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Investigation of microbial diversity in thermal springs with molecular techniques

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Thermal springs are geologically distinct regions and microorganisms which are inhabitants of such extreme temperatures are known as thermophiles. Thermophilic microbial mat communities are largely temperature-defined and are of great interest due to their ability to produce thermostable enzymes such as DNA polymerases, polyphosphate kinases, other heat stable bioactive compounds with therapeutic properties and also toxins. Typically 99% of the naturally occurring microorganisms could not be cultured by standard culture techniques. Therefore, culture-independent molecular techniques have become a more powerful tool for comprehensive study of microbial diversity in thermal springs. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of PCR amplified 16S rRNA gene sequences based on differences in nucleotide sequence has been proven as a useful tool in profiling the microbial mat communities. Hence, the present study focused on identification of microbial diversity in five thermal springs in Sri Lanka using DNA amplification and DGGE separation of 16S rRNA gene fragments.

Environmental DNA was directly extracted from the water samples collected from thermal springs of Rangiriulpotha, Kanniya, Nelumwewa, MahaOya, Wahawa and Kapurella. Polymerase chain reaction (PCR) was performed for the amplification of 16S rRNAspecific sequences of eubacteria, cyanobacteria and archaea. The resulting PCR products were analyzed using DGGE technique and the bands were excised, re-amplified and were directly sequenced. Five water samples collected from thermal springs revealed the presence of eubacteria yielding the amplicon size of 1500 bp, while two water samples from Nelumwewa and Wahawasprings revealed the presence of archaea with an amplicon size of 750 bp. Also five thermal spring samples gave positive PCR amplification product (450 bp) with cyanobacterial specific primers. DNA sequences of 16S rRNA genes confirmed the presence of cyanobacterial species belonging to the genera *Chroococcidiopsis, Oscillatoria, Calothrix, Leptolyngbya* and *Xenococcus*. In addition *Burkholderia* species belonging to the proteobacteria were recorded from MahaOya and Wahawa thermal springs.

In conclusion, partial 16S rRNA sequence analysis confirmed the presence of eubacteria, cyanobacteria and archaea in five thermal springs. These findings extend our understanding of the vast diversity of microorganisms present in thermal springs of Sri Lanka.

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