

A comparison of conventional and miniprimer PCR to elucidate bacteria diversity in Malaysia Ulu Slim hot spring using 16S rDNA clone library

ABSTRACT

The diversity of the bacterial community in Ulu Slim hot spring was examined using a 16S ribosomal DNA culture-independent approach. A total of 144 different 16S rDNA sequences were cloned and analyzed. The majority of sequences were found to be within the Betaproteobacteria and Gammaproteobacteria while the remaining sequences belonged to the Bacteroidetes, Acidobacteria, Chloroflexi, Nitrospira and candidate divisions. Some of the bacteria 16S sequences could be novel and indigenous to this hot spring as their sequences are low in similarity when compared with known sequences. The 16S ribosomal DNA clone library was assembled using conventional PCR amplification with degenerate 27F and 1492R primers specific for bacterial rDNA. A new miniprimer PCR assay was also used to construct a library. When compared, we conclude that the latter approach which using mutagenized Dynamo-II polymerase utilizing 10-mer primers was a better approach than the conventional PCR as the environmental humid acid inhibition effect was less. Ideally, to best understand the overall prokaryote diversity in the hot spring, it is better to use a combined conventional and miniprimer PCR approach.

Keyword: Prokaryote biodiversity; Thermophiles; Hot springs; Restriction fragment length polymorphism