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Microbial Enzymes and Bioactivities from Soil and Rhizosphere Metagenome

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Molecular analysis of microbial communities has indicated that microbial diversity of soil and plant rhizosphere is much underestimated because over 99% of microbes in soil are not culturable by a standard culture technique. Although the microbial diversity in soil has been estimated by means of culture-independent methods, it is uncertain whether it would be possible to find a universal way to cultivate and characterize most bacteria present in natural ecosystems. Many attempts to search valuable microbial products from microorganisms employ pure culture of microorganisms and culture-based activity screening. However, a recently developed strategy employs cloning of the total microbial genome, the so-called 'metagenome', directly isolated from natural environments in culturable bacteria such as *Escherichia coli*. Although foreign genes should be expressed in a heterologous host, the metagenomic approach has proven to be technically feasible for exploring novel microbial resources from the unculturable microbes. Several studies have demonstrated that the metagenomic approach allows for searches of various microbial products such as microbial enzymes and natural products.

In an attempt to search for novel biocatalysts and bioactivities from unculturable bacteria, we constructed metagenomic libraries from forest topsoil and rhizosphere soil using a fosmid and microbial DNA directly extracted from soils. Restriction analysis and random fosmid end DNA sequence analysis of the constructed library revealed high diversity of cloned genes in a fosmid. The libraries, consisting of over 500,000 clones, were constructed from 4 different soils including forest topsoil, cave soil, upland soil and plant rhizosphere with average DNA insert size of 35 kb. Seventeen unique lipolytic active clones were obtained from the 78,000-member library on the basis of tributyrin hydrolysis. Subsequently, secondary libraries in a high-copy-number plasmid were generated to select lipolytic subclones and to characterize the individual genes responsible for the lipolytic activity. DNA sequence analysis of six genes revealed that the enzymes encoded by the metagenomic genes for lipolytic activity were similar to those in the hormone-sensitive lipase family with 33 - 46% deduced amino acid identities (Fig. 1). The six predicted gene products were highly expressed in *E. coli* and were successfully secreted into the culture broth. Most of the secreted enzymes hydrolyzed *p*-nitrophenyl butyrate (C_4) but not *p*-nitrophenyl palmitate (C_{16}).

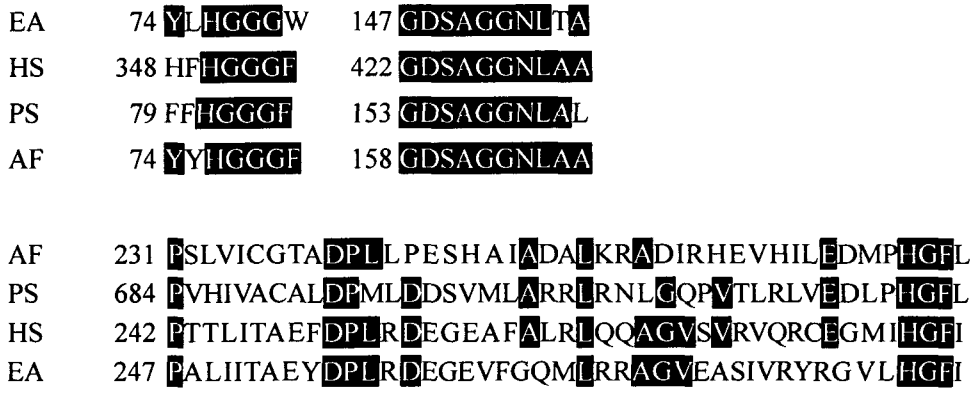

Fig. 1. Alignment of the conserved regions containing enzyme active sites of lipases from the forest topsoil metagenome (EA), from human (hormone sensitive lipase, HS), from cold-adapted *Pseudomonas* sp. B11-1 (PS), and from hyperthermophilic *Archaeoglobus fulgidus* (AF). Asterisks indicate the catalytic triads (serine, aspartate, histidine) of the hormone-sensitive lipase family.

In order to search for novel bioactivities, we have adopted double agar layer method using *Saccharomyces cerevisiae* for antifungal activity and *Agrobacterium tumefaciens* for antibacterial activity. By screening forest topsoil metagenomic libraries, one antifungal active clone and an antibacterial active clone were selected. *In vitro* mutational analysis and DNA sequence analysis of the antifungal fosmid clone revealed the genes involved in the biosynthesis of antimicrobial secondary metabolite. The antifungal active clone produced active diffusible antifungal compounds. Isolation and structure determination of the antifungal activity is in progress. Our progress in this study suggested that tapping soil and plant rhizosphere metagenome provide a unique opportunity to search for novel microbial products.

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