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Isolation of Lipase Genes Based on Expression in *Escherichia coli* from Metagenomic Library

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Majority of soil microorganisms are not culturable and the genetic resources of the unculturable bacteria were not extensively characterized. We have constructed metagenomic libraries in *Escherichia coli* using a fosmid vector and soil DNA directly extracted from various soils. The libraries were subject to search for lipolytic activity from unculturable soil bacteria. Lipolytic active metagenome clones were selected based on tributyrin hydrolysis showing the clear zone due to heterologous expression and secretion of the enzymes in *E. coli*. A total of 13 lipolytic clones were selected from 128,000-member library. Subcloning and DNA sequence analysis of previously isolated clones revealed several novel lipolytic enzymes such as GDSL family lipases/esterases, hormone sensitive lipases and one which is not similar to any known protein in database. Our result suggested that simple expression based screening of metagenomic library is valuable to obtain novel enzymes from uncultured soil microorganisms. The lipolytic clones will be subjected to investigate the degradation of toxic chemicals.

Key words: Lipase, metagenome, unculturable bacteria

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Formulations of Biocontrol bacteria *Bacillus licheniformis* N1 and *Burkholderia cepacia* CH-67 to Control Strawberry Anthracnose

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We previously selected two bacterial strains, *Bacillus licheniformis* N1 and *Burkholderia cepacia* CH-67, exhibiting good biocontrol activity against strawberry anthracnose. To provide effective formulations of these bacteria for the biocontrol of the disease, conditions for the mass cultivation of these bacteria were determined in flask cultivation condition. Mineral minimal medium (MMM) was used as a basal medium to investigate the effect of various carbon sources and nitrogen sources to provide the highest cell density. Biji medium containing 5% Biji powder in MMM and rice oil medium containing 3% rice oil and $(NH_4)_2SO_4$ in MMM were finally selected for the mass cultivation of *B. licheniformis* N1 and *B. cepacia* CH-67, respectively. Various formulation of two bacteria were prepared under the determined cultivation conditions by mixing various carriers, protectants, and additonal nutrients.

Key words: Biocontrol, mass cultivation, strawberry anthracnose