



UNIVERSITI PUTRA MALAYSIA

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

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By

MEHRNOUSH HADADDZADEH SHAKIBA

**Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in
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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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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 ijazah Master Sains

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

Oleh

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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 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 mengkodkan 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 dituliskan 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 dituliskan 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 β-mercaptoethanol. Walaubagaimanapun, aktiviti enzim direncat oleh SDS, EDTA, dan 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 hidrolase 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.



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APPROVAL

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

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