PCR AMPLIFICATION OF CATECHOL 2,3- DIOXYGENASE GENE FROM OIL DEGRADING BACTERIA

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PCR AMPLIFICATION OF CATECHOL 2,3-DIOXYGENASE GENE FROM OIL DEGRADING BACTERIA

By

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the requirements for the degree of
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PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: PCR AMPLIFICATION OF CATECHOL 2,3-DIOXYGENASE GENE FROM OIL DEGRADING BACTERIA oleh Chin Sing Siew, no. matrik: UK8508 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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LIST OF ABBREVIATIONS

bp	Basepair
$CaCl_2$	Calcium Chloride
C23DO	Catechol 2,3-Dioxygenase
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
G+C	Guanine and Cystosine Content
Kb	Kilo Base
$MgCl_2$	Magnesium Chloride
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
Tm	Melting Temperature
ηg	Nano gram
NCBI	National Centre for Biotechnology Information
nt	Nucleotide
OD	Optical Density
TAE	Tris-Acetate-EDTA
U	Unit

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ABSTRACT

Aromatic ring destabilized via oxidation by multicomponent enzyme system to produce catechol follows either *ortho*-cleavage pathway or *meta*-cleavage pathway. *Meta*-cleavage dioxygenase or catechol 2,3-dioxygenase is more capable than *ortho*-cleavage dioxygenase in degrading aromatic hydrocarbons. The Polymerase Chain Reaction (PCR) method was used to amplify catechol 2,3-dioxygenase gene (C23DO) from extracted genomic DNA and plasmid of *Pseudomonas* sp. strain AR3. Four sets of primer combinations (CDO-F1+CDO-R1, CDO-F2+CDO-R2, 23CAT-F+23CAT-R and DEG-F+DEG-R) were used to amplify the C23DO gene. The primer combinations 23CAT-F+23CAT-R and DEG-F+DEG-R were unable to produce the expected product of 238 bp from *Pseudomonas* sp. strain AR3. This could be either be due to high variation existed in C23DO gene sequences from *Pseudomonas* sp. strain AR3 compare to C23DO gene sequences of other oil degrading species or C23DO gene was not present in *Pseudomonas* sp. strain AR3. Two putative bands of 640 bp (CDO1) and 460 bp (CDO2) were obtained from PCR products with primer combinations CDO-F1+CDO-R1 and CDO-F2+CDO-R2 respectively. These putative bands were excised from gel and recovered with purification kits. Both of the putative bands were determined as self-amplified products through PCR reamplification.

PCR AMPLIFIKASI GEN CATECHOL 2,3-DIOXYGENASE DARIPADA BAKTERIA DEGRADASI MINYAK

ABSTRAK

Cincin aromatik dinyahstabil melalui pengoksidaan oleh sistem pelbagai komponen enzim untuk menghasilkan catechol dan seterusnya menjalani laluan tindakbalas *ortho-* atau *meta*-pemisahan. *Meta*-pemisahan dioksigenase atau catechol 2,3-dioksigenase lebih berkeupayaan mendegradasi hidrokarbon aromatik daripada *ortho*-pemisahan dioksigenase. Teknik Polymerase Chain Reaction (PCR) digunakan untuk mengamplifikasi gen catechol 2,3-dioksigenase (C23DO) dalam genomik DNA dan plasmid yang diekstrak daripada *Pseudomonas* sp. strain AR3. Gen tersebut diamplifikasi dengan menggunakan empat kombinasi primer (CDO-F1+CDO-R1, CDO-F2+CDO-R2, 23CAT-F+23CAT-R dan DEG-F+DEG-R). Kombinasi primer 23CAT-F+23CAT-R dan DEG-F+DEG-R gagal mengamplifikasi serpihan bersaiz 238 bp daripada *Pseudomonas* sp. strain AR3. Ini kemungkinan disebabkan oleh variasi yang ketara wujud antara jujukan gen C23DO *Pseudomonas* sp. strain AR3 dengan bacteria degradasi minyak yang lain atau *Pseudomonas* sp. strain AR3 tidak mempunyai gen C23DO. Dua putatif jalur serpihan yang bersaiz 640 bp (CDO1) dan 460 bp (CDO2) diperolehi dengan gabungan pencetus CDO-F1+CDO-R1 dan CDO-F2+CDO-R2 masing-masing daripada PCR. Jalur putatif tersebut ditulen dengan menggunakan kit penulenan. Kedua-dua jalur putatif tersebut disahkan sebagai produk amplifikasi sendirian dalam PCR.