



Investigation of the microbial community in the Odisha hot spring cluster based on the cultivation independent approach



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ABSTRACT

Deulajhari hot spring is located in the Angul district of Odisha. The significance of this hot spring is the presence of the hot spring cluster adjacent to the cold spring which attracts the attention of microbiologists to understand the role of physio-chemical factors of these springs on bacterial community structure. Next-generation sequencing technology helps us to depict the pioneering microflora of any ecological niche based on metagenomic approach. Our study represents the first Illumina based metagenomic study of Deulajhari hot spring DH1, and DH2 of the cluster with temperature 65 °C to 55 °C respectively establishing a difference of 10 °C. Comprehensive study of microbiota of these two hot springs was done using the metagenomic sequencing of 16S rRNA of V3-V4 region extracting metagenomic DNA from the two hot spring sediments. Sequencing community DNA reported about 28 phyla in spring DH1 of which the majority were Chloroflexi (22.98%), Proteobacteria (15.51%), Acidobacteria (14.51%), Chlorobi (9.52%), Nitrospirae (8.54%), and Armatimonadetes (7.07%), at the existing physiochemical conditions like; temperature 65 °C, pH 8.06, electro conductivity 0.020 dSm⁻¹, and total organic carbon (TOC) 3.76%. About 40 phyla were detected in cluster DH2 at the existing physiochemical parameters like temperature 55 °C, pH 8.10, electro conductivity 0.019 dSm⁻¹, and total organic carbon (TOC) 0.58% predominated with Chloroflexi (41.98%), Proteobacteria (10.74%), Nitrospirae (10.01%), Chlorobi (8.73%), Acidobacteria (6.73%) and Planctomycetes (3.73%). Approximately 68 class, 107 order, 171 genus and 184 species were reported in cluster DH1 but 102 class, 180 order, 375 genus and 411 species in cluster DH2. The comparative metagenomics study of the Deulajhari hot spring clusters DH1, and DH2 depicts the differential profile of the microbiota. Metagenome sequences of these two hot spring clusters are deposited to the SRA database and are available in NCBI with accession no. SRX1459734 for DH1 and SRX1459735 for DH2.

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Specifications [standardized info for the reader]

Organism/cell line/tissue	Deulajhari hot spring cluster metagenome.
Sex	Not applicable
Sequencer or array type	Illumina Genome Analyzer Iix
Data format	Raw data: FASTA Q file
Experimental factors	Environmental samples
Experimental features	Amplification of the 16s rRNA genes from the Deulajhari hot spring cluster metagenome using Illumina sequencing platform and further bacterial community structure study using QIIME pipeline and Krona tool.
Consent	Not applicable.
Sample source location	Soil sediment sample, Deulajhari hot spring cluster, Odisha.

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1. Direct link to deposited data

www.ncbi.nlm.nih.gov/sra/SRX1459734

www.ncbi.nlm.nih.gov/sra/SRX1459735

2. Experimental design, materials and methods

Metagenomics analysis of microbial community DNA has enabled its comprehensive study of the diversity existing within it [1]. Next generation sequencing has allowed the explosion in the sequencing of metagenome thus enabled the complete characterization of the microbiota of any ecological niche [2]. Odisha possesses four hot springs located in different geographical locations with variations in the physiochemical parameters namely, Atri hot spring in Khurda, Tarabalo in Nayagarh, Taptapani in Ganjam and Deulajhari in Angul and thus expected possesses diverse microbiota respective to their locations and physiochemical parameters affecting the community [3]. Various global studies on the thermal springs helped in the discovery of the various

Table 1
Showing the physiochemical parameters of the Deulajhari hot spring cluster.

Sample	Temperature	pH	Electro conductivity	TOC
Deulajhari hot spring (Ref. [3])	69 °C	8.09	0.025 dSm ⁻¹	0.36%
S1 (Ref. [7])	43 °C	7.83	0.019 dSm ⁻¹	3.80%
S2 (Ref. [7])	55 °C	7.14	0.019 dSm ⁻¹	0.97%

novel microbes which find their role in various biotechnological applications [6]. Deulajhari hot spring cluster (Latitude 20.74199N, Longitude 84.49206E) has a number of springs with variable temperature range. Our previous study in the different cluster of the Deulajhari hot spring with the temperature of 69 °C [3], 43 °C (S1) and 55 °C (S2) [7] showed the varied physiochemical parameters mentioned in Table 1 and differential occurrence of microflora.

Spring with the temperature of 69 °C was dominated by Proteobacteria (88.12%), followed by Bacteroidetes (10.76%), Firmicutes (0.35%), Spirochetes (0.18%), and Chloroflexi (0.11%). While hot spring cluster S1 under the mentioned physiochemical parameters showed

the dominance of Chloroflexi (27.65%), Proteobacteria (27.60%), Chlorobi (8.32%), Acidobacteria (6.49%), Spirochetes (5.94%) and S2 spring were dominated with Chloroflexi (39.39%), Proteobacteria (19.53%), Chlorobi (10.83%), OP1 (6.16%) and Nitrospirae (4.96%) respectively at the phylum level.

In our present experiment the sediment samples from the Deulajhari hot spring main source DH1 (65 °C) and the outlet DH2 (55 °C) were collected and were immediately sent to the lab for various physiochemical parameters analysis viz. pH 8.06, electro conductivity 0.020 dSm⁻¹, and total organic carbon (TOC) 3.76% for DH1, and pH 8.10, electro conductivity 0.019 dSm⁻¹, and total organic carbon (TOC) 0.58% for DH2 and were further processed for the metagenomics DNA extraction [4]. 50 ng of the metagenomic DNA of each cluster was further used for the amplification of the V3–V4 region of the 16S rRNA using the primers 341F, 5’CCTACGGGAGCGCAG-3’ and 518R, 5’-ATTACCGGGTCTGG-3’. PCR products were further purified using the Minelute column (QIAGEN, India) and were further processed for the 150 nucleotides paired and multiplex sequencing using the Illumina GAIIx sequencer at the genotypic technology, Pvt. Ltd. (Bangalore,

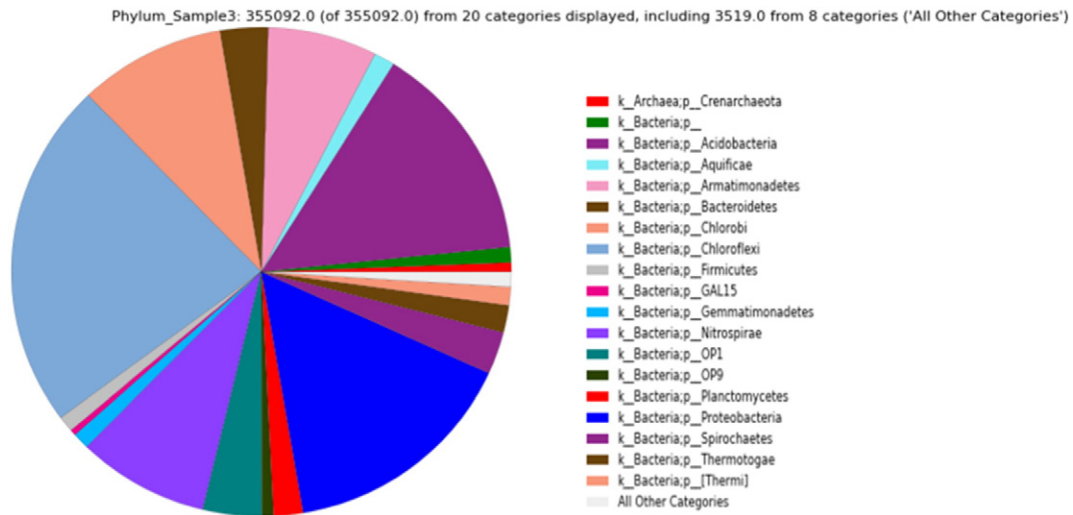


Fig. 1. Microbial diversity of Deulajhari hot spring DH1 at the phylum level.

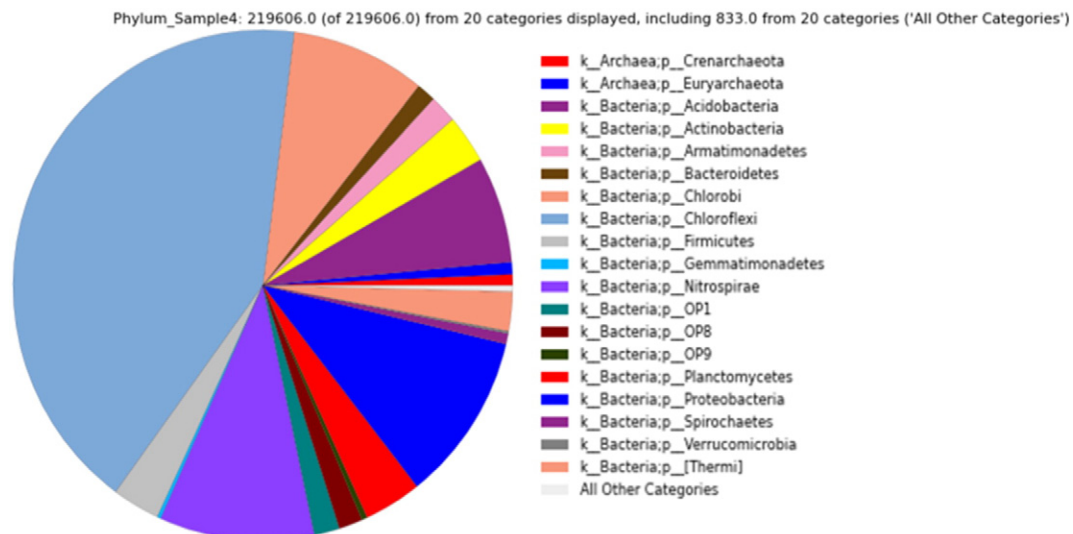


Fig. 2. Microbial diversity of Deulajhari hot spring DH2 at the phylum level.

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