



Complete metagenome sequencing based bacterial diversity and functional insights from basaltic hot spring of Unkeshwar, Maharashtra, India



Gajanan T. Mehetre ^{a,b}, Aditi S. Paranjpe ^a, Syed G. Dastager ^{a,b}, Mahesh S. Dharne ^{a,b,*}

^a National Collection of Industrial Microorganisms (NCIM), CSIR-National Chemical Laboratory (NCL), Pune, India

^b Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

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ABSTRACT

Unkeshwar hot springs are located at geographical South East Deccan Continental basalt of India. Here, we report the microbial community analysis of this hot spring using whole metagenome shotgun sequencing approach. The analysis revealed a total of 848,096 reads with 212.87 Mbps with 50.87% G + C content. Metagenomic sequences were deposited in SRA database with accession number (SUB1242219). Community analysis revealed 99.98% sequences belonging to bacteria and 0.01% to archaea and 0.01% to Viruses. The data obtained revealed 41 phyla including bacteria and Archaea and including 719 different species. In taxonomic analysis, the dominant phyla were found as, Actinobacteria (56%), Verrucomicrobia (24%), Bacteriodes (13%), Deinococcus-Thermus (3%) and firmicutes (2%) and Viruses (2%). Furthermore, functional annotation using pathway information revealed dynamic potential of hot spring community in terms of metabolism, environmental information processing, cellular processes and other important aspects. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of each contig sequence by assigning KEGG Orthology (KO) numbers revealed contig sequences that were assigned to metabolism, organismal system, Environmental Information Processing, cellular processes and human diseases with some unclassified sequences. The Unkeshwar hot springs offer rich phylogenetic diversity and metabolic potential for biotechnological applications.

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Specifications	
Organism/cell line/tissue	Unkeshwar hot spring metagenome
Sex	not applicable
Sequencer or array type	Illumina Hi seq 2500
Data format	raw data: fastq
Experimental factors	environmental sample
Experimental features	shotgun metagenome sequencing followed by microbial community and taxonomic analysis using KEGG pathways
Consent	not applicable
Sample source location	water sample, Unkeshwar hot spring, Maharashtra State, India

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/sra/SRX1499016>

* Corresponding author at: Academy of Scientific and Innovative Research (AcSIR), New Delhi, India.

E-mail address: ms.dharne@ncl.res.in (M.S. Dharne).

2. Experimental design, materials and methods

Thermopiles have been discovered in geothermal features all over the world, leading to the discovery of many novel environmental microorganisms with important applications in biotechnology, medicine, and bioremediation [1]. Microbes inhabiting hot springs are dictated by environmental physicochemical characteristics such as pH, redox potential, temperature and concentration of trace elements [2,3]. Among these factors, water temperature is the major factor in controlling microbial distribution within hot springs [4]. Hot springs microorganisms thrive under multiple environmental stresses and to survive under such stresses, microbial communities use mutualistic or communalistic symbiotic relationships [5].

Metagenomic analyses using high throughput sequencing have been extremely a valuable tool for describing microbial community structure and function in extreme ecosystems [6,7]. Using such high throughput techniques most of the terrestrial hot springs all over the world, hosting diverse thermophilic microorganisms has been investigated. Majorly studied regions such as Yellowstone National Park, USA [8], Great Basin, USA [4], Philippines [9], Canada and New Zealand [10] and China [11] revealed many novel microbial lineages with promising applications in biotechnology. Metagenome sequencing techniques

are also useful for identifying novel genes with novel bioactive molecule production capabilities [12,13].

India has some high as well as low temperature geothermal springs in different geographic regions. In India thermal springs are found scattered throughout the country and occur either as solitarily or in groups up to 3000 M above sea level [14]. From hot springs of basaltic region of Deccan plateau of India, scanty and superficial reports have been documented [15,16]. Therefore we investigated microbial diversity of Unkeshwar hot spring located in such region in Maharashtra state of India. Unkeshwar is located at geographical South East Deccan Continental basalt of India (19°34'–19°40'N and 78°22'–78°34'E) from mean sea level. The hot springs in this area emerge through basalt. The main Unkeshwar hot spring is within Unkeshwar temple in the form of kund. The water has sulphurous smell with feeble gaseous activity and discharge through the jointed Deccan basalts. Water samples were collected in sterile containers from Mukhya kund location of Unkeshwar hot spring during November 2012. The discharged water (10,000 l/h) is used by visitors to take bath for a therapeutic belief. Samples were processed for metagenomic and physico-chemical analysis.

Temperature and pH were measured for the sample at the site. The physiochemical parameters of the samples were analysed at a certified chemical testing laboratory (Accurate Analytical Laboratory Pvt. Ltd., Pune, India) using standard methods of American Public Health Association [17]. The temperature of water sampled was between 50 °C–60 °C and the pH 7.3. The physiological parameters of the water sample tested shown that the total dissolved solids (0.0507%), total volatile solids (0.0180%), Phosphorus (0.008724%), and Sulphates (SO_4^{2-}) (0.004375%) were present in higher amount as compared to other

elements. Other elements like Calcium, Cobalt, Iron, and Copper were not detected in the tests. Unlike other hot springs, Unkeshwar hot springs content high Phosphorus and Sulphates content. The chemical stress like availability of high phosphorus, sulphur concentrations and slightly higher organic content enriches the microbial diversity of this hot spring.

One litre of water sample was filtered using a 0.45 μm (to remove debris) followed by 0.22 μm filter membrane (MOBIO, USA). The 0.22 μm filter membrane was then sliced and subjected to simultaneous metagenomic DNA and RNA extraction using RNA PowerSoil® Total RNA Isolation Kit (MO BIO), according to the manufacturer's protocol. The quantitative analysis of the DNA was done using a Nanodrop and the integrity of DNA samples was checked on 1% agarose gel using a gel documentation system (Protein Simple). The low abundant metagenomic DNA was enriched by Multiple Annealing and Looping Based Amplification Cycles (MALBAC) amplification protocol as described earlier [18]. Enriched metagenomic DNA was extracted and analysed by whole shotgun metagenome analysis for microbial community structure and functional annotations.

Whole metagenome shotgun sequencing of 12UM sample was performed using the Illumina HiSeq 2500 sequencer (Illumina, USA). Metagenome sample library quantification was done using Bioanalyzer 2000 (Agilent, USA). For sequencing, a dual-indexed Paired-End sequencing (2×251 base pairs) strategy with a total of 250 cycles, six bp index sequence was used. The entire sequencing run was completed in 39 h. Based on quality report of fastq files sequence reads were trimmed wherever necessary to only retain high quality sequence for further analysis. Assembled contigs size >150 bp was considered for

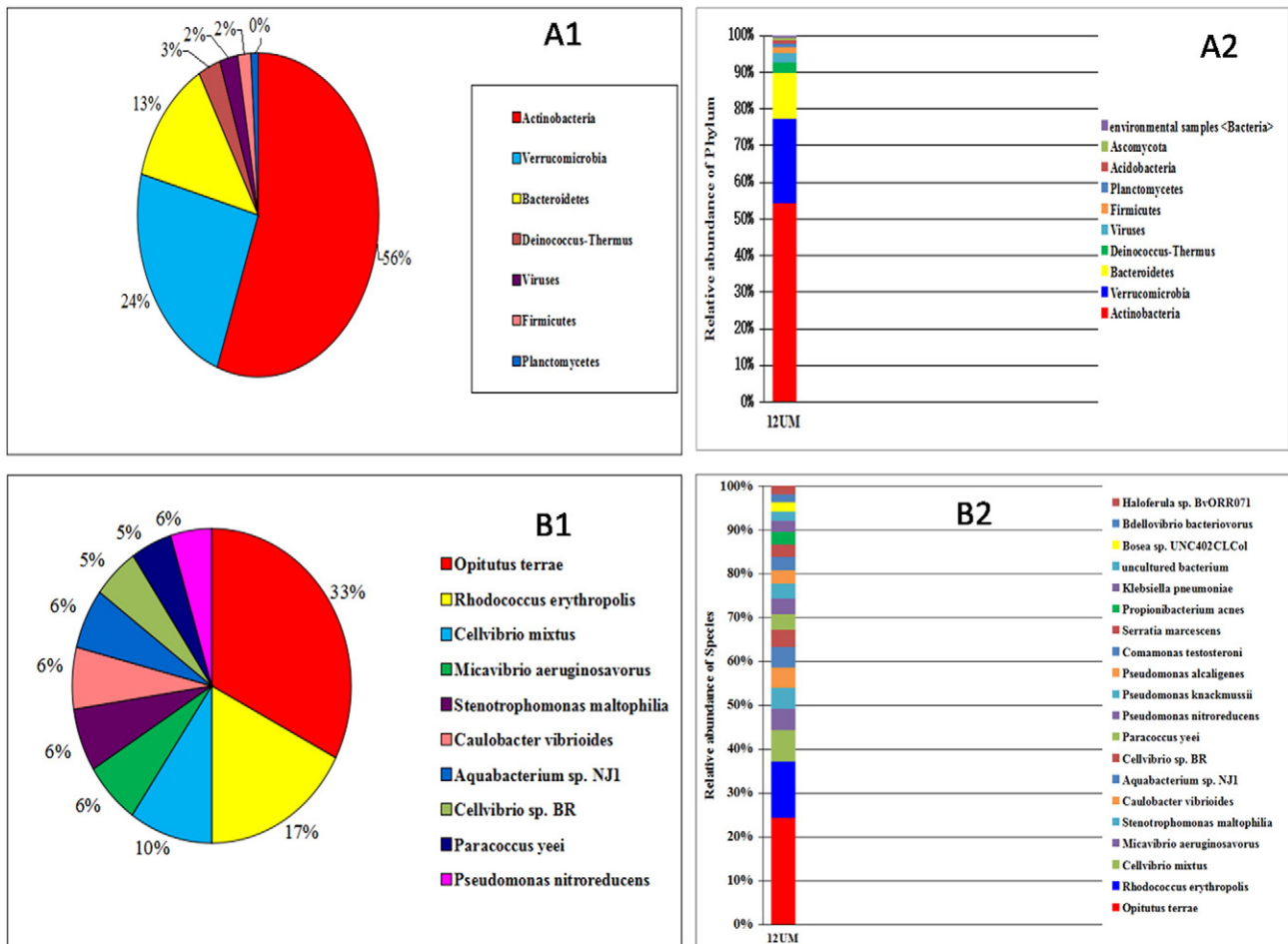


Fig. 1. Taxonomic profiling and microbial community structure in Unkeshwar hot spring using NCBI taxonomy data sets A1: Phylum abundance; A2: Relative abundance phylum of samples; B1: Species abundance; and B2: Relative abundance of species of hot springs (Only top 10 species hits were highlighted).

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