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PURIFICATION AND CHARACTERIZATION OF RECOMBINANT LIPASE FROM *Arthrobacter* sp. 3B

SITI NADZIRAH PADRILAH

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SITI NADZIRAH BINTI PADRILAH

163988

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Faculty of Biotechnology and Biomolecular Sciences

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SITI NADZIRAH BINTI PADRILAH

163988

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

UNIVERSITI PUTRA MALAYSIA

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "PURIFICATION AND CHARACTERIZATION OF RECOMBINANT LIPASE FROM *Arthrobacter* sp. 3B" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh SITI NADZIRAH BINTI PADRILAH (163988) sebagai syarat untuk kursus BCH4999 Projek.

Disahkan oleh,

..... Tarikh : .....

(Dr. Mohd Shukuri Mohamad Ali)
Penyelia projek
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

Disahkan oleh,

..... Tarikh : .....

(Prof. Dato' Dr. Abu Bakar Salleh)
Ketua Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

ABSTRACT

A recombinant lipase from *Arthrobacter* sp. 3B, was successfully purified and characterized. The lipase was purified using affinity chromatography with Nickel sepharose as a resin. The molecular weight of the pure protein was estimated to be 66.2 kDa by SDS-PAGE. The enzyme exhibited maximum activity at 60ºC and was stable at the temperature lower than 60ºC. The enzyme indicated that the optimum pH for the enzyme activity and stability was pH 7. Lipase 3B has broad substrate specificity, which tend to hydrolyze most natural oils that contain medium and long chain fatty acid, with the highest activity in canola oil (C18:1). Lipase activity was enhanced in the presence of metal ions, especially K^{+}, Ca^{2+}, and Mg^{2+} ions in 1 mM concentration, but it was inhibited by Ni^{2+} ion. The activity of the purified enzyme was slightly decreased in the present of organic solvents. These properties suggest that the lipase may find potential applications in industrial and biotechnology applications.

ABSTRAK

Lipase recombinan dari *Arthrobacter* sp. 3B, telah ditulenkan dan ciri-cirinya telah ditentukan. Lipase 3B telah ditulenkan melalui kolum kromatografi affiniti dengan menggunakan *Nickel Sepharose* sebagai resin. Berat molekul bagi Lipase 3B yang tulen adalah dalam anggaran 66.2 kDa. Enzim lipase dari *Arthrobacter* ini menunjukkan aktiviti maksimum pada suhu yang unik iaitu 60°C dan stabil pada suhu kurang daripada 60°C. Enzim menunjukan bahawa pH optima untuk aktiviti dan kestabilan adalah pada pH 7. Lipase 3B mempunyai substrat pengkhususan yang luas dan boleh menghidrolisiskan kebanyakkan minyak semula jadi yang mempunyai rantai lemak sederhana dan panjang (C12 ke C18) dengan mencapai aktiviti maksima dengan minyak canola (C18:1). Lipase 3B juga berkebolehan menghidrolisis rantai lemak pendek (C4:0). Aktiviti lipase telah dipertingkatkan dengan kehadiran logam bercaj terutama, K^{+}, Ca^{2+} dan Mg^{2+} dengan kepekatan 1 mM tetapi aktiviti enzim telah dihalang oleh Ni^{2+}. Aktiviti enzim tulen menurun dengan kehadiran pelarut organik. Ciri- ciri ini menunjukkan bahawa Lipase 3B berpotensi dalam aplikasi industri dan bioteknologi.

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LIST OF ABBREVIATIONS

%	Percent
°C	Degree celcius
µl	Micro liter
µmole	Micro mole
v/v	Volume per volume
w/v	Weight per volume
kDa	KiloDalton
rpm	Revolutions per minute
M	Molar
mg	Milligram
mg/ml	Milligram per mililiter
FFA	Free fatty acid
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
LB	Luria Bertani
ml	Milliliter
mM	milliMolar
HCl	Hydrochloric acid
NaCl	Sodium chloride
DMSO	Dimethyl sulfoxide
CD	Circular dichroism
U	Unit of activity
nm	nanometer
Ni-sepharose	Nickel sepharose

CHAPTER 1

INTRODUCTION

Lipases (glycerol-ester-hydrolases, E.C. 3.1.1.3) are a class of enzymes that catalyze the hydrolysis of triacylglycerol, diacylglycerol, free fatty acids and glycerol. Lipases have many special features that make them very valuable in the production of optically active compounds such as the selection of regional and stereo and stability in organic solvents. Besides, the temperature and pH stability of lipases are the most important characteristics for use in industry.

Microbial lipases are currently receiving much attention because of their biotechnology potential, for instance, broad substrate specificity, stable in organic solvents, high yield and low cost production (Jaeger and Reetz, 1998). Lipases belong to the group of enzymes which may be found in some Gram-positive and Gram-negative bacteria (Saxena *et al.*, 2003). Thus, many researches have been done to find microbial lipases that produce high productivity for industrial applications such as biodiesel production, organic synthesis, food, pharmaceuticals and detergents.

Microbial enzymes are now well established and it is preferred over other sources because these microbes can be easily cultivated. Currently, psychrophilic lipases have received high intention because of their potential in detergent and therapeutic applications, lower energy cost, lower cost production and lower microbial contamination in industrial processes. To date, some psychrophilic microorganisms, including *Pseudomonas* sp., *Aeromonas* sp., *Pseudoalteromonas* sp., and *Candida antarctica* was found to produce cold active lipases. However, they have low productivity and too many protein

contaminants from culture medium which makes hard to obtain pure lipases for further research on the characteristics of the enzyme. Thus, the recombinant expression of lipases is a great tool to solve the problem.

Previously, a gene encoding lipases was isolated from *Arthrobacter* sp. 3B, and was expressed in *Escherichia coli* expression system. Lipase 3B that have been expressed in *E. coli* BL21 (DE3) could be efficiently produced in high yield and it was further purified and characterized under certain optimization. In this study, the recombinant Lipase 3B biochemical features will be determined.

This was achieved through the following objectives:

1. To purify the recombinant lipase from *Arthrobacter* sp. 3B.
2. To characterize the recombinant lipase from *Arthrobacter* sp. 3B.

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