UNIVERSITI PUTRA MALAYSIA

CLONING, PURIFICATION AND CHARACTERIZATION OF A NOVEL COLD-ADAPTED GDSL ESTERASE FROM PHOTOBACTERIUM SP. J15

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FBSB 2014 9

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By

MEHRNOUSH HADADDZADEH SHAKIBA

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2014

DEDICATION

To my dearly beloved father and mother for their endless love, support, care and encouragement**.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

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Chairman: Adam Leow Thean Chor, PhD

Faculty: Biotechnology and Biomolecular Sciences

The studies of characteristics of cold-adapted enzymes have become a growing interest for researchers as these enzymes have a range of structural properties that confer a high level of flexibility, low-activation enthalpy, low-substrate affinity, and high specific activity at low temperatures compared to thermostable homologs. These features can be extremely useful in various applications such as detergent additives, textile and food industry, and bioremediation. These enzymes are both innovative and invaluable. Therefore, due to these important applications, a cold adapted esterase from family II of bacterial classification (GDSL family) was chosen to clone, purify and characterize.

In current study, a novel cold-adapted GDSL esterase was isolated from *Photobacterium* sp. strain J15. The open reading frame (ORF) of the GDSL esterase J15 was 1044 bp in length coding for 347 amino acids with 19 amino acid residues predicted as signal peptide. The mature GDSL esterase gene was amplified by PCR and then cloned into pET-32b(+) expression vector and expressed as a His-tagged fusion protein. Due to three Leu, one Arg, and one Ile rare codons in the GDSL esterase, the recombinant plasmid was transformed into *E. coli* strain Rosetta-gami(DE3)pLysS expression host to enhance the expression of the GDSL esterase. To optimize over-expression of GDSL esterase J15, parameters such as inducer, temperature and induction time were taken into consideration. A final esterase activity of 4.318 U/ml was detected when the recombinant *E. coli* culture was induced with 0.1 mM IPTG at 20 °C for 16 h.

The His-tagged recombinant GDSL esterase was purified in two steps chromatography; affinity chromatography using Cobalt Sepharose resin and ion exchange (IEX) chromatography using Q-Sepharose resin. After the first purification step, Trx-tag was removed from the recombinant GDSL esterase using thrombin. The protein yield from the first purification step and the final step was 57.4% and 38.5%,

respectively. The purified GDSL esterase is highly active at 20 °C and pH 8. It has a half-life of 5 h at 10 °C. The activity of the enzyme was increased when added with Mg^{2+}, Ca^{2+}, Sr^{2+}, Fe^{3+}, Tween-20, Tween-60, Tween-80, Triton-100, DTT and β-mercaptoethanol. However, SDS, EDTA and PMSF inhibited the enzyme activity. In addition, the enzyme activity was increased when 1.5 M of NaCl was added to the enzyme preparation. Gene analysis of GDSL esterase showed that GDSL esterase J15 is belonged to the members of SGNH hydrolase superfamily with 31% identity with *Fischerella* sp. JSC-11. In the predicted model of GDSL esterase, Ser31, Asp321, and His324 were situated in close proximity, most probably representing the active site of the enzyme. Therefore, the study of the GDSL esterase J15 revealed that the enzyme is a novel cold-adapted esterase from family II with high specific activity at low temperature and other specific characteristics.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazahMaster Sains

PENGKLONAN, PENULENAN DAN PENCIRIAN ESTERASE TAHAN SEJUK BARU DARIPADA *PHOTOBACTERIUM* SP. J15

Oleh

MEHRNOUSH HADADDZADEH SHAKIBA

Mei 2014

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Kajian berkaitan pencirian enzim tahan sejuk telah mendapat perhatian yang menggalakkan di kalangan penyelidik-penyelidik berikutan struktur enzim yang mengandungi ciri fleksibiliti, entalpi pengaktifan yang rendah, affiniti terhadap substrat yang rendah, dan aktiviti spesifik yang tinggi pada suhu yang rendah berbanding enzim tahan panas yang lain. Ciri-ciri ini adalah sangat berguna untuk pelbagai aplikasi seperti penambah dalam pencuci, tekstil dan industri makanan, serta bioremediasi.Enzim-enzim ini adalah inovatif dan dan tidak ternilai.Oleh itu, disebabkan oleh kepentingan aplikasi ini, esterase daya tahan sejuk dari keluarga II klasifikasi bakteria telah dipilih untuk diklon, ditulen dan dicirikan.

Dalam kajian yang dijalankan, enzim GDSL esterase baru yang tahan sejuk telah dipencilkan daripada *Photobacterium* sp. strain J15. Rangka bacaan terbuka (ORF) bagi GDSL esterase mengandungi 1044 bp yang mengekodkan 347 asid amino dan 19 residu diramal sebagai ‘signal peptide’. Gen matang GDSL esterase telah digandakan melalui PCR dan diklon ke dalam pET-32b(+) vektor ekspresi dan diekspres sebagai protein gabungan His-tag. Untuk meningkatkan tahap pengsekspresan GDSL esterase, plasmid rekombinan tersebut telah di pindahkan ke dalam hos pengekspresan *E. coli* strain Rosetta-gami(DE3)pLysS berikutan terdapat tiga Leu, satu Arg, dan satu Ile yang ditafsirkan sebagai ‘rare codon’. Parameter seperti pengklonan, suhu, dan masa induksi telah dipertimbang semasa pengoptimuman tahap ekspresi. Aktiviti esterase akhir yang dikesan di dalam medium kultur adalah 4.318 U/mL apabila kultur rekombinan *E. coli* dicetus dengan 0.1 mM IPTG pada 20 °C selama 16 jam.

Rekombinan His-tag GDSL esterase telah ditulenkan melalui dua langkah kromatografi; kromatografi afiniti menggunakan resin Cobalt Sefarose, dan kromatografi pertukaran ion (IEX) menggunakan resin Q-Sepharose. Selepas langkah kromatografi pertama, tag Trx telah dikeluarkan daripada rekombinan GDSL esterase menggunakan thrombin.Hasil protein yang diperoleh adalah 57.4% dan 38.5%, masing-masing, bagi langkah penulenan pertama dan terakhir.GDSL

esterase yang telah ditulenkan adalah sangat aktif pada suhu 20 °C dan pH 8.Separuh hayat enzim tersebut adalah 5 jam pada suhu 10 °C. Aktiviti enzim meningkat dengan kehadiran Mg2+, Ca2+, Sr2+, Fe3+, Tween-20, Tween-60, Tween-80, Triton-100, DTT and ß-mercaptoethanol. Walaubagaimanapun, aktiviti enzim direncat oleh SDS, EDTA, adan PMSF.Selain itu, aktiviti enzim meningkat apabila 1.5 mM NaCl ditambah semasa penyediaan enzim. Analisis gen GDSL esterase menunjukkan GDSL esterase J15 tergolong dalam superfamili SGNH hydrolase melalui 31% identiti terhadap *Fischerella* sp. JSC-11. Berdasarkan struktur ramalan GDSL esterase, Ser31, Asp321 dan His324 berada dalam kedudukan yang berdekatan, berkemungkinan membentuk tapak aktif enzim tersebut.Oleh itu, kajian mengenai GDSL esterase J15 mendedahkan bahawa esterase enzim tahan sejuk daripada keluarga II mempunyai aktiviti yang tinggi pada suhu yang rendah dan ciri spesifik yang lain.

ACKNOWLEDGEMENT

First and foremost I wish to express my deepest respect, profound gratitude, and great appreciation to Dr. Adam Leow Thean Chorfor his valuable guidance, help, encouragement and constructive advice as my mentors, which made possible the successful completion of this work.

I would also like to express many thanks to Prof. Raja Noor Zaliha Raja Abd Rahman and Dr. Mohd Shukuri Mohamad Ali for valuable suggestions I received during my work. Their suggestions and encouragement helped greatly in venturing into and successfully accomplishing my work.

I would also like to thank to the laboratory technician Mrs. Sharipah Samah for her constant assistance all these years. I would also like to thank all my friends and labmates especially Dr. Malihe Masomian, Randa Abdelkareem, and Sahar Abbasiliasifor their friendship, support and encouragement for this work.

Last but not least, I wish to thank my beloved parents, sister and brotherfor their continuous moral support and encouragement. By remaining close to me through constant contact and being there when I need them, they made me feel as if I am at home with them even though they are far away from me.

APPROVAL

I certify that an Examination Committee has met on date of viva toconduct the final examination of Mehrnoush Hadaddzadeh Shakiba on her degreeof Master of Science thesis entitled **"CLONING, PURIFICATION AND CHARACTERIZATION OF A NOVEL COLD-ADAPTEDGDSL ESTERASE FROM *PHOTOBACTERIUM* SP. STRAIN J15"** in accordance with Universiti PertanianMalaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981.The Committee recommends that thestudent be awarded the (Name of relevant degree).

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This thesis was submitted to the Senate of 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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DECLARATION

Declaration by graduate student

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