DESIGN, SYNTHESIS, AND CLONING OF INDUSTRIAL LIPASE

JUAN PABLO BIFANI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
(BIOCHEMISTRY)

IN
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
1993

Thesis Title Design, Synthesis, and Cloning of Industrial Lipase
Name Juan Pablo Bifani
Degree Master of Science (Biochemistry)

Thesis Supervisory Committee
Yongyuth Yuthavong, D. Phil.
Prayad Komaratat, Ph.D.
Prapon Wilairat, Ph.D.

Date of Graduation 16 July B.E. 2536 (1993)

ABSTRACT

Two different approaches have been pursued in the search for a lipase with potential industrial applications. One approach concentrated on the design, synthesis, assembly, and cloning of an industrial lipase, while the second involved an extensive search, identification, and purification of a lipase-secreting thermophile and its lipase protein from endogenous sources in Thailand.

A synthetic lipase gene (SLG) pJPUCLipI was designed and synthesized based on the reported amino acid sequence of *Pseudomonas fragi* IFO 3458 and IFO 12049. The construct pJPUCLipI has 32 regularly-spaced unique restriction sites allowing for specific and rapid cassette mutagenesis. The entire gene has been transformed and split into two vectors, but not joined together into a single insert. The first clone pFLip3/4 is 311 nucleotides long, including the conserved region Ser^{83} and 24 unique restriction sites. The fidelity of the insert was tested by restriction analysis, sequencing, and colony hybridization. The second clone pFLip5/6 consists of 108 nucleotides and 8 restriction sites. It was generated by polymerase chain reaction (PCR) and cloned as a blunt end insert.

The wild-type lipase model from *P. fragi* 3458 and another highly-homologous gene from *P. fragi* IFO 12049 were amplified by PCR, cloned, and expressed in *E. coli.*

A simple protocol for the screening, identification, and isolation of lipase-secreting thermophiles was developed. Thirteen thermophilic bacteria displaying esterase activity with potential lipolytic characteristics were isolated from hot-water springs from Chiangmai, Thailand. The growth of one of the isolates, strain TL71, was enhanced and suppressed through the regulation of culture conditions, growth medium, pH, triglycerides, free fatty acids, and most notably, polysaccharides. Through temperature regulation of cell growth, it was determined that the polysaccharide concentration required for maximum lipase secretion was finite. Cell arrest resulting from either the addition of potassium cyanide or low temperature, both of which counter-balanced the enhancement effect of polysaccharides, lent support to the "detachment theory".

Partially-purified lipase concentrate from strain TL71 was subjected to thermostability determinations. Lipase TL71-2c proved to be highly thermostable as compared with other reports, thus displaying a very promising potential for industrial applications.

A simple protocol involving extraction, purification, hydrolysis, ligation, and cloning of genomic DNA was used to obtain three positive clones of the lipase gene of thermophile TL71. Screenings of the desired clones were performed by consecutive assays with tributyrin agar plates, *p*-nitrophenylpalmitate assay, and a combination of olive oil-rhodamine, olive oil-calcium chloride or olive oil-saturated copper techniques.