

Selective phylogenetic analysis targeted at 16S rRNA genes of (hyper)thermophiles in deep-subsurface geothermal environments

Hiroyuki Kimura[1]; Satoshi Hanada[2]; Kenji Kato[1]

[1] Institute of Geoscience, Shizuoka University; [2] Natl. Inst. of Adv. Ind. Sci. & Tech. (AIST)

<http://www.ipc.shizuoka.ac.jp/~shkimur/index.html>

Deep-subsurface samples obtained by deep drilling are likely to be contaminated with mesophilic and psychrophilic microorganisms in the drilling fluid, and this could affect determination of the community structure of the geothermal microflora using 16S rRNA gene clone library analysis. To eliminate possible contamination by PCR-amplified 16S rRNA genes from mesophiles and psychrophiles, a combined thermal denaturation and enzyme digestion method, based on a strong correlation between the G+C content of the 16S rRNA gene and the optimum growth temperatures of most known prokaryotic cultures, was developed prior to the clone library construction.

To validate this technique, river water (14C), hot spring fluid (76C), surface seawater (30C) and deep-sea hydrothermal fluid (117C) were used to mimic a deep subsurface sample contaminated with drilling fluid. After DNA extraction and PCR amplification of 16S rRNA genes from individual samples separately, bacterial 16S rRNA genes amplified from river water were observed to be denatured at 82C and completely digested by exonuclease I (Exo I), while bacterial 16S rRNA genes from hot spring fluid remained intact after denaturation at 84C and restriction enzyme digestion with Exo I. DNA extracted from river water and hot spring fluid were mixed and used as template for the amplification of bacterial 16S rRNA genes. The amplified rRNA genes were denatured at 84C and digested with Exo I before the clone library construction. Results indicated that 16S rDNA sequences from the river water were almost completely eliminated, whereas those from the hot spring fluid remained. Further, clone analysis targeted at archaeal 16S rRNA genes in surface seawater and deep-sea hydrothermal fluid also indicated the similar results. This method is useful for phylogenetic analyses targeted at thermophiles and hyperthermophiles in geothermal or hydrothermal samples, e.g., deep-subsurface cores obtained in the process of deep drilling that may be contaminated with abundant mesophilic microorganisms in the drilling fluid.

Reference: *Appl. Environ. Microbiol.* 72: 21-27 (2006)