HETEROLOGOUS EXPRESSION OF LIPASE GENE (*LIP*) AND PHA SYNTHASE GENE (*PHAC1*) FROM *PSEUDOMONAS* SPP. IN *ESCHERICHIA COLI* FOR THE BIOSYNTHESIS OF POLYHYDROXYALKANOATES (PHA)

AUNG SHUH WEN

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AUNG SHUH WEN

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INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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Abstract

Polyhydroxyalkanoates (PHA) are natural biodegradable and biocompatible plastic materials which potentially have a wide range of applications. The potential commercial value of these polyesters prompts a demand for intensive studies to maximise their production and applications. Generally, PHAs are produced and accumulated intracellular by numerous bacteria as energy storage. The accumulation of medium-chain-length (mcl) PHA in bacteria is dependent on the type of carbon source available to the bacteria. It has been reported that palm oil, palm kernel oil and their derivatives were suitable carbon source for the production of mcl-PHA. However, there are few bacteria strains that are able to synthesis PHA by utilising oil because they can produce lipase enzyme. This study describes the construction of a recombinant strain of *Escherichia coli* that is able to digest palm oil for the biosynthesis of PHA. A lipase gene (*lip*) from *Pseudomonas fluorescens* and a PHA synthase gene (*phaC1*) from *Pseudomonas putida* were cloned, separately and together, into *fab B*$^{-}$ *E. coli* using the pBAD-TOPO vector. The constructed *fab B*$^{-}$ *E. coli* strain LS_pT-*phaC1* which harboured the *phaC1* gene only, and *fab B*$^{-}$ *E. coli* strain LS_M3 which harboured both the *lip* and *phaC1* genes were tested for the accumulation of PHA. The results revealed that up to 7.8% cell dry weight of PHA was detected in *fab B*$^{-}$ *E. coli* strain LS_M3 when it was cultivated in medium containing palm kernel oil (PKO) as the sole carbon source. No PHA was detected in *fab B*$^{-}$ *E.coli* strain LS_pT-*phaC1* grown with PKO as the sole carbon source. This showed that both the *lip* and *phaC1* genes were successfully cloned and expressed in the *fab B*$^{-}$ *E. coli* strain LS_M3.

Abstrak

Polihidrosialkanoat (PHA) merupakan plastik semulajadi yang dapat dibiodegradasikan dan dapat digunakan secara serasi dalam sistem biologi. PHA mempunyai pelbagai aplikasi dan nilai komersial yang tinggi. Oleh itu, penyelidikan dalam bidang penghasilan dan penggunaan PHA telah menarik banyak perhatian. Pada umumnya, PHA dihasilkan oleh pelbagai jenis bakteria dalam sel-sel sebagai simpanan tenaga. Penghasilan polimer berantai sederhana (mcl-PHA) dalam bakteria bergantung kepada jenis bekalan karbon yang boleh didapati oleh bakteria tersebut. Minyak kelapa sawit dan hasil sampingannya telah dilaporkan sesuai untuk digunakan sebagai sumber karbon dalam penghasilan mcl-PHA. Namun demikian, hanya terdapat beberapa jenis bakteria yang boleh menghasilkan PHA dengan menggunakan minyak sebagai substrak kerana bakteria-bakteria ini mampu menghasilkan enzim lipase. Kajian ini menghuraikan pembinaan strain rekombinan *Escherichia coli* yang berkemampuan mencerna minyak kelapa sawit dan menggunakannya dalam penghasilan PHA. Gen lipase (*lip*) daripada *Pseudomonas fluorescens* dan gen PHA sintase (*phaC1*) daripada *Pseudomonas putida* telah diklonkan ke dalam *fab* B^{-} *E. coli* dengan menggunakan vektor pBAD-TOPO. Strain LS_pT-*phaC1* merupakan rekombinan *fab* B^{-} *E. coli* yang mengandungi gen *phaC1* sahaja, manakala strain LS_M3 merupakan rekombinan *fab* B^{-} *E. coli* yang mengandungi kedua-dua gen *lip* dan gen *phaC1*. Penghasilan PHA dalam kedua-dua strain rekombinasi tersebut telah diuji. Sebanyak 7.8% berat kering sel PHA telah dihasilkan daripada strain LS_M3 apabila ditumbuhkan dalam medium yang mengandungi minyak isi kelapa sawit atau “palm kernel oil” (PKO) sebagai sumber karbon tunggal. Ini menunjukkan bahawa kedua-dua gen *lip* dan gen *phaC1* telah berjaya diklonkan dan diekspreskan dalam strain LS_M3 tersebut.

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LIST OF ABBREVIATIONS

cAMP		3'-5'-cyclic adenosine monophosphate
fadA		3-ketoacyl-CoA thiolase gene
phaG		3-hydroxyacyl-ACP-CoA
fadB		3-hydroxyacyl-CoA dehydrogenase gene
A		absorbance
Amino acid	A (Ala)	alanine
	D (Asp)	aspartate
	C (Cys)	cycteine
	G (Gly)	glycine
	H (His)	histidine
	L (Leu)	leucine
	V (Val)	valine
ABC		ATP-binding cassatte
ACP		acyl carrier protein
AMP		adenosine monophosphate
Amp^R		ampicillin resistant
ATCC		American Type Culture Collection
bp		base pair
β		beta
$CaCl_2$		calcium chloride
C		carbon
cells /ml		cells per millilitre

DTT	dithiothreitol
CDW	cell dry weight
CoA	coenzyme A
cfu/µg	colony forming unit per microgram
cDNA	complementary deoxyribonucleic acid
Da	Dalton
°C	degree Celsius
DNase	deoxyribonucleas
DNA	deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'-triphosphate
DTT	dithiotreitol
EDTA	ethylenediamine tetraacetic acid
GC	gas chromatography
g	gram
T_g	glass-transition temperature
g	gravity
h	hour
Kan^R	kanamycin resistant
kb	kilo base pair
kDa	kilo dalton
lip	lipase gene
L	litre
LB	Luria Bertani
MPa	megapascals

Mg	magnesium
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
MCL	medium chain length
T_m	melting temperature
mRNA	messenger ribonucleic acid
m	metre
mg	milligram
µg/µL	microgram per microlitre
µg/mL	microgram per millilitre
µL	microlitre
µM	micromolar
µU	microunit
µV	microvolt
mm	millimetre
mM	millimolar
mU/mL	milliunit per millilitre
m	minute
M	molar
ng/µL	nanogram per microlitre
ng/mL	nanogram per millilitre
nM	nanomolar
N	nitrogen
OD	optical density

ORF	open reading frame
O	oxygen
PKO	palm kernel oil
pg	pictogram
%	percentage
P	phosphorus
PCL	poly-ε-caprolactone
PGA	poly-glycolic
P(3HB)	poly(3-hydroxybutyrate)
P(3HB-co-4HB)	poly(3-hydroxybutyrate-co-4-hydroxybutyrate)
P(3HB-co-3HV)	poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)
P(3HHx-co-3HO)	poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate)
PHA	polyhydroxyalkanoate
phaC1	polyhydroxyalkanoate synthase C1 gene
phaC2	polyhydroxyalkanoate synthase C2 gene
phaZ	polyhydroxyalkanoate depolymerase gene
PHAs	polyhydroxyalkanoates
PHB	polyhydroxybutyrate
PLLA	poly-L-lactides
PLA	poly-lactic acid
PCR	polymerase chain reaction
KCl	potassium chloride
RT	reverse transcriptase
RT-PCR	reverse transcriptase polymerase chain reaction

RBS	ribosome binding site
rpm	revolutions per minute
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid
SPKO	saponified palm kernel oil
s	second
SCL	short chain length
Na_2CO_3	sodium carbonate
NaCl	sodium chloride
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel
H_2SO_4	sulphuric acid
TAE	tris-acetic EDTA
U	unit
U/µL	unit per microlitre
U/mL	unit per millilitre
V	volt
vol	volume
vol/vol	volume per volume
H_2O	water
wt	weight
wt/vol	weight per volume
wt/wt	weight per weight

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