

ABSTRACT

Hot springs harbour diverse groups of microorganisms adapted to extreme conditions. Due to limitations in the culture-dependent approach, most thermophiles remain uncultured and unexplored worldwide. Hence, this study was conducted to understand the bacterial and archaeal diversity of six hot springs namely Mahapelessa, Wahawa, Mahaoya, Nelumwewa, Rankihiriya, and Kanniya with a normal spring at Digana (26.9 °C) in Sri Lanka using both culture-dependent and culture-independent approaches. The surface temperatures of these geothermal springs range from 32.6 °C to 54.6 °C, and the pH varies from 6.1 to 8.9. 16S rRNA Sanger sequencing of bacteria revealed the presence of 13 genera belonging to two phyla in Mahapelessa, Kanniya, and Rankihiriya: Proteobacteria (89%) and Firmicutes (11%) and the most abundant genera being *Klebsiella* and *Pseudomonas*. *Pigmentiphaga* was isolated from Mahapelessa, and to the best of our knowledge, this is the first record of this genus from a hot spring. Sanger sequencing of cyanobacterial 16S rRNA revealed the presence of 9 genera belonging to genera *Gloeocapsa*, *Gloeocapsopsis*, *Pseudanabaena*, *Leptolyngbya*, *Mastigocladus*, *Cyanothece*, *Pantanalinema*, *Calothrix*, and *Synechocystis*. Twenty-four bacterial phyla representing 165 species and four archaeal phyla representing 23 species were revealed by metagenomics sequencing analysis using V3-V4 and V5-V6 regions respectively. The bacterial community from Mahapelessa, Wahawa, Mahaoya, and Nelumwewa were dominated by Proteobacteria (>46%). The phylum Euryarchaeota (0.09-0.9%) was the most abundant in Mahapelessa and Wahawa. Metagenomics sequencing revealed the sulfate-reducing bacterium *Desulfotomaculum reducens*, which was dominant and unique in Mahapelessa. Cyanotoxin-producing *Microseira wollei* was only observed in Wahawa. With a high temperature, Mahaoya was primarily dominated by biological