Isolation and Sequencing of a Lipase Gene from a Fat Contaminated Soil through Metagenomic Approach

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Metagenomes of uncultured microbial communities are potentially rich sources of novel biocatalysts. Lipases (triglycerol acyl-hydrolases, EC 3.1.1.3) act on ester bonds and have a wide range of biotechnological applications. In the present work, a lipase gene was isolated from a fosmid metagenomic library of 500,000 clones constructed with the total DNA from a fat-contaminated soil collected in an industrial wastewater treatment plant. These clones were subjected to screening for lipolytic activity on Luria Bertani agar (LA) containing 1% (v/v) tributyrin or tricaprylin. A total of 127 clones were active on tricaprylin. In order to identify true-lipase producing clones, they were screened on LA containing 1% triolein. Thirty-two clones were able to hydrolyze triolein. One clone, Lip1AH10, exhibited the highest lipolytic activity on triolein and was further characterized. Lip1AH10 was fragmented and subcloned into pUC18, producing a subclone library of 1344 clones with an average insert size of 2 kb. The inserts of fifteen subclones that expressed lipolytic activity were fully sequenced and assembled into a contig of 3220 bp. An ORF of 882 bp encoding a lipase of 294 amino acids was identified. The complete assembled sequence was analyzed *in silico* using the tools BLASTN, BLASTX and ORF-finder (NCBI). Through amino acid sequence alignment, the lipase gene showed 72% identity to a lipase of Yersinia enterocolitica subsp. enterocolitica 8081. The biochemical characterization of the purified lipase will be presented.

Key words: metagenome; lipase; biocatalysts.

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