UNIVERSITI PUTRA MALAYSIA

IMPROVEMENT OF T1 LIPASE OF Geobacillus zalihae BY DIRECTED EVOLUTION

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By

ABDUL RAHIM B ABDUL RACHMAN

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

February 2014

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DEDICATION

SPECIAL DEDICATION TO MY BELOVED PARENTS

ABDUL RACHMAN BIN MANGKUTO AND FARIDAH BINTI MAD YATIM

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

IMPROVEMENT OF T1 LIPASE OF *Geobacillus zalihae* BY DIRECTED EVOLUTION

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ABDUL RAHIM ABDUL RACHMAN

February 2014

Chairman: **Adam Leow Thean Chor, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Directed evolution is one of the methods to improve the characteristic of an enzyme such as activity, optimum temperature, pH, stability, substrate specificity and enantioselectivity. Directed evolution relies on random mutation and high throughput screening. This study is mainly conducted to improve the characteristic of native recombinant T1 lipase by directed evolution by using two methods in directed evolution which are DNA shuffling and error prone polymerase chain reaction (PCR) to create potential mutant for further studies. No reported so far about improvement of T1 lipase by using directed evolution method. In addition, lack understanding on the factor affecting specific activity and other characteristic of T1 lipase became the reason why this method was chosen. UPM-1 lipase and T1 lipase gene were digested randomly by using Dnase I and reassambled together before error prone PCR were taken place. Reassambled products were amplified using specific primers of T1 lipase. Full length of mutated gene was cloned into pTrHis TOPO TA expression vector and transformed into *Escherichia coli* TOP 10 host. More than 1500 colonies, only eleven transformants gave rise to clearing zone on trybutyrin agar plate. Eleven putative colonies were proceed for preliminary lipase activity to screen the best among eleven colonies. Only M1 and M3 chimeric lipases with lipase activity above 5 U/ml were subjected to further study. M1 and M3 chimeric lipases were purified with 1.48 fold and 2.36 fold higher specific lipase activity than native T1 lipase, respectively. Random mutations on M3 chimeric lipase increased the optimum temperature from 65°C to 70°C, but the optimum pH was shifted from pH 9 to pH 8 as compared to native T1 lipase. About 194 mutations on M1 chimeric lipase significantly decreased the optimum temperature from 65°C to 55°C but had high lipase activity at pH 6 until 9 with wide pH range compared to native T1 lipase. Interestingly, M1 chimeric lipase has twenty percent lipase activity at pH 4 and pH 5 while no sign of lipase activity for native T1 lipase and M3 chimeric lipase were detected. Substrate specificity for both mutants was not changed as compared to native T1 lipase whereby long carbon chain length substrates were preferable. Improvement of lipase activity for both chimeric lipase (M1 chimeric and M3 chimeric) were closely

related with distance of serine-histidine-aspartic acid catalytic center. Distance between histidine and serine were shorten from 3.5Å (native T1) into 3.2Å (M1 chimeric) and 3.0Å (M3 chimeric). Proline addition at position 87 in M3 chimeric protein sequence became main reason why distance of serine-histidine catalytic center for M3 chimeric lipase was shortened. M1 chimeric lipase protein structure showed 194 mutations at N-terminal and C-terminal in protein sequence without change three amino acid catalytic center (serine-aspartic-histidine). Combination of mutation make M1 chimeric lipase characteristic such as optimum temperature, protein stability and pH profile were altered compared to native T1 lipase. As conclusion, directed evolution as been successfully altered and improved characteristic of T1 lipase such as optimum temperature, enzyme stability and pH profile.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBAIKAN T1 LIPASE DARIPADA *Geobacillus zalihae* SECARA EVOLUSI BERARAH

Oleh

ABDUL RAHIM ABDUL RACHMAN

Februari 2014

Pengerusi: **Adam Leow Thean Chor, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Evolusi berarah adalah salah satu kaedah yang digunakan untuk memperbaiki ciri-ciri enzim seperti aktiviti, suhu optimum, pH, kestabilan, spesifikasi substrat dan enantioselektif. Evolusi berarah bergantung kepada mutasi rawak dan penabiran celusan tinggi. Tujuan utama kajian ini adalah untuk memperbaiki ciri-ciri rekombinan T1 lipase asli melalui evolusi berarah dengan menggunakan dua kaedah (campur aduk DNA dan reaksi rantaian polimerase (PCR) berkecenderungan ralat) untuk menghasilkan mutan berpotensi untuk kegunaan kajian selanjutnya. Tiada laporan setakat ini tentang pembaikan T1 lipase secara evolusi berarah. Tambahan pula, kurang pemahaman tentang faktor yang memberi kesan kepada spesifik aktiviti dan sifat yang lain bagi T1 lipase menyebabkan cara ini telah dipilih. UPM-1 lipase dan T1 lipase gen telah dicerna secara rawak dengan menggunakan Dnase I dan diatur semula bersama sebelum PCR kecenderungan ralat mengambil alih. Hasil diatur semula bersama telah digandakan menggunakan primer tertentu T1 lipase. Panjang keseluruhan gen bermutasi telah diklonkan ke pTrHis TOPO TA vektor expresi dan ditransformasikan ke dalam *Escherichia coli* TOP 10. Daripada 1500 koloni, hanya sebelas transforman menghasilkan zon jelas pada plat agar tributirin. Sebelas koloni yang dijangkakan mempunyai aktiviti lipase telah dipilih untuk saringan awal aktiviti lipase. Saringan dilakukan untuk mendapatkan koloni dengan aktiviti lipase yang terbaik. Hanya M1 kimera lipase dan M3 kimera lipase dengan aktiviti lipase melebihi 5 U/ml dipilih untuk ujian selanjutnya. M1 kimera lipase dan M3 kimera lipase telah ditulenkan dengan masing-masing mempunyai spesifik aktiviti lipase 1.48 kali ganda dan 2.36 kali ganda lebih tinggi daripada T1 lipase asli. Mutasi secara rawak ke atas M3 kimera lipase telah meningkatkan suhu optimum daripada 65°C ke 70°C, tetapi menurunkan pH optimum daripada pH 9 ke pH 8 berbanding T1 lipase asli. Sekurang-kurangnya, 194 mutasi pada M1 kimera lipase telah menurunkan suhu optimum daripada 65°C kepada 55°C tetapi mempunyai aktiviti lipase tinggi pada pH 6 hingga 9 dengan julat pH yang luas berbanding dengan T1 lipase asli. Menariknya, M1 kimera lipase mempunyai dua puluh peratus aktiviti lipase pada pH 4 dan pH 5 manakala tiada tanda-tanda aktiviti lipase

untuk T1 lipase asli dan M3 kimera lipase dikesan. Spesifikasi substrat untuk kedua-dua mutan tidak berubah berbanding dengan T1 lipase asli di mana karbon substrat berantai panjang adalah pilihan utama. Peningkatan aktiviti lipase untuk kedua-dua kimera lipase (M1 dan M3 kimera lipase) berkait rapat dengan jarak antara serine-histidine-aspartik asid pusat bermangkin. Jarak antara histidine dan serine telah memendekkan daripada 3.5Å (T1 asli) ke 3.2Å (M1 kimera) dan 3.0Å (M3 kimera).Penambahan proline pada kedudukan 87 dalam M3 urutan asid amino kimera menjadi sebab utama mengapa jarak serine-histidine pusat bermangkin untuk M3 kimera lipase memendek. Kimera struktur protein lipase M1 menunjukkan 194 mutasi di N-terminal dan C-terminal dalam struktur protein tidak merubah kedudukan tiga asid amino pusat bermangkin (serine-aspartik-histidine). Gabungan mutasi membuatkan ciri M1 lipase kimera seperti suhu optimum, kestabilan dan profil pH protein telah berubah berbanding T1 lipase asli. Kesimpulannya, evolusi berarah telah berjaya mengubah dan memperbaiki ciri T1 lipase seperti suhu optimum, kestabilan enzim dan profil pH.

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I certify that a Thesis Examination Committee has met on 5 February 2014 to conduct the final examination of Abdul Rahim b Abdul Rachman on his thesis entitled "Improvement Of T1 Lipase Of *Geobacillus zalihae* By Directed Evolution" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the Degree of Master of Science. The members of the Supervisory Committee are as follows:

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