

Direct detection and discovery of gene resources from the environment

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Although many cultivable microorganisms, including thermophiles and other extremophiles, were isolated, it is generally said that over 99 % of total microbes are now still uncultivable. These uncultivable microbes are expected to influence to the surrounding environment, e. g. effect to gas concentration or metal selection. Thus, it can be thought that identification and monitoring of such uncultivable microbes alive in the environment are necessary for recognition of their influences.

For detection of uncultivable microorganisms, the shotgun libraries were constructed from the environmental DNA prepared from Southern Eastern Pacific Rise (S-EPR), Suiyo seamount and Beppu hot spring in Japan. End sequencing of the randomly selected clones in each library indicated that there is no sequence in the public database identical to the end sequences, revealing that all sequences isolated from the environment were novel and unique. As results of determination of entire nucleotide sequence and detection of protein-coding regions, the clones with eukaryotic features, containing the intron-like sequences or tetraplet repetitive sequences, were dominantly detected in the S-EPR library. Analyses of entire sequences of clones from Beppu hot spring and Suiyo seamount indicated that the microorganisms were dominant in both sampling places. The aminoacyl-tRNA synthetase genes isolated indicated the evidence that novel archaea and bacteria are present in both the thermal places. To investigate thermostability of each gene products, the ORFs isolated on the clones from Suiyo seamount were used for construction of expression vector in *E. coli*. 50 % of gene products were recovered as soluble proteins, then the maximum temperatures stable after treatment at different temperature were analyzed. The results indicated that the proteins encoded on the same DNA fragment exhibited similar features of thermostability.

To identify the novel genes from the environment, shotgun library with 4 kb insert fragment was constructed using DNA prepared from Kirishima hot spring. Each DNA molecule prepared was stored in the independent wells in 96-well plates. It was revealed that the DNA molecules mixed in each line, column and plate were useful for searching novel genes from the environment, by checking of identification of genes homologous to the well-characterized ones.