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Diversity of Firmicutes bacterial communities 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 metabolisms can be found within Firmicutes. Different species of Firmicutes show various ecological optima. Sri Lanka harbors many hot springs, the majority of which are not yet explored and require comprehensive studies to unravel the inhabitant microbial diversity. This study was focused into uncover the resident thermophilic Firmicute bacterial community 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 phylum Firmicute, 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 isophthalicicum, 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. *Anaerosolibacter 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 in bacterial species belonging to phylum Firmicutes in hot water springs than the natural springs. More comprehensive studies on these Firmucutes are needed to identify their potential to be used in industrial and biotechnological applications.