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### **Diversity of Firmicutes in selected hot water springs of Sri Lanka by 16S metagenomic sequencing**

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Thermophiles have the ability to survive in environments with very high temperatures. Hot springs provide unique natural environments for these thermophilic microorganisms. In recent years, hot water springs and thermophilic microorganisms have gained attention due to their industrial and biotechnological importance. Among the thermophilic bacteria, a large number of metabolites can be found in the phylum Firmicutes. Different species of Firmicutes show various ecological optima. Even though Sri Lanka harbors many hot springs, majority of them are not yet explored and require comprehensive studies to unravel the inhabitant microbial diversity, specially the phylum Firmicutes. This study was focused into uncovering the resident thermophilic Firmicutes which could be unculturable and their diversity in four major hot springs namely, Mahapelessa, Wahawa, Maha Oya and Nelumwewa using 16S rRNA metagenomic sequencing. Water samples were collected from the four hot springs in which the surface temperature ranged from 44.2 to 53.8 °C. Further, a water sample from a natural spring located in Digana (26.9 °C) was used as the control. Genomic DNA was extracted from the water samples using modified Boom's method and was subjected to 16S rRNA metagenomic sequencing (16S V3-V4 region) using Illumina platform. The results were analyzed using GAIA: Metagenomics data analysis software to identify Firmicute bacteria (Operational taxonomic units/ OTU) and to determine their relative abundance. OTU analysis was carried out with a cut-off similarity value at 97%. The relative abundance of Firmicutes in each spring is as follows: Mahapelessa (23.7%), Wahawa (0.8%), Maha Oya (0.9%), Nelumwewa (5.8%) and Digana natural spring (4.2%). The class Clostridia and Bacilli were the most abundant classes observed in hot springs. A total of twenty-one species were affiliated with the phylum Firmicutes, among which 18 species were only detected in hot water springs while the rest of the three species (*Clostridium* sp., *Flintibacter butyricus* and *Oscillibacter* sp.) were only detected in the natural spring. Nine different bacterial species were unique to Mahapelessa hot spring including; *Bacillus licheniformis* and *Bacillus thuringiensis* belonging to class Bacilli, *Caldanaerocella colombiensis*, *Desulfotomaculum reducens*, *Geosporobacter* sp., *Geosporobacter subterraneus*, *Pelotomaculum isophthalicum*, *Salimesophilobacter vulgaris* and *Tepidibacter* sp. belonging to the class Clostridia. The two bacterial species; *Carboxydocella manganica* and *Faecalibacterium prausnitzii* were only recorded from Maha Oya while *Clostridium islandicum* was reported from Maha Oya and Nelumwewa hot springs. *Bacillus pumilus* and *Sporacetigenium mesophilum* were present in Mahapelessa and Wahawa hot springs. *Anaerolibacter carboniphilus* was found in both Mahapelessa and Nelumwewa while *Anaerobacterium chartisolvens* was detected in all hot water springs except Wahawa. *Bacillus* sp. and *Paenibacillus* sp. were common in all the hot water springs. When compared to the natural spring, the four hot water springs showed high bacterial species diversity (85%). Results from this study confirm the uniqueness of bacterial species belonging to phylum Firmicutes in hot water springs than in the natural springs. More comprehensive studies on these Firmicutes are needed to identify their potential to be used in industrial and biotechnological applications.

**Keywords:** Hot springs, 16S metagenomics, Firmicutes