【Workshop 5】Perspectives of Metagenomics in Agricultural Research

Retrieving a Full-length of Functional Genes from Soil by PCR-DGGE and Metagenome Walking

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Microorganisms in the environment are a treasure house of potentially useful genes. Such genetic resources have been exploited mainly by culture-dependent methods. However, traditional culture-dependent methods are available only for a tiny fraction of microorganisms in the environment. Recently, to overcome availability limitations of culture-dependent methods, metagenomic approaches that investigate DNA extracted directly from environmental samples have attracted considerable attention. We present here a new strategy for retrieving a full-length of functional genes from soil without any cultivation steps, using a combination of PCR-denaturing gradient gel electrophoresis (DGGE) and metagenome walking. Benzoate 1,2-dioxygenase alpha subunit gene (*benA*) involved in the degradation of 3-chlorobenzoate (3CB) was used as the model target for evaluating our strategy. Partial fragments (358 bp) of *benA* derived from various bacteria were amplified by PCR from soil DNA extracted from a 3CB-dosed soil. Resulting PCR products were separated by DGGE. One of the DGGE bands that intensified with 3CB-dose was chosen as the target for metagenome walking, because the band was most likely to represent a functional 3CB-degrading gene in the soil. Using the nested primers designed from the band with the random primers, we performed metagenome walking to retrieve the flanking regions of the target band from the soil DNA. As the result, we succeeded in retrieving a 2161-bp fragment, which contained not only the full-length of target *benA* but also its downstream gene, benzoate 1,2-dioxygenase beta subunit gene (*benB*). Amino acid sequences of the retrieved genes showed 71～74% identity with those of the known genes on the database. We confirmed that our strategy was applicable also to the case of targeting a different gene (*tfdC* encoding chlorocatechol 1,2-dioxygenase). PCR-DGGE is helpful to screen for target genes based on their potential for degrading contaminants in the environment. This feature provides our strategy with a notable advantage over other existing methods for retrieving complete genes from metagenome.