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DEVELOPMENT AND CHARACTERISATION OF A RECOMBINANT Escherichia COLI FOR THE PRODUCTION OF POLYHYDROXYALKANOATES

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DEVELOPMENT AND CHARACTERISATION OF A RECOMBINANT *Escherichia* COLI FOR THE PRODUCTION OF POLYHYDROXYALKANOATES

By

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DEVELOPMENT AND CHARACTERISATION OF A RECOMBINANT *Escherichia* COLI FOR THE PRODUCTION OF POLYHYDROXYALKANOATES

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March 2013

Chairman : Professor Mohd Ali Hassan, PhD

Faculty : Biotechnology and Biomolecular Sciences

Polyhydroxyalkanoates (PHA) are biodegradable polymers synthesized by a variety of microorganisms as an intracellular carbon and energy storage materials. The nature of *Escherichia coli*, which is devoid of PHA-degrading enzymes, and the extensive genome studies on this microbe that can be easily manipulated and improved have led it to be a powerful host for PHA accumulation. In order to overcome the low cell density, instability of PHA production and inability of glucose uptake for wild type, *Comamonas* sp. EB172, a high acid-tolerant strain isolated from POME sludge in the open digester, recombinant *E. coli* was constructed. In this study, the unknown PHA biosynthesis genes of *Comamonas* sp. EB172 were successfully isolated. It was found that the isolated acetyl-CoA acetyltransferase ($phaA_{Co}$), acetoacetyl-CoA reductase ($phaB_{Co}$) and PHA synthase, class I ($phaC_{Co}$) genes were clustered together as $phaC_{Co}$-$phaA_{Co}$-$phaB_{Co}$. The modified pGEM' vector with PHA biosynthetic genes express under the control of native promoter from *Cupriavidus necator*. Recombinant *E. coli* harbouring isolated PHA biosynthesis

genes were then compared with recombinant *E. coli* harbouring *C. necator* PHA biosynthesis genes as a control in shake flask fermentation. Approximately 41% of PHA content was detected in recombinant *E. coli* JM109 harbouring pGEM'-phaCAB$_{Co}$ and it was comparable to that recombinant *E. coli* JM109 containing *phbCAB$_{Re}$* with 46% PHA content (as a control). The effect of carbon and nitrogen on cell growth and PHA accumulation of recombinant *E. coli* was evaluated. The use of glucose can increase cell growth and PHA accumulation with nitrogen supplementation. However, mixed organic acids failed to increase cell growth but the PHA accumulated in the cell can be improved by supplying the nitrogen. However, *E. coli* JM109 transformant harbouring pGEM'-phaCAB$_{Co}$ successfully polymerised P(3HB-*co*-3HV) when fed with mixed organic acids without nitrogen source. Overall, supplementation of nitrogen source in the medium improved the cell dry weight with glucose as carbon source, and increased the 3HV monomer for the polymer produced from mixed organic acids. Fed-batch fermentation not only improved the cell growth using 20 g/L glucose and 1 g/L $(NH_4)_2SO_4$, but also improved the productivity using mixed organic acids for P(3HB-*co*-3HV) copolymer production. The productivity with 0.1 g PHA/(L.h) was achieved using 10 g/L mixed organic acids as feeding carbon source. The constant feeding of 10 g/L mixed organic acids triggered 3HV monomer formation started after 16 h and enhanced PHA content to more than 70% and P(3HB-*co*-3HV) copolymer with about 2 mol% 3HV monomer. The fed-batch cultivation increased the cell growth, PHA accumulation and improved the molecular weight of polymer in the range of 850 - 1490 kDa. The overall results in this study indicated that the isolated PHA biosynthesis genes from wild type bacteria can serve as PHA production system using glucose and mixed fatty acids. This finding can contribute towards the accumulation of PHA using natural or renewable carbon sources.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBENTUKAN DAN REKOMBINAN *Escherichia* COLI BAGI PENGHASILAN POLIHIDROKSIALKANOAT

Oleh

YEE LIAN NGIT

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Polihidroksialkanoat (PHA) adalah polimer biourai yang disintesis oleh pelbagai mikroorganisma sebagai bahan simpanan karbon di dalam sel dan bahan-bahan penyimpanan tenaga. *Escherichia coli* yang rekombinan adalah salah satu bio-kilang utama yang digunakan untuk menghasilkan PHA. Sifat semulajadi *E. coli* yang tanpa enzim degradasi PHA dan banyak kajian genomik yang terperinci tentang mikrob ini boleh memudahkannya dimanipulasi dan diperbaiki untuk menjadi bakteria yang terbaik untuk penghasilan PHA. Dalam usaha untuk mengatasi kepadatan sel rendah, ketidakstabilan pengeluaran PHA dan ketidakupayaan pengambilan glucose untuk bakteria tempatan, *Comamonas* sp. EB172, bakteria yang menunjukkan tahap toleransi yang tinggi terhadap asik organik dan terpencil daripada enapcemar sisa kilang kelapa sawit (POME) dalam tangki terbuka, *E. coli* rekombinan telah dibina. Dalam kajian ini, gen biosintesis PHA yang tidak diketahui bagi *Comamonas* sp. EB172 telah berjaya dikenalpastikan. Gen yang didapati terdiri daripada asetil-CoA acetyltransferase ($phaA_{Co}$), acetoacetyl-CoA reductase ($phaB_{Co}$) dan PHA sintase,

kelas I ($phaC_{Co}$) gen adalah berkelompok bersama-sama sebagai $phaC_{Co}$-$phaA_{Co}$-$phaB_{Co}$. Gen baru $phaCAB_{Co}$ telah berjaya diklonkan ke dalam vektor pGEM' yang diubahsuai di bawah kawalan promoter daripada *C. necator*, dan transformasi ke dalam *E. coli* JM109 untuk mengenalpasti fungsinya dalam kelalang goncang. Kira-kira 41% kandungan PHA telah dikesan dalam *E. coli* JM109 yang rekombinan mengandungi pGEM'-phaCAB$_{Co}$ dan kandungn PHA ini setanding dengan *E. coli* JM109 rekombinan mengandungi $phbCAB_{Re}$ dengan kandungan PHA sebanyak 46% (sebagai kawalan). Kesan karbon dan nitrogen kepada penghasilan PHA oleh E. coli JM109 transformants yang terpilih telah dikaji. Penggunaan glukosa boleh meningkatkan pertumbuhan sel dan penghasilan PHA dengan penambahan nitrogen. Walaubagaimanapun, campuran asid organik gagal untuk meningkatkan pertumbuhan sel tetapi PHA yang dihasilkan di dalam sel boleh bertambah dengan membekalkan nitrogen. Walaubagaimanapun, *E. coli* JM109 transformant mengandungi pGEM'-phaCAB$_{Co}$ berjaya menghasilkan poli (3-hidrosibutirat-ko-3-hidrosivalerat), P (3HB-*ko*-3HV) apabila penggunaan campuran asid organik tanpa sumber nitrogen berbanding dengan hanya P (3HB) daripada glukosa. Secara keseluruhan, penambahan sumber nitrogen dalam media boleh meningkatkan berat kering sel dengan glukosa sebagai sumber karbon dan monomer 3HV bagi polimer yang dihasilkan daripada campuran asid organik. Produktiviti volumetrik dengan 0.1 g PHA/(L.h) telah dicapai dengan suapan 10 g/L campuran asid organik sebagai sumber karbon dalam fermentasi suapan sesekelompok. Fasa penambahan berterusan dengan 10 g/L campuran asid organik telah mencetuskan pembentukan kandungan 3HV bermula selepas 16 h dan mempertingkatkan kandungan PHA kepada lebih daripada 70% dan kopolimer P (3HB-*ko*-3HV) dengan kira-kira 2% mol kandungan 3HV. Kaedah fermentasi suapan sesekelompok telah meningkat pertumbuhan sel,

penghasilan PHA dan meningkatkan berat molekul polimer dalam julat 850-1490 kDa. Secara keseluruhan, kajian ini menunjukkan bahawa gen biosintesis PHA terpencil daripada bakteria boleh bertindak sebagai sistem penghasilan PHA dengan menggunakan glukosa dan campuran asid organik. Penemuan ini boleh menyumbang ke arah penghasilan PHA dengan penggunakan sumber karbon semulajadi atau boleh diperbaharui.

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I certify that a Thesis Examination Committee has met on 11th March 2013 to conduct the final examination of Yee Lian Ngit on her PhD thesis entitled "Development And Characterization of a genetically engineered *Escherichia coli* JM109 for the production of polyhydroxyalkanoates" in accordance with 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

YEE LIAN NGIT

Date: 13 March 2013

TABLE OF CONTENTS