CLONING AND SEQUENCING OF PUTATIVE
β-LACTAM GENE FROM SELECTED
AMOEBA ISOLATE

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FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
2007

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Cloning and sequencing of putative B-Lactam gene from selecte amoeba isolate / Lim Wei Chun.

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CLONING AND SEQUENCING OF PUTATIVE β-LACTAM GENE FROM SELECTED AMOEBA ISOLATE

By

Lim Wei Chun

Research Report submitted in partial fulfillment of
the requirements for the degree of
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Department of Biological Sciences
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A	Adenine
ACVS	Aminoadipyl-Cysteinyl-Valine Synthetase
AK	Amebic Keratitis
ATP	Adenosine Triphospahate
bp	Basepair
C	Cytosine
$CaCl_2$	Calcium Chloride
CNS	Control Nervous System
CoA	Coenzyme A
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
E.coli	*Escherichia coli*
G	Guanine
GAE	Granulomatous Amebic Encephalitis
H_20	Water
IAT	Isopenicillin N Acyltransferase
IPN	Isopenicillin N
IPNS	Isopenicillin N Synthase
LB	Luria-Bertani
LLD-ACV	δ-(L-α-aminoadipyl)- L-cysteinyl-D-valine
L-α-AAA	L-α-Aminoadipic acid
M	Marker
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium Chloride
Mn^{2+}	Manganase ion
mtDNA	Mitochondrial DNA
NaCl	Natrium Chloride

NADH	Nicotinamide Adenine Dinucleotide Hydrogenase
OD	Optical Density
ORF	Open Reading Frame
PAM	Primary Amoebic Meningoencephalitis
PCR	Polymerase Chain reaction
pH	Potential of Hydrogen
RNA	Ribonucleotide Acid
Sp	Species
T	Thiamine
TAE	Tris-bes EDTA
U	Unit

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ABSTRACT

Penicillin is useful for the treatment of bacterial infections. Microorganism such as *Acanthamoeba castelanii* that inhibit the biosynthesis of the bacterial cell wall are believe to produce penicillin antibiotics. Previous research by Chuah Huey Shuan had identified twelve specific bands using primer combinations of IPNS-F1/IPNS-R2, IPNS-F2/IPNS-R2 and IPNS-F2/IPNS-R1 with gradient PCR technique from ten isolates of amoeba. The MA1 fragment (850 bp) was selected for cloning, sequencing and analyzing. The MA1 fragment was reamplified using combination of IPNS-F1/IPNS-R2 primer with PCR technique. The purified PCR product was cloned into pGEM-T Easy Vector. The colonies from recombinant clones were screened using Colony-PCR technique with the combination of PGT-T7/PGT-SP6 primer. Positive plasmid was sent for DNA sequencing and was analyzed. Results show that 850 bp fragment of purified PCR product was inserted correctly into the pGEM-T vector in the cloning process. Eleven out of fourteen colonies of bacteria shows positive recombinant banding pattern with the size of 1030 bp. Sequence of pMA1 shows unreadable sequence. The weak and noisy sequence cause the analysis cannot be made.

PENGKLONAN DAN PENJUJUKAN GEN PUTATIF β-LACTAM DARIPADA ISOLASI AMEBA YANG TERPILIH

ABSTRAK

Penisilin adalah penting dalam rawatan untuk jangkitan bakteria. Mikroorganisma seperti *Acanthamoeba castelanii* yang menghalang biosintesis dinding sel bakteria dipercayai menghasilkan antibiotik penisilin. Kajian sebelum ini oleh Chuah Huey Shuan telah mengenal pasti dua belas jalur yang spesifik dengan menggunakan gabungan pencetus IPNS-F1/IPNS-R2, IPNS-F2/IPNS-R2 dan IPNS-F2/IPNS-R1 dengan teknik kecerunan PCR daripada sepuluh isolasi ameba. Jalur MA1 (850 bp) telah dipilih untuk pengklonan, penjujukan dan penganalisaan. Jalur MA1 diamplifikasi menggunakan gabungan pencetus IPNS-F1/IPNS-R2 dengan teknik PCR. Produk PCR yang tulen diklon ke dalam vektor pGEM-T. Koloni daripada klon rekombinan disaring dengan menggunakan teknik PCR-koloni dengan gabungan pencetus PGT-T7/PGT-SP6. Plasmid yang positif dihantar untuk penjujukan DNA dan seterusnya dianalisa. Keputusan menunjukkan bahawa fragmen bersaiz 850 bp daripada produk PCR yang tulen telah dimasukkan dengan betulnya ke dalam vektor pGEM-T melalui proses pengklonan. Sebelas daripada empat belas koloni bakteria menunjukkan corak penjaluran rekombinan yang positif dengan saiz 1030 bp. Jujukan pMA1 menunjukkan jujukan yang tidak boleh dibaca. Jujukan yang lemah dan berterabur menyebabkan analisa tidak boleh dijalankan.