UNIVERSITI PUTRA MALAYSIA

PURIFICATION, CRYSTALLIZATION AND MOLECULAR MODELING OF RECOMBINANT A3 LIPASE FROM AN ANTARTIC PSEUDOMONAS SP. STRAIN AMS 3

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan yang bertajuk penulenan, penghabluran dan pemodelan molekul recombinan lipase A3 asal daripada *Pseudomonas sp.* strain AMS 3 Antartika telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh Leong Mun Hong (161696) sebagai syarat untuk kursus BCH 4999 (Projek).

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ABSTRACT

A recombinant A3 lipase from *Pseudomonas sp.* strain AMS 3 was previously expressed in *E. coli*. In present study, recombinant A3 lipase was successfully purified through Nickel-Sepharose Fast Flow affinity chromatography. This purification recovered 58.47% of yield with the purification fold 3.69. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight of purified A3 lipase. A3 lipase was screened for crystal under crystallization condition of Crystal Screen and Crystal Screen 2 from Hampton Research in MRC 2 well crystallization plate (Swissci) with the aid of crystallization robot, Oryx 8 for crystallization hits from the screening. The best A3 lipase crystal being observed in the formulation of 0.5 M ammonium sulfate, 0.1 M HEPES pH 7.5 and 30% v/v (+/-)-2-methyl-2,4-pentanediol. 3D structure of A3 lipase was predicted from RaptorX and analyzed by YASARA software. The predicted 3D structure of A3 lipase contained catalytic triad covered with 2 lid subunits, 2 metal ions binding site and glutathione-s-transferase located at the N-terminal.

ABSTRAK

A3 lipase recombinan sebelum ini telah berjaya diekspreskan dalam *E. coli* daripada *Pseudomonas sp.* strain AMS 3. Dalam kajian ini, A3 lipase rekombinan telah berjaya ditulenkan dengan menggunakan turus kromatografi afiniti *Nickel-Sepharose Fast Flow*. Hasil perolehan penulenan adalah sebanyak 58.47% dan faktor penulenan 3.69 telah didapati. Berat molekul A3 lipase telah ditentukan dengan aplikasi natrium dodesil sulfat poliakrilamida gel elektroforesis. Lipase A3 dihablurkan dalam plat penghabluran MRC 2 perigi (Swissci) dengan formulasi penghabluran *Crystal Screen* dan *Crystal Screen 2* daripada Hampton Research melalui aplikasi robot penghabluran, *Oryx 8*. A3 lipase berjaya dihablurkan dalam formulasi 0.5 M ammonium sulfat, 0.1 M HEPES pH 7.5 and 30% v/v (+/-)-2-metil-2,4-pentanediol. Struktur 3D lipase A3 telah berjaya diramalkan dengan RaptorX dan dianalisis dengan perisian YASARA. Menurut struktur 3D A3 lipase yang diramalkan, ia mengandungi tapak pemangkinan yang diliputi oleh 2 subunit penutup, 2 tapak pengikatan ion logam dan glutathione-s-transferase pada hujung terminal-N.

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
%	Percent
µl	Micro liter
µmole	Micro mole
U/ml	Unit per milliliter
v/v	Volume per volume
w/v	Weight per volume
kDa	KiloDalton
rpm	Revolutions per minute
M	Molar
mg	Milligram
mg/ml	Milligram per milliliter
FFA	Free fatty acid
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
LB	Luria Bertani
mM	milliMolar
ml	Milliliter
HCl	Hydrochloric acid
NaCl	Sodium chloride
U	Unit of activity
nm	Nanometer
Ni-sepharose	Nickel sepharose
YASARA	Yet Another Scientific Artificial Reality Application
dH_2O	Distilled water

CHAPTER 1

INTRODUCTION

The role of enzyme in catalyzing different processes is known more than a century ago. Microbial enzyme production is more preferable and economic as compared to plants or animals sources. The microbial enzyme is faster and simpler way to produce enzyme in a large quantities. Furthermore it can be genetically manipulated based on the requirement (Demain and Vaishnav, 2009).

The cold adapted microbial enzymes had become the aim of researchers to explore as they carry the enzyme properties of catalyzing process under the low temperature condition where the mesophiles or thermophiles are eventually showed little or no activity (Joseph et al., 2008). The cold adapted enzymes are widely used in industrial application such as in detergent, pharmaceutical, food, flavour industry, wastewater treatment, textiles and more (Hasan et al., 2006).

Protein crystallography is a loop from the virtual to the visual. Crystallization of proteins develops an approach to the fundamental understanding of the molecular study. The self-organization of protein molecules in an order way and give a crystal lattice that can be beam by the X-ray provide a clear view of protein structure that can be further research for its properties (McPherson, 2004).

Structural biology plays an important role in molecular studies. The molecular modeling is a method to predict the structure of obtained sequence by comparative method so called homology modeling. The predicted model can be used as template for the further research before the crystal structure is solved (Ginalski, 2006).

Previously, a *Pseudomonas sp.* strain AMS 3 was screened to have lipid hydrolysis (lipase) property. Then, the lipase gene *Pseudomonas sp.* strain AMS 3 was isolated and cloned then expressed in *E. coli* BL21 (DE3) for high yield production. The biochemical properties of A3 Lipase were determined in previous research to have an optimum temperature at 50 ^{o}C and most stable in pH 8. The lipase A3 was able to function in broad range temperature and pH. These special features call up the study of the structure of lipase A3. The following were the objectives of the study:

1) To purify the recombinant A3 Lipase from recombinant *E. coli*
2) To crystallize the purified A3 Lipase
3) To predict 3D structure of A3 Lipase by computational approach

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