Thesis Title	Screening, Purification and Characterization of a Thermophile Lipase
Name	Amonlaya Jaturapat
Degree	Master of Science (Biochemistry)

Thesis Supervisor Committee

Prayad Komaratat, Ph.D.

Pichit Tosukhowong, Ph.D.

Prapon Wilairat, Ph.D.

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ABSTRACT

Thermophilic microorganisms producing lipase were screened from hot springs in northern Thailand. Seven strains exhibiting lipase activity were isolated. Among these, the strain TP404 produced the highest lipase activity (55 unit/liter culture medium) and was chosen for further study. The crude lipase had an optimum temperature at 60°C, pH stability 7-10 and was relatively thermostable since 80% of initial activity remained after 24 hr at 60°C.

Purification of the thermostable lipase was performed on the enzyme produced by *E.coli* cloned lipase gene of TP404 (pUCTL4). The enzyme was purified to homogeneity as judged by SDS-PAGE and

isoelectric focusing. The purification included ammonium sulfate fractionation and sequential column chromatographies on DEAE-Sephadex A-25, Sephadex G-100 and phenyl Sepharose CL4B. The purified enzyme was found to be a monomeric protein with molecular weight of 43 kDa by SDS-PAGE and 40 kDa by gel filtration. The optimum temperature was at 60°C when p-nitrophenylpalmitate was used as substrate. The pH and temperature stabilities were similar to those of native TP404. The enzyme was activated by Ca^{2+} but was inhibited by various cations including $Zn^{2+}$and Fe^{2+}. The enzyme was deactivated in the presence of organic solvents, among those tested, acetonitrile and acetone showed markedly effect. In the presence of low concentration of detergents (0.1-0.5%), CHAPS, Triton X-100 and Brij W-1 had the stimulatory effect whereas AOT, DOC and SDS showed inhibitory effect. The enzyme showed high lipolytic activity toward trimyristin and trilinolein and preferentially hydrolyzed ester bond of 1- and 3-position of triolein. In the form of immobilized enzyme on celite, the lipase was able to catalyze transesterification of trimyristin and soybean oil in organic solvent system. As judged from its properties, this lipase would be useful for biotechnological application.