Discovery and Application of the novel Genes from the Genomic information and the Environment

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Purpose of study: To make a sustainable development, transfer from the present chemical process in production of the most chemicals to the biological process, in which extreme enzymes might be utilized. The enzymes are originally possessed by the extremophilic microorganisms, that are living in the extreme environment. It is thought that two main and individual approaches for obtaining the extreme enzymes were present, the one is isolation from genomic data of extremophiles and the other is discovery of novel genes from the extreme environment through direct cloning of the environmental DNA. To evaluate these two possibilities, the two independent trial experiments were performed.

Methods: The novel genes indicating the similarity with the known genes were amplified from the DNA of the *Sulfolobus tokodaii* atrain7 (1) and cloned into the expression vector pET vector for *E. coli*. The resulting soluble and thermostable proteins were used for functional analyses. To identify the novel genes from environment, DNA prepared from the geo-thermal environment was randomly fragmented and cloned into plasmid vector for construction of the environmental DNA library. The plasmid DNAs prepared were stored independently in each well of the 96 wells plates. The prepared DNAs were mixed according to the line, column and plate and used for PCR searching of the novel useful gene as template.

Results: In approximately 350 expression vectors comprehensively constructed, over the 150 soluble and thermostable proteins were successfully expressed. A couple of thermostable functions correlating to the carbohydrate methabolism were analyzed and confirmed (2, 3). These enzymes indicated the flexible recognition of substrates and flexible utilization of many metal ions as cofactors. As the resources for searching the novel genes, the library was constructed by using the DNA prepared from the geo-thermal environment. It was shown that this library was good resource for obtaining the novel useful gene, because the clone encoding the full-sized novel DNA polymerase was identified from this library.

Conclusions: These results indicated that the novel enzymes or proteins, which are expected to be useful for application, are identified from the genomic data and environmental samples. So I can conclude that these approaches are powerful for obtaining the novel gene resources.