

Genomic Analysis of microbial Community in the Hydrothermal Environment

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It was indicated by microscopic observation or comparison of 16S rDNA sequence that many microbes were surviving in many extreme environments including the hot water. But it is generally said that over 99 % of total microbes are now still uncultivable. It is expected that these unidentified and uncultivable microbes should have the useful functions for industry. Thus, we planned to identify unidentified and uncultivable microbes through direct sequencing of environmental DNA.

At first, shotgun plasmid libraries were directly constructed with the DNA molecules prepared from mixed microbes collected from low-temperature hydrothermal water at RM24 in the Southern East Pacific Rise (S-EPR). It was shown that all clones were completely novel, because no identical sequences to these sequences were recognized within the public databases. Analysis of sequence for the entire insert fragments indicated that sequences of some number of clones were similar to the intron in eukaryote, because ORFs were not identified on these sequences by often appearance of stop codons for all six frames. The tandem repetitive sequences were isolated on some number of clones, which were identified in some human familiar diseases as amplifying units during illness. The results indicated that many microorganisms with eukaryotic feature were present in low temperature water from hydrothermal vent in S-EPR.

Secondly, shotgun plasmid libraries were constructed with the DNA molecules prepared from mixed microbes collected from 300C and 80C high-temperature hydrothermal water at a Suiyo seamount with a depth of 1,300 m. Construction of the library for 300C high-temperature environment was not succeeded. It was thought that the contaminated metal ion in high-temperature water should be precipitated on the filter and these metal should inhibit the enzymatic reactions for construction of library. However, shotgun plasmid library could be constructed for the 80C high-temperature vent water. The ORFs were easily extracted from the entire sequences of some number of clones.

Thirdly, shotgun plasmid libraries were constructed from the environmental DNA prepared from Beppu hot springs and from 80C middle-temperature hydrothermal water at Suiyo seamount. It was shown that no identical sequences to these sequences were recognized within the public databases. The ORFs were easily extracted from the entire sequences of some number of clones. The results indicated that microbes, including bacteria and (or) archaea, were present in hot spring and middle-temperature hydrothermal vent water.

In some clone from hot spring and middle-temperature hydrothermal vent water, aminoacyl-tRNA synthetase, which is generally present in all organisms, was isolated by similarity. The phylogenetic analysis of Aminoacyl-tRNA synthetase identified indicated that novel and unidentified microorganisms should be present in hot spring and Suiyo seamount. Our work indicates that environmental genomics, direct cloning and sequencing of environmental DNA, is powerful approach to collect novel uncultivable microbes or novel genes.