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EXPRESSION, CHARACTERIZATION AND HOMOLOGY MODELLING OF A NOVEL HORMONE-SENSITIVE LIPASE LIKE-ESTERASE FROM Glaciozyma antarctica PI12

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By

HIRYAHAFIRA BTE MOHAMAD TAHIR

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Chairman : Associate Professor Mohd Shukuri Mohamad Ali, PhD
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Microbial lipolytic enzyme, especially from extreme cold places such as Antarctica, has gained attention to study on their characterization and structure properties. Furthermore, there are many reported information on the isolation and production of an esterase from psychrophile which also useful in the industrial application. However, there are less reported enzyme from Antarctica especially from psychrophilic yeast due to low production level of wild strain and the cost to grow the wild strain at low temperature is quite expensive. Therefore, this project is conducted to focus on cloning, optimization expression, purification, biochemical characterization and structural prediction of esterase from psychrophilic yeast. Previously, a psychrophilic yeast was isolated from Antarctic sea ice and was identified as *Glaciozyma antarctica* PI12 and the genome was successfully sequenced and annotated. Several low identity gene encoding putative lipolytic enzyme were identified. These genes include a putative esterase that belongs to HSL like-esterase known as GlaEst12. Gene analysis reveals that GlaEst12 encoded for 399 of amino acids and has 30 % identity with chain A of bacterial hormone-sensitive lipase of E40 and belongs to H group since it has conserved motif HGGG and GDSAG at the amino acid sequences. GlaEst12 was expressed in the form of inclusion bodies and successfully purified via one-step Ni-Sepharose affinity chromatography with the final yield and fold of 38.91 and 1.72 respectively. The biochemical characterization of GlaEst12 showed novel properties since the optimal temperature range 50-60 °C and stable at alkaline pH condition. Unlike another HSL like-esterase, this esterase showed higher activity towards medium-chain ester substrates rather than shorter chain ester. Besides that, this esterase was activated when treated with metal ions (Na+, K+, Ca2+ and Mn2+) and stabilized when incubated with 1-propanol and toluene. Homology modelling of GlaEst12 was performed by Robetta software and using crystal structure of esterase 40 from HSL family as template. The 3D structure was predicted as a homodimer in which each monomer composed of N-terminal and

C-terminal domain and have a typical α/β hydrolase fold with the catalytic residues found at Ser232, Glu341 and His371. The analysis of the structure and characterization of this esterase may provide new insights about the enzyme characteristics, especially from HSL like-esterase of psychrophilic yeast.

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PENGEKSPRESIAN, PENCIRIAN DAN PEMODELAN HOMOLOGI BAGI HORMONE-SENSITIF LIPASE (HSL) SEPERTI-ESTERASE NOVEL DARI *Glaciozyma Antarctica* PI12

Oleh

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Enzim lipolitik daripada sumber mikrob terutamanya dari tempat terlampau sejuk seperti Antartika, telah mendapat perhatian untuk pengkajian mengenai ciri-ciri biokimia dan struktur enzim. Tambahan pula, terdapat banyak laporan mengenai informasi pengasingan dan pengeluaran esterase daripada psikrofilik yang juga berguna dalam aplikasi perindustrian. Walaubagaimanapun, sedikit laporan mengenai enzim daripada Antartika terutamanya daripada ragi psikofilik kerana tahap pengeluaran asli yang rendah dan kos untuk mengkultur ragi pada suhu rendah agak mahal. Oleh itu, projek ini dijalankan untuk memberi tumpuan kepada pengklonan, ekspresi pengoptimuman, penulenan, pencirian biokimia dan ramalan struktur HSL dari yis psikrofilik. Sebelum ini, yis dari psikrofilik telah diasingkan dari laut Antartika dan dikenal pasti sebagai Glaciozyma antarctica PI12 dan genom berjaya dijujukan dan didepositkan. Sejumlah enzim lipolitik yang rendah identiti dengan enzim yang lain telah dikenal pasti. Enzim-enzim itu termasuklah bakal jaluran esterase yang dimiliki oleh kumpulan esterase HSL yang dikenali sebagai GlaEst12. Analisis gen mendedahkan bahawa GlaEst12 dikodkan untuk 399 asid amino dan mempunyai identiti 30 % dengan rantai A lipase sensitif hormon bakteria E40 dan tergolong dalam kumpulan H kerana ia mempunyai motif HGGG dan GDSAG pada urutan asid amino. GlaEst12 diekspresi dalam bentuk (inclusion bodies) dan berjaya ditulenkan melalui kromatografi afinitas Ni-Sepharose langkah tunggal dengan hasil akhir dan lipatan masing-masing 38.91 dan 1.72.. Pencirian biokimia GlaEst12 menunjukkan sifat-sifat baru kerana suhu optimum 50-60 °C dan stabil pada keadaan pH alkali. Tidak seperti HSL yang lain, esterase ini menunjukkan aktiviti yang lebih tinggi ke arah ester rantaian carbon yang sederhana dan bukannya ester rantaian carbon yang pendek. Selain itu, esterase ini diaktifkan apabila dirawat dengan ion logam (Na+, K+, Ca2+ dan Mn2+) dan stabil apabila diinkubasi dengan 1-propanol dan

toluena. Pemodelan homology GlaEst12 dilakukan menggunakan perisian Robetta dan menggunakan struktur kristal esterase 40 dari keluarga HSL sebagai templat. Struktur 3D diramalkan sebagai homodimer yang setiap monomernya terdiri daripada domain terminal N dan terminal C dan mempunyai tipikal hydroxyase α/β dengan residu-residu tapak aktif yang terdapat di Ser232, Glu341 dan His371. Analisis struktur dan pencirian esterase ini dapat memberi gambaran baru tentang ciri enzim, terutama daripada HSL yis psikrofilik.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
°C	Degree celsius
%	Percentage
A_{600nm}	Optical density at wavelength 600 nanometer
µL	Microliter
µm	Micrometer
µmoles	Micromoles
bp	Base pair
DNA	Deoxyribonucleic acid
g	Gram
hrs	Hours
kb	Kilobase
L	Litre
LB	Luria-Bertani
M	Molar
Min	Minute
Sec	Second

CHAPTER 1

INTRODUCTION

Antarctica is recognized as an extreme place in the earth because of its severe cold temperature in surroundings, higher exposure to the ultra-radiation, high atmospheric pressure and limited nutrient availability (Carpenter et al., 2000; Nadzir et al., 2018). Due to its disadvantages and misfortune condition, only the survival of the fittest of the organism capable of inhabiting this kind of place such as psychrophilic microorganism. Psychrophilic is one of microbial extremophile that found in the nature that can adapt and lives in extremely cold temperature which nearly around - 20 °C for the bacteria that live in sea ice (D'Amico et al., 2006). It had been reported that to maintain and adapt under "inhospitable" environment, the microorganism need to have diverse adaptive mechanisms such as producing ice-binding protein (AFP) or antifreeze protein to control the ice crystal growth, the presence of flexible structure of enzyme especially in the catalytic site to increase activity at low temperature and modification of the membrane composition of the lipid bilayer to maintain the fluidity of membrane at lower temperature (De Maayer et al., 2014; Feller & Gerday, 2003).

The lipolytic enzyme consists of esterase (EC 3.1.1.1) and lipase (EC 3.1.1.3) that catalyze both the cleavage and formation of ester bonds (Jensen et al., 2016). Although esterase and lipase have similar secondary structures, i.e., α/β hydrolase fold, esterase prefers to hydrolyze fatty-acids esters with an acyl chain with less than ten carbon atoms. In contrast, lipase can hydrolyze long-chain fatty acids with more than ten carbon atoms (Fojan, 2000). Based on the sequence similarity, this protein has been classified into four groups: (i) C (cholinesterase and fungal lipase); (ii) L (lipoprotein and bacterial lipase); (iii) H (mammalian hormone-sensitive lipase and hormone-sensitive lipase [HSL]-like family); and (iv) X (α/β hydrolase and does not belong to any of the other groups) (De Simone et al., 2004). Among of these group, HSL is a known enzyme that is mostly found in mammalian tissue and stimulated by several hormones, such as catecholamines, Adrenocorticotropic (ACTH) and glucagon, to hydrolyze the triglyceride into free fatty acids and glycerol, which makes it plays a pivotal role in providing the primary source of energy for most tissues (Lampidonis et al., 2011; Langin et al., 1993). Interestingly, HSL enzyme also presents in microorganism which their C-terminal domain containing catalytic triad homologous to the mammalian HSL counterpart and known as hormone-sensitive lipase like family. Several HSL-like enzymes have been reported from microbial sources, such as RmEstB from the thermophilic fungus *Rhizomucor miehei* (Yan et al., 2014), PMGL2 from a permafrost bacterium *Psychrobacter cryohalolentis* (Petrovskaya et al., 2017) and SaHSL from *Salinisphaera* sp. P7-4 (Kim et al., 2019).

Currently, study on enzyme especially in cold environment such as in Antarctica has gained interest among researcher to investigate the structure-function relationship and their characteristics. However, there are less reported enzyme from Antarctica especially from psychrophilic yeast due to low production level of wild strain and the cost to grow the wild strain at low temperature is quite expensive. Therefore, the main objective of this project to overexpress the novel HSL esterase using heterologous expression and to study the characteristics of the esterase. Thus, the sub-objectives of the project were performed as follow:

I. To clone and overexpress recombinant GlaEst12 in *E. coli* host system
II. To purify and characterize the enzyme using a biochemical approach
III. To predict the structure of GlaEst12 *in silico*

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