

Analysis of Lipolytic Activity of Soil Samples and Soil Metagenome Library Screening

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The purpose of the experiments was to determine lipolytic activity in selected soil samples and biomass from enriched soil samples to create a soil metagenome library for heterologous expression and screen the library for lipolytic activity. eDNA was isolated by the direct method (bead-beating), using different process parameters to obtain high quality DNA. A metagenome library was constructed using the expression vector pET*Blue-2* (Novagen). All obtained clones and soil samples were tested for lipolytic activity by selection on agar medium with tributyrin and in microtiter plate with *p*-NP butyrate as a substrate.

The lipolytic activity determined in the soil samples ranged from 0.1 to 0.4 $\mu\text{mol o-NP}$ per mg of soil. The highest lipolytic activity was found in the samples of peat soil which were used in further experiments.

Enrichment cultures were obtained by incubation of soil in M9 medium with the addition of vegetable oil and resulted in 2-times increased lipolytic activity compared to that obtained from the raw soil.

The metagenomic library contained 27,000 clones and the ligation efficiency was over 45 %. Screening of the metagenome library for lipolytic activity was performed for about 90 % of the obtained clones, which represents approximately 4.5 Mb, and no lipase activity was detected.

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