YSCMR 2021

Proceedings of the Young Scientists' Conference on Multidisciplinary Research - 2021

Virtual International Conference

21st October 2021

"Multidisciplinary Research for Tomorrow's Challenges"

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ISSN 2815-0260

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Published by:

Young Scientists' Association (NIFS-YSA), National Institute of Fundamental Studies, Hanthana Road, Kandy, Sri Lanka www.nifs.ac.lk Tel: +94 (0) 812 232 002 Email: ysa@nifs.ac.lk

Cover page design & Page setup

Buddhika Karunarathne, Mahesh Senarathna, Hiran Kankanamge



Preliminary study of human pathogenic bacteria in the hot springs of Sri Lanka

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Background: Thermal springs are one of the extreme environments which harbour natural microbial populations including potential pathogens. High-throughput sequencing provides a more comprehensive insight into human bacterial pathogens in the environment.

Objectives: The current study investigated potential human pathogenic bacteria in the thermal springs of Sri Lanka through a metagenomic approach.

Methods: The water samples (n=2, totalling 3 L from each spring) were collected from Mahapelessa (44.5 °C), Wahawa (42.8 °C), Maha Oya (53.8 °C) and Nelumwewa (44.2 °C). Composite water samples (100 ml) from each hot spring were used for genomic DNA extraction (6 replicates) by modified Boom's method. The extracted DNA was subjected to 16S rRNA metagenomic sequencing (V3-V4 region) using the Illumina platform. The raw data were analysed to identify microorganisms (Operational taxonomic units/OTU) and their relative abundance. OTU analysis was carried out with a cut-off similarity of 97%.

Results: Most of the potentially pathogenic bacteria identified from hot springs belonged to the phyla, Proteobacteria and Firmicutes. *Burkholderia* (11.93%) and *Pseudomonas* (0.52%) were the most common pathogenic bacterial genera in all thermal springs. Based on the bacterial composition, the highest proportion of potential pathogens was from Mahapelessa (12.87%), followed by Maha Oya (6.15%), Wahawa (1.93%) and Nelumwewa (0.57%). The highest abundance of bacterial pathogens has been reported from Mahapelessa and Maha Oya, with temperatures varying from 45 °C to 55 °C. Diverse bacterial pathogens dominated the microbial community in investigated hot springs, including *Acinetobacter* sp. (2.29%), *Bacillus licheniformis* (0.07%), *Bacillus pumilus* (0.24%), *Burkholderia cepacia* (9.72%), *Enterobacter asburiaein* (0.19%), *Pseudomonas aeruginosa* (0.21%) and *Vibrio fluvialis* (0.06%) from Mahapelessa, *Bosea* sp. (0.42%) from Wahawa, *Delftia tsuruhatensis* (5.44%) from Maha Oya and *Burkholderia multivorans* (0.20%), and *Bosea* sp. (0.19%) from Nelumwewa.

Conclusion: The present investigation reports different dominant bacterial pathogens in geothermal springs in Sri Lanka and the significant differences in relative abundance could be observed for the diverse temperature ranges between hot springs.

Keywords: Hot springs, Metagenomics, Pathogenic bacteria