbon and Kjeldahl nitrogen [3] that provides ample nutrition to flourish the more cultivable bacteria than the lake water [4].

Lonar Lake is a remarkable analogue for studying basaltic impact crater structures that are common on the surfaces of Mars [5] and the Moon [6]. A culture-dependent study from the lake has revealed the substantial phylogenetic diversity of bacteria, including Firmicute, and α-, β-, and γ-Proteobacteria [4,7,8]. Cloning based studies were reported few new phyla, including Actinobacteria, Planctomycetes, Cyanobacteria, Deinococcus-Thermus, Spirochetes, Cytophagas-Flavobacterium-Bacteroidetes, Euryarchaeota and Crenarchaeota [9,10]. In addition, three novel bacterial genera [11–13] and four novel species [14–17] from the lake have been isolated by a culture-dependent method. Fungal ITS genes retrieved from Lonar lake sediment indicated the rich fungal diversity [18]. Therefore, the Lonar soda lake is a bioprosperity sink of various novel biotechnological important extremophiles. We probe the prokaryotic community structure in the sediment of the lake by a metagenomic approach in the present work.

Sediment samples were collected from a 3 m depth of Lonar soda lake (19°59′N, 76°31′E), India.
Metagenomic DNA was isolated by the soil DNA isolation kit PowerMaX™ (MO BIO Laboratories, Inc. CA, USA). Metagenome sequencing was done by the 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) method [19]. The pyrosequence output contains 5093 reads with 2,531,282 bp size and 53 ± 2% G + C content. Further, sequences were processed and analyzed with an MG-RAST on-line server [20].

At the domain level, 83.1% bacteria and 10.5% archaea were counted in metagenome. In the bacterial domain, 14 bacterial phyla including unclassified category were obtained in metagenome. Prominent phyla were Proteobacteria (18%), unclassified (16%) and Planctomycetes (15%). A total of 57 species of bacteria were documented with dominant presence of *Coxiella burnetii* (17%), *Fibrobacter intestinalis* (12%) and *Candidatus Cloacamonas acidaminovorans* (11%) (Fig. 1). While metagenome contained two archaeal phyla Euryarchaeota and Thaumarchaeota with 24 species. Dominating archaeal species were *Methanosaeta harundinacea* (35%), *Methanoculleus chikugoensis* (12%) and *Methanolinea tarda* (11%) (Fig. 2). The existence of methanogen, methylotrophic methanogen, hydrogenotrophic methanogens, ammonia-oxidizing archaea (AOA), Phototrophic archaea and haloalkaliphilic archaea indicated the vital role into lake biogeochemical cycles.

The occurrence of diverse groups of bacteria and archaea illustrates the lake sediment harbor complex microbial food webs that interconnect various biological cycles via redox coupling [21]. Detection of the few animal and human gut bacteria suggests the urgent need for protection from anthropogenic forces. Occurrence of the microaerophilic and anaerobic bacteria and archaea in sediment is due to the anoxic condition in sediment.

**Nucleotide sequence accession number**

Metagenome sequence data are available at NCBI BioProject accession no. PRJNA218849.

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Fig. 1. Bacterial community structure of Lonar soda lake sediment metagenome.
Fig. 2. Archaeal community structure of Lonar soda lake sediment metagenome.

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