



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF MUTATION OF HELIX TERMINI ON STABILITY AND
ACTIVITY OF GEOBACILLUS SP. STRAIN ARM LIPASE***

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ACTIVITY OF *GEOBACILLUS* SP. STRAIN ARM LIPASE**

By

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Chairman: Professor Dato' Abu Bakar Salleh, PhD

Faculty : Biotechnology and Biomolecular Sciences

A thermophilic *Geobacillus* sp. strain ARM was found to produce a thermostable lipase. The *Geobacillus* sp. strain ARM lipase amino acid sequence was used to predict its corresponding three dimensional structure thus providing the means for protein engineering. The common characteristics of the mutated *Geobacillus* sp. strain ARM lipases were studied and compared to the recombinant wild type enzyme to determine the new properties developed upon mutation.

More than 20 years ago, dipole moment was studied towards the stability of protein but the correlation between dipole moment and protein is not fully understood. Rational design was used to create mutations at helix termini based on the dipole moment theory. The two point mutations were predicted by computer modeling to investigate the

relationship between dipole and protein stability. Subsequently, the thermostable *Geobacillus* sp. strain ARM lipase and its variants were subjected to molecular dynamic simulations in order to analyze their structural conformation with regards to stability, flexibility and the interaction between amino acid residues within the structure. Experimental studies were carried out to validate the in silico studies. The lipase variants showed a marked difference from the wild-type in the interaction of residues within the protein interior and exterior with slight changes in the stability and flexibility. The overall stability of the modeled *Geobacillus* sp. strain ARM lipase was altered by the mutations and different dynamical behaviour was observed in the molecular dynamic simulations.

The recombinant *Geobacillus* sp. strain ARM lipase and the A79D lipase variant were found to function optimally at 60°C, whilst, the S236R lipase was found to hydrolyze the substrate actively at 55°C. In spite of the reduced activity of both variant lipases, substitution of serine to arginine decreased the thermostability of the S236R lipase to 45°C as opposed to 50°C for the wild-type *Geobacillus* sp. strain ARM and the A79D lipase during 30 min of incubation time. In contrary, the half-life of S236R lipase was 4 h, the same as the half-life of *Geobacillus* sp. strain ARM lipase whereas the A79D lipase has a half life of only 2 h.

The optimum pH for activity of the variants also shifted one unit where the optimal pH for both variants, A79D and S236R were at pH 9 instead of 8 (wild-type), whereas for the wild-type *Geobacillus* sp. strain ARM lipase and both the variant lipases maintained their pH stability within the range of 6 to 9. Meanwhile, the mutations showed no effects in activity using different natural oils as a substrate.

A residue substitution at the helix terminal did cause dynamical changes in the structural conformation due to position of both mutated amino acid located at the surface of the enzyme. Thus, it is suggested the replacement of one amino acid can contribute in altering the structure stability, flexibility and re-arrangement.



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**KESAN MUTASI PADA TERMINI HELIX TERHADAP KESTABILAN DAN
AKTIVITI *GEOBACILLUS* SP STRAIN ARM LIPASE**

Oleh

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Geobacillus sp. termofilik strain ARM didapati menghasilkan lipase tahan panas. Jujukan asid amino ARM lipase telah digunakan untuk meramalkan struktur tiga dimensi sekaligus membawa peluang kepada kejuruteraan protein untuk menghasilkan enzim yang mempunyai ciri-ciri novel. Ciri-ciri umum mutasi ARM lipase telah dikaji dan dibandingkan dengan jenis rekombinan liar untuk menentukan sifat-sifat baru yang dibangunkan menggunakan kaedah mutasi.

Lebih 20 tahun lalu, kesan momen dipole terhadap kestabilan protein telah disiasat tetapi, hubungan diantara momen dipole dan kestabilan protein tidak dapat difahami sepenuhnya. Reka bentuk rasional telah digunakan untuk menghasilkan mutasi pada hujung helis berdasarkan teori momen dipole. Titik mutasi telah diramal menggunakan

pemodelan computer untuk menyiasat hubungan antara dipole dan kestabilan protein. Selepas itu, ARM tahan panas lipase dan mutan diuji dalam simulasi molekul dinamik untuk menganalisis kestabilan, fleksibel dan interaksi dalam bentuk struktur masing-masing. Kajian telah dijalankan untuk mengesahkan penkajian in silico. Mutan menunjukkan perbezaan yang ketara daripada jenis liar dalam interaksi pedalaman protein dan luas permukaan dengan perubahan yang sedikit dalam kestabilan dan fleksibiliti. Ternyata, kestabilan keseluruhan ARM lipase yang dimodelkan telah diubah oleh mutasi, tetapi kelakuan dinamik yang berbeza diperhatikan di kalangan simulasi dinamik molekul.

ARM rekombinan lipase dan A79D mutan telah didapati menghidrolisis secara optimum pada 60°C. Sementara S236R lipase telah didapati menghidrolisis substrat secara aktif pada 55°C. Walaupun mutasi penggantian mengurangkan aktiviti kedua-dua lipase varian, pertukaran serin kepada arginin telah mengurangkan thermostabiliti S236R lipase kepada 45°C dan bukannya 50°C seperti untuk ARM dan A79D lipase selama 30 minit masa pengeraman. Sebaliknya, separuh hayat bagi S236R lipase adalah 4 jam, sama seperti separuh hayat ARM lipase manakala A79D lipase hanya berjaya untuk mengekalkan aktiviti sehingga 2 jam.

pH optimum bagi aktiviti varian juga beralih satu unit di mana pH pilihan bagi kedua-dua varian, A79D dan S236R adalah pada pH 9, manakala ARM lipase dan kedua-dua lipase varian mengekalkan kestabilan pH dalam julat 6 hingga 9. Sementara itu, mutasi tidak memberikan perubahan dalam aktiviti apabila substrat minyak asli digunakan.

Satu penggantian residu di terminal heliks telah menyebabkan perubahan dinamik menurut spesifikasi struktur disebabkan kedudukan asid amino bermutasi terletak dipermukaan struktur enzim tersebut. Oleh itu, ia mencadangkan penggantian satu asid amino dapat menyumbang dalam mengubah struktur kestabilan, fleksibiliti dan susunan sesuatu enzim.



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I certify that an Examination Committee has met on date of viva to conduct the final examination of Norhayati binti Zakariya @ Zainuddin on her degree of Masters of Science thesis entitled “Effect of Mutations of Helix Termini on Enzyme Stability” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Degree of Masters of Science.

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DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



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