Functional Selection of Metagenome Genes Derived from Soil Microbial Diversity

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Over 99% of soil bacteria are not culturable by a standard cultivation method. Thus, one may expect that exploitation of the unculturable microbial resources would provide the unique opportunity to obtain novel microbial resources. Metagenome approach was developed to tap the unculturable microbial resources. Metagenome is a collective microbial genome directly extracted from natural microbial ecosystem and it can be cloned in a surrogate host bacterium to constitute metagenome library. The cloned library can be used for analyzing microbial community by direct sequencing of the whole community and can be also used for selecting novel genetic resources from the majority of unculturable bacteria. Either expression-dependent or sequence homology based screening of the constructed library can be performed to obtain novel resources.

Forest soil and plant rhizosphere soils were our target microbial ecosystems for metagenome approach. Culture independent analysis of the microbial ecosystem of Jindong Valley forest soil revealed that our metagenome contained abundant DNA from phylum *Acidobacteria* which has only few cultured representatives. Directly isolated DNAs from the forest soil and plant rhizosphere soil were cloned in a fosmid vector for the construction of metagenomic libraries. Lipolytic enzymes, which serve as a model enzyme, for exploring more diverse from diverse microbial resources were screened from the constructed library. Selected lipolytic enzymes included novel lipases and esterases exhibiting stereoselective activity of chiral mixtures and were found to be belonging to a new family of esterases. One of clones revealed apparent esterase activity but did not match to any enzymes present in the database except several hypothetical proteins. Therefore, we proposed re-annotation of the similar hypothetical proteins into members of a novel family of esterases. When one considers the limitation of functional screening of metagenome clones in *Escherichia coli* and lipolytic enzymes as one of the most well studied enzymes from cultured bacteria, our discovery of a new family of esterases suggests that there are still more enzymes to be explored from uncultured microbial diversity.

Several metagenome clones with biological activities were also selected by the functional screening of constructed libraries. These included clones involved in producing pigment antibiotics, unknown antifungal activity and porphyrin intermediates. The respective clones were analyzed by subcloning, transposon insertional mutagenesis and DNA sequence analysis to determine the gene clusters and genes responsible for the relevant activity production. One of the antifungal clones contained genes for core bacterial type II polyketide synthase and their regulatory proteins as a cluster. Comparisons of the identified genes and gene cluster

indicated that identities to homologous genes in GenBank were generally low. Our results suggested that the expression based screening of metagenome is still feasible to attempt, despite the limited heterologous expression of cloned genes in *E. coli.* Improvement in the host-vector system used for metagenome library construction and more sensitive high-throughput screening methods would be necessary for exploring the uncultured bacterial resources more efficiently. Nevertheless, the expression based screening of metagenomic library has made it possible to identify the metagenomic genes and their expression products simultaneously.