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Screening and characterization of a novel thermostable lipase with detergent-additive potential from the metagenomic library of a mangrove soil

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Abbreviations: r*Lip906*, recombinant *Lip906*; EDTA, ethylene diamine tetraacetic acid; CTAB, cetyltrimethyl ammonium bromide; DMSO, dimethyl sulfoxide; IPTG, isopropyl β-D-1-thiogalactopyranoside; ORF, open reading frame; NCBI, national center of biotechnology information; MEGA, Molecular Evolutionary Genetics Analysis; PCR, polymerase chain reaction; AGE, agarose gel electrophoresis; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; *p*-NPE, *p*-nitrophenyl ester; *p*-NPM (C14), *p*-nitrophenyl myristate (C14).

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Abstract One clone (*Lip906*) exhibiting lipase activity was screened from a metagenomic library by using a medium containing tricaprylin. A novel lipase gene from the inserted fragment of Lip906 was obtained by sequencing. The phylogenetic analysis of *Lip906* lipase exhibited 34% and 32% homologue to lipases from *Streptomyces* sp. MspMP-M5 and *Rhodopirellula europaea*. This gene was expressed in *Escherichia coli* (*E. coli*) BL21 (DE3), and the recombinant protein was purified and characterized. The best substrate of the recombinant *Lip906* lipase was *p*-nitrophenyl myristate (C14). The lipase expressed maximum activity at 74ºC and pH 7.8, and it was found to be stable at pH values and temperatures ranging from 6.0–8.0 and 4–78ºC, respectively. Furthermore, the lipase was found to be highly resistant to commercial detergent, DMSO, and EDTA, whereas its activity was stimulated in the presence of methanol and ethanol at low concentrations. The lipase showed enhanced activity in the presence of Hg^{2+}, whereas the presence of the metal ions Fe^{2+}, Ca^{2+}, Co^{2+}, and Mg^{2+} inhibited the activity. These beneficial characteristics of *Lip906* lipase provide some advantages for its potential application in industry.

Keywords: Gene expression · Metagenomic library · Lipase · Enzyme stability

1. Introduction

Lipases (triacylglycerol lipases, EC 3.1.1.3) are enzymes that hydrolyze or synthesize esters by acting on ester bonds, depending on the amount of water in the reaction medium (Jaeger et al., 1999; Jaeger et al., 2002; Gupta et al., 2004). Lipases act on triacylglycerols that contain long-chain fatty acids (Glogauer et al., 2011) and are the most versatile and multifunctional enzymes used in various industries (Khan and Jithesh, 2012). Because of their useful features, such as stability in organic solvents, broad substrate specificity, stereoselectivity and regioselectivity, lipases are useful in industrial applications such as detergents, food, leather, medicine and biological material (Hasan et al., 2006). Lipases are considered to be the third largest enzyme group after proteases and carbohydrases. Although a large number of lipases are isolated from bacteria, fungi, plants, and higher animals as