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DNA SHUFFLING OF LID REMOVED T1 LIPASE AND PHAZ6

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DNA SHUFFLING OF LID REMOVED T1 LIPASE AND PHAZ6

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan yang bertajuk Shuffling DNA antara T1 lipase yang penutup dikeluarkan dengan phaZ6 telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh Ngan Kian Ann (162430) sebagai isyarat untuk kursus BCH 4999 (Projek).

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ABSTRACT

Due to the impressive progress of enzyme technology, our ability to create new and efficient enzyme has become possible. One of the methods to create a new enzyme is through DNA shuffling. In the present study, both enzymes T1 lipase and Poly(3-hydroxybutyrate) depolymerase (phaZ6) are hydrolases which have a similar active sites but with different substrate specificity. In this experiment, lid removed T1 lipase was shuffled with phaZ6. The lid of T1 lipase was removed so that the active site is exposed. The enzymes were fragmented by DNase and assembled through polymerase chain reaction amplification. A new enzyme was created which had a total number of 153 base pairs. It is 10X smaller in size compared to T1 lipase and phaZ6. It shows positive result in lipolytic activity screening test. However, the new enzyme shows negative result in Polyhydroxybutyrate screening test. In conclusion, a shuffled fragment has been obtained which retains the lipolytic activity but not the depolymerase activity. Therefore, the characterization and specificity of the new enzyme should be carried out.

ABSTRAK

Dengan kemajuan teknologi enzim, keupayaan kami untuk mewujudkan aktiviti enzim baru dan cekap menjadi semakin mungkin. Salah satu cara until mewujudkan satu enzim yang baru adalah melalui shuffling DNA. Dalam kajian ini, kedua-dua enzim lipase T1 dan poli(3-hydroxybutyrate) depolymerase adalah hydrolase yang mempunyai tapak aktif yang sama tetapi dengan substrat kekhususan yang berbeza. Dalam eksperimen ini, lipase T1 yang penutup dikeluarkan telah shuffled dengan phaZ6. Enzim telah dipecahkan dengan DNASE dan dipasang balik melalui polymerase chain reaction penguatan. Enzim baru yang telah dicipta mempunyai seramai 153 pasangan asas. Ia adalah 10X lebih kecil berbanding dengan lipase T1 dan phaZ6. Ia menunjukkan keputusan yang positif dalam ujian Polyhydroxubutyrate. Kesimpulannya, enzim yang diperolehi telah mengekalkan aktiviti lipolytic tetapi tiada untuk aktiviti depolymerase. Oleh itu, pencirian dan kekhususan enzim yang baru perlu diuji.

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
%	Percent
w/v	Weight Over Volume Percentage
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
PHA	Polyhydroxyalkanoate
kDa	kilo Dalton
ChE	Cholinesterase
Ca	Calcium
Zn	Zinc
HCl	Hydrochloric acid
PHB	Polyhydroxybutyrate
kDa	Kilo Dalton
DEAE	Diethylaminoethanol
M	Molar
mg	Miligram
mM	Milimolar
µL	Microliter
NaOH	Sodium hydroxide
pH	-log concentration of H+ ion
ng/uL	Nanogram over microliter
nm	Nanometer
U/uL	Enzyme unit over microliter
rpm	Revolutions per minute
LB	Luria Broth

CHAPTER 1

INTRODUCTION

Due to the impressive progress of enzyme technology, enzymes have evolved as an important molecule that has been widely used in different industrial and therapeutical purposes. Creating a new enzyme is currently acquiring much attention with rapid development of enzyme technology. One of the methods to create a new enzyme is through DNA shuffling. DNA shuffling is a method of recombination of pools of selected mutant genes by random fragmentation and polymerase chain reaction (PCR) assembly in *in vitro* homologous (Coco *et al*., 2001). With the combination of different DNA species, it is able to create a new enzyme with new catalytic site and different specificity. T1 lipase is an enzyme purified from *Geobacillus zalihae*. It acts as a hydrolase to catalyze the hydrolysis of triglycerides from long chain fatty acids. PhaZ6 is one of the five polyhydroxyalkanoate (PHA) depolymerase structural genes that is equipped in *Pseudomonas lemoignei*. It also acts as hydrolase to catalyze the hydrolysis of polyester. Both enzymes have Serine-Histidine-Aspartate motif as the catalytic triad residues but the position of the motif and specificity of both enzymes are different. Through DNA shuffling of both enzymes, we aim to create a new enzyme that has a different specificity compared to T1 lipase and phaZ6.

1.1 OBJECTIVE

The main objective of this study is to create a new enzyme (hydrolase) with different specificity.

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