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Metagenomic assessment of archaeal diversity in surface waters of Mahapelessa and Wahawa hot springs of Sri Lanka

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Archaea are well known for their adaptations to extreme environments such as submarine volcanic vents, solfataric hot springs, or soda lakes. There are several hot springs in Sri Lanka; however, most of them have not yet been explored for the presence of archaea and require comprehensive studies to unravel their unknown and untapped phylogenetic and functional diversity. The objective of this study was to perform a metagenomic analysis on two major hot springs in Sri Lanka to uncover the resident archaeal diversity. Water samples (n=2 from each well) were collected from Mahapelessa (44.5 °C, pH=8.14) and Wahawa (42.8 °C, pH=7.17) hot springs located at Hambantota and Ampara districts, respectively. Genomic DNA was extracted from the water samples (6 replicates) using modified Boom's method and was subjected to 16S rRNA metagenomic sequencing using ARC 787F 5'-ATTAGATACCCSBGTAGTCC-3' and ARC 1059R 5'-GCCATGCACCCWCCTCT-3' primers on an Illumina platform at Macrogen Inc., South Korea. The results were analyzed using EzBioCloud: Metagenomics data analysis software to identify archaeal diversity (Operational taxonomic units/ OTU), their relative abundance and alpha diversity indices in each hot spring. OTU analysis was carried out with a cut-off similarity of 97%. The 16S rRNA gene amplicon of V5-V8 region metagenome sequencing uncovered a unique taxonomic diversity of the resident thermophilic archaeal communities in these hot springs. A total of three archaeal phyla were observed, and among them, Thaumarchaeota (88.78%) was dominant in Mahapelessa, and Euryarchaeota (99.19%) was dominant in Wahawa hot spring. The low abundance of phylum Bathyarchaeota (0.1%) was detected in Mahapelessa hot spring. Genera *Halalkalicoccus*, *Halobacterium*, *Methanobacterium*, and *Methanosarcina* were unique to Mahapelessa, while genera *Halorubrum*, *Methanolinea*, and *Methanosaeta* were unique to Wahawa. Genus *Methanocella* was common to both hot springs. All identified genera belonged to the phylum Euryarchaeota. Among them, *Methanocella arvoryzae* (6.697%) and *Methanosarcina acetivorans* (0.002%) were identified up to species level from Mahapelessa and *Methanocella paludicola* (42.276%) was identified from Wahawa hot spring. This study gives insight into the vast archaeal diversity present in Mahapelessa and Wahawa hot springs.

Key Words: Metagenomic, archaeal, diversity, surface waters, hot springs

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