



Bacterial Diversity in a Sri Lankan Geothermal Spring Assessed by Culture-Dependent and Culture-Independent Approaches

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Abstract

Hot springs harbour diverse and interesting groups of microorganisms adapted to extreme conditions. However, due to limitations in the culture-dependent approach, most of such thermophiles remain uncultured and unexplored. Hence, this study was conducted to gain a comprehensive understanding of the bacterial diversity of Mahapelessa hot spring, Sri Lanka using both culture-dependent and culture-independent approaches. The in situ temperature of the water sample was 44.5 °C and the pH was 8.14. 16S rRNA Sanger sequencing of DNA extracted from the 18 bacterial isolates revealed the presence of eight genera belonging to two phyla: Proteobacteria (84%) and Firmicutes (16%) and the most abundant genus being *Klebsiella*. A total of 23 bacterial phyla representing 80 classes, 43 orders, 123 families, 205 genera and 83 species were detected by 16S rRNA V3-V4 region by amplicon metagenome sequencing of DNA extracted from water samples, where the most abundant phylum was the Proteobacteria (57.39%), followed by Firmicutes (23.7%) and Chloroflexi (4.14%). The three phyla Actinobacteria, Planctomycetes and Bacteroidetes were also detected less than 3% in abundance while 4.48% of bacteria could not be fit into any known phylum. The most abundant genera were *Burkholderia* (14.87%), *Desulfotomaculum* (7.23%) and *Stenotrophomonas* (6.1%). Four strictly anaerobic bacteria, *Anaerolibacter carboniphilus* (0.71%), *Bellilinea caldifistulae* (0.04%), *Salimesophilobacter vulgaris* (0.1%), *Anaerobacterium chartisolvans* (0.12%); two potential plant growth-promoting bacteria, *Azospirillum halopraeferens* (0.04%) and *Bradyrhizobium liaoningense* (0.16%) and one potential alkali tolerant and sulphate-reducing bacterium, *Desulfovibrio alkalitolerans* (0.45%) were recorded. *Pigmentiphaga* sp. was isolated from Mahapelessa hot spring and to the best of our knowledge, this is the first record of this genus from a hot spring. This study gives insight into the vast bacterial diversity present in the Mahapelessa hot spring from the culture-independent approach which could not be identified using standard culturing techniques.

Introduction

Many extreme environments, such as hot springs, deserts, saline and/or alkaline lakes and, ocean beds are too harsh for common life to exist. Natural geothermal springs, including terrestrial hot springs, are widely spread in various parts of the world. These hot springs are primarily associated with tectonically active zones in areas where the Earth's crust is relatively thin [1, 2]. These geothermal springs contain new sources of fascinating microorganisms that adapted to those

extreme environments [2]. The adaptations to these harsh habitats make thermophiles and their thermostable proteins suitable for various industrial and biotechnological applications [3]. Thermophiles can be defined as organisms that are adaptable to grow optimally at higher temperatures. These thermophiles are classified into several groups: facultative thermophiles (survive below 45 °C); moderate thermophiles (optimum growth temperature between 45 °C and 60 °C); strict thermophiles (optimum growth temperature between 60 °C and 90 °C); extreme thermophiles or hyperthermophiles (grow best at temperatures greater than 90 °C) [4, 5]. Most thermophilic microorganisms are prokaryotes (Domain Bacteria and Domain Archaea) surviving in extreme heat, pH, salinity, and pressure conditions [6].

Microbial communities in hot springs are widely explored and microbial diversity in some hot springs has been studied using both culture-dependent and culture-independent approaches. In recent years, the application of the

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