OPTIMIZATION OF LIPASE PRODUCTION USING RESPONSE SURFACE METHODOLOGY BY A NEWLY ISOLATED, *Bacillus* sp. UMK-HS6

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Optimization Of Lipase Production Using Response Surface Methodology By A Newly Isolated, *Bacillus* sp. UMK-HS6

by

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LIST OF ABBREVIATIONS

+ve	positive
3-D	3-dimensional
AA	arachidonic acid
ANOVA	Analysis of Varians
ATP	Adenosine tri-phosphate
BCL	*Burkholderia cepacia* lipases
BLAST	Basic Local Alignment Search Tool
C5	Carbon-5
CoA	Coenzyme A
CVL	*Chromobacterium viscosum* lipases
DAG	diacylglycerol
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
g	gram
g/l	gram per liter
GLA	gamma linolenic acid
h	hour
H^+	hydrogen

HGL	Human gastric lipase
HMDS	hexamethyldisylazane
HPL	human pancreatic lipase
kb	kilobase
LB	Luria Bertani
M1	medium 1
M2	medium 2
M3	medium 3
MAG	monoacylglycerol
MBT	*Mycobacterium tuberculosis*
mg/ml	milligram per milliliter
ml	milliliter
mm	millimeter
MR-VP	Methyl Red-Voges Proskauer
MVA	mevalonic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
OD	optical density
PCR	polymerase chain reaction
PFL	*Pseudomonas fluorescens* lipases
PUFA	polyunsaturated fatty acids
RM	Ringgit Malaysia
RNA	ribonucleic acid

ROL	*Rhizopus oryzae* lipases
rpm	rotation per minute
rRNA	ribosomal ribonucleic acid
RSM	Response Surface Methodology
SEM	scanning electron microscope
SHW	slaughterhouse wastewater
sp.	Species
TAG	triacylglycerol
TLL	*Thermomyces lanuginosus* lipases
TSS	total suspended solids
U/mg	Unit per milligram
U/ml	Unit per milliliter
v/v	volume per volume
-ve	negative
w/v	weight per volume

LIST OF SYMBOLS

%	percent
°C	degree Celcius
α	alpha
γ	gamma
μ	micron
M	molar

Pengoptimuman Penghasilan Lipase Menggunakan Kaedah Gerak Balas Permukaan (*Response Surface Methodology - RSM*) Oleh Pencilan Baru, *Bacillus* sp. UMK-HS6

ABSTRAK

Sampel diperolehi daripada pelbagai sumber di sebuah kilang kelapa sawit yang terletak di Kulim, Kedah. Melalui ujian saringan menggunakan plat agar tributirin, bakteria yang menghasilkan enzim lipase akan membentuk zon hidrolisis. Kesemua 18 pencilan telah dikenalpasti berdasarkan kriteria seperti ciri morfologi, koloni dan sifat biokimia. Pencilan UMK-HS6 telah menghasilkan lipase yang maksimum (627 U/ml) dan dikenalpasti sebagai *Bacillus cereus*, sejenis bakteria Gram positif berbentuk rod melalui analisis 16S rRNA. Komposisi medium pertumbuhan yang optimum diperolehi daripada aplikasi Kaedah Gerak Balas Permukaan (*Response Surface Methodology - RSM*) ialah sebanyak 54.99g pepton, 2.92g natrium klorida serta 5.48ml minyak zaitun untuk penyediaan 1 liter yang boleh menghasilkan aktiviti lipase sebanyak 687 U/ml. Keadaan pengkulturan optimum dicapai apabila kepekatan inokulum *Bacillus cereus* UMK-HS6 yang diinokulasi ke dalam medium adalah sebanyak 7% pada keadaan pH 6.9 dengan kelajuan goncangan 234 psm selama 24 jam. Aktiviti lipase didapati meningkat melebihi 30% (1020 U/ml) menggunakan medium dan keadaan pengkulturan yang optimum. Enzim lipase ekstrasel kasar menunjukkan aktiviti yang maksimum pada nilai pH 6.0 dan suhu setinggi 30°C serta mempunyai kestabilan pada keadaan yang sama apabila dieramkan selama 25 jam.

Optimization Of Lipase Production Using Response Surface Methodology By A Newly Isolated, *Bacillus* sp. UMK-HS6

ABSTRACT

Samples were collected from different sources in palm oil mill factory located in Kulim, Kedah. Screening of lipase producing bacteria was done on Tributyrin agar plate and formation of zone of hydrolysis was observed. Total of 18 isolates were characterized and identified based on morphological properties, colony characteristics and biochemical properties. Isolate UMK-HS6 was selected for its maximum yield of lipase (627 U/ml) and identified as *Bacillus cereus* via 16S rRNA analysis, a Gram positive bacilli. The optimum media composition derived from Response Surface Methodology (RSM) was consisted of (per liter): peptone, 54.99g; NaCl, 2.92; olive oil, 5.48ml with the lipase activity was 687 U/ml. Optimum culture conditions was observed when 7% of *Bacillus cereus* UMK-HS6 inoculated in the media containing above composition at pH 6.9 with agitation speed of 234 rpm for 24 hours. The lipase activity had increased more than 30% (1020 U/ml) upon the exposure in the optimum media and culture condition. Crude extracellular lipase enzyme was characterized for its activity and stability profile towards pH and temperature and both pH 6.0 and temperature, 30°C were observed to be the best condition for enzyme in 25 hours incubation.

CHAPTER 1

INTRODUCTION

- Research Background

Enzymes are protein and are considered as potential biocatalysts for a large number of reactions. Enzyme is a biological catalyst that regulates the rate of a chemical reaction in a living organism. In living systems, enzymes function in the transformation of macromolecules to energy and new materials, besides for growth, repair and maintenance of cells. Thus, all living things, particularly animal, plant and microorganisms are sources of enzymes. However, for commercial applications of industrial enzymes, microorganisms are the most important source of various enzymes. The production of enzymes from microorganisms can be controlled physiologically and physico-chemically. Furthermore, the enzyme quantities produced by microorganisms are far higher than that of the plant and animal sources. Enzymes have played an increased role in industrial processes since the scientific understanding of their catalytic function in the late nineteenth century up to now and the most popular enzymes are proteases, amylase and lipases for their application in daily and industrial used. For instance, many household cleaners use enzymes to speed up the breakdown of protein or starch stains on clothes.

A market research on world enzyme conducted by Freedonia Group Incorporated has reported that the world market for enzymes will recover from a difficult 2009 to reach $7 billion in 2013 based on continued strong demand for specialty enzymes, as well as above average growth in the animal feed and ethanol production markets.

From a regional perspective, the developed economies of North America and Western Europe will achieve healthy gains, while the fastest growth will continue to come from the more rapidly developing economies of the Asia/Pacific and Africa/Mideast regions, as well as Latin America and Eastern Europe. With the world in a global economic downturn in 2009, however, the market for enzymes has become much more challenging, and growth will moderate significantly going forward.

Through 2013, world enzyme demand will average annual gains of 6.3 percent per year, led by pharmaceutical and biocatalyst enzymes, both of which will be less susceptible to the effects of lowered economic activity. Diagnostics enzyme demand will also fair well due to expanded access to medical care in developing countries, and the drive to achieve nearly universal health care in the United States.

In Malaysia, we have spent millions importing enzymes for industrial uses in variety products including detergent formulation, dairy products, shampoos, conditioners, cosmetics and pharmaceuticals. For example in 2003 alone, Malaysia purchased RM 28.3 million worth of enzymes as mentioned by Mhd Fairos Asillam, Head of Space Science Research, National Space Agency

to New Straits Time. It is possible for Malaysia to gain profit from practical applications of the global industrial enzyme market which worth RM 8 billion ($ 3.3 billion) a year.

Lipolytic enzymes is one the industrial enzymes that was extensively researched on mainly on its application as catalysts in lipid modification. Lipolytic enzymes is a group of enzymes which is responsible for the hydrolysis of fats and oil consisting of lipase, esterase, phospholipase, and lipoprotein lipase. Today, lipases stand amongst the most important biocatalysts carrying out novel reactions in both aqueous and non-aqueous media (Shivareddy *et al.*, 2010). According to Gao *et al.*, (2000), lipases catalyze the hydrolysis of various fatty esters are widely distributed in animals, plants and microorganisms and microbial lipases is the one that find immense application since microbes can be easily cultivated and their lipases can catalyze a wide variety of hydrolytic and synthetic reaction. Lipases perform essential roles in the digestion, transport and processing of dietary lipids such as triglycerides, fats, oils in most, if not all, living organisms.

Presence of lipase is important to maintain optimal cell membrane permeability; this allows adequate nutrient supply into the cells and wastes to flow out. The biochemical function of lipase is to split fats into their components, specifically to remove two or all three fatty acids from their glycerol base in order to transport the individual components through the intestinal wall.

Interest of lipase has grown significantly in recent years (Fadiloglu & Erkmen, 2002) and widely used in industrial applications due to the wealth of reaction they catalyze and its versatility made them a unique heterogeneous catalyst for transesterification reactions (Nasratun *et al*., 2009) and having lot of commercial applications (Shivareddy *et al.*, 2010).

An ever greater industrial application of these enzymes would depend on the development of low-cost processes for the production of lipases (Elisa *et al*., 2005). Lipases play the role in the postmortem quality deterioration of seafood (other foodstuffs) during handling, chilling frozen storage, and widely used for biotechnological applications in such dairy industry, oil processing etc. (Islam *et al.*, 2009). Lipases are also used in the synthesis of polymers, agrochemical leather textile, baking pharmaceutical and paper industry.

Most of the lipases used in industry are microbial enzymes, of both fungal and bacterial origin (Arpigny & Jaeger, 1999) as they are more stable compared to plant and animal lipases and can be obtained in bulk at low cost (Jyoti & Avneet, 2006). Lipolytic enzymes are currently attracting enormous attention because of their biotechnological potential (Arpigny & Jaeger, 1999). Lipases are widely use nowadays as they can be obtained from various sources.

In order to yield a better method of microbial lipases, Saxena *et al*., (2003) have used a different strategy for the purification of bacterial, yeast and fungal lipases. Lipases find use in a variety of biotechnological fields such as food and dairy (cheese ripening, flavour development), detergent,

pharmaceutical (naproxen, ibuprofen), agrochemical (insecticide, pesticide) and oleochemical (fat and oil hydrolysis, biosurfactant synthesis) industries.

Enzymatic modification of fats and oils which are the triglycerides provides ambient reaction conditions unmatched in many chemical processes, for production of high-value products from cheap and plentiful raw material. Currently, the industrial hydrolysis of oils or fats employs alkaline high pressure steam splitting. This involves high energy utilization and yields a product requiring costly purification. The oxidation and polymerization of high value unsaturated fatty acids are unavoidable under these process conditions. Hence, it is not suitable for the splitting of the sensitive triglycerides or high value polyunsaturated oils. On the other hand, the applications of enzymes to oil or fat hydrolysis may create significantly less burden on the environment in terms of energy consumption and waste produced (Gan *et al*., 1998). The dynamic nature of lipolytic enzyme technology has resulted active and continuous research in enzyme production systems using new and unique microorganisms, industrial enzyme applications and protein engineering.