CLONING AND SEQUENCING OF PUTATIVE ANHYDROTETRACYCLINE OXYGENASE (ANTH) GENE FROM ANCEBA (*Actinomadura* sp.)

SAIT YING TU

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
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CLONING AND SEQUENCING OF PUTATIVE ANHYDROTETRACYCLINE OXYGENASE (AHTM) GENE FROM AMOEBA (*Acanthamoeba* spp.)

By

Saw Ying Yu

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Department of Biological Sciences
Faculty of Science and Technology
UNIVERSITY MALAYSIA TERENGGANU
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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: CLONING AND SEQUENCING OF PUTATIVE ANHYDROTETRACYCLINE OXYGENASE (AHTM) GENE FROM AMOEBA (*Acanthamoeba* sp.) oleh SAW YING YU, no. matrik: UK10189 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, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

.....

Penyelia Utama / *Main Supervisor*

Nama: **DR. CHA THYE SAN**
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Cop Rasmi:

Tarikh: 29/4/07

.....

Penyelia Kedua (jika ada) / *Co-Supervisor (if applicable)*

Nama: PROF. MADYA DR. NAKISAH MAT AMIN
Timbalan Dekan
Pusat Pengajian Siswazah
Universiti Malaysia Terengganu (UMT)
Aras 2, Bangunan Canselori dan Pentadbiran
21030 Kuala Terengganu

Cop Rasmi

Tarikh: 3/5/2007

.....

Ketua Jabatan Sains Biologi /*Head, Department of Biological Sciences*

Nama:

Cop Rasmi: DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 7/5/2007

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LIST OF ABBREVIATIONS

AHTM	Anhydrotetracycline oxygenase
AnPRT	Anthranilate phosphoribosyltransferase
DNA	Deoxyribonucleic Acid
dNTP	Aeoxynucleotide Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
Kb	Kilo Base
LB	Luria Bertani
$MgCl_2$	Magnesium Chloride
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nt	Nucleotide
OD	Optical Density
ORF	Open Reading Frame
PCR	Polymerase Chain Raction
TAE	Tris-Acetate-EDTA
U	Unit
Tm	Melting Temperature

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ABSTRACT

Acanthamoeba spp. was believed to possess various antimicrobial peptides and proteins to combat bacterial growth inside their phagosomes. Tetracycline belongs to a group of antibiotics that contain antimicrobial and pharmacokinetic properties. Anhydrotetracycline oxygenase (anhydrotetracycline monooxygenase or AHTM) is the enzyme that involve in the reaction of biosynthesis pathway of *Streptomyces* spp. in the production of tetracycline. Previous studies had successfully identified six putative fragments by using PCR technique. The putative fragment of AHTM5 was selected and re-amplified and cloned into pGEM-T® Easy Vector. The putative recombinant colonies were selected for plasmid extraction. The presence of inserted DNA was confirmed by using PCR technique. The complete nucleotide sequence consists of 243bp while the deduced amino acids from the complete nucleotide sequence are 81. The translated amino acid sequence of clone pAHTM5 shows 81%-85% of positive similarity and 64%-67% identity to anthranilate phosphoribosyltransferase of the gene bank but with different organisms. These organisms are *Burkholderia cenocepacia AU 1054, Streptomyces coelicolor A3 (2), Ralstonia eutropha H16, Ralstonia eutropha JMP134* and *Pseudomonas aeruginosa PAO1*.

PENGKLONAN DAN PENJUJUKAN PUTATIF ANHYDROTETRACYCLINE OXYGENASE (AHTM) GEN DARI AMOEBA (*Acanthamoeba* spp.)

ABSTRAK

Acanthamoeba spp. dipercayai mempunyai pelbagai antimikrob peptida dan protein untuk menghalang pertumbuhan bakteria di dalam vakuol fagositosik. Tetrasiklin tergolong dalam kumpulan antibiotik yang mengandungi potensi antimikrob dan farmakokinetik. Anhidrotetrasiklin oksigenase (Anhidrotetrasiklin monooksigenase atau AHTM) merupakan enzim yang terlibat dalam tindakbalas biosintesis *Streptomyces* spp. dalam penghasilan antibiotik tetrasiklin. Kajian sebelum ini telah berjaya mengenalpasti 6 fragmen putatif dengan menggunakan teknik PCR. Dalam kajian ini pula, pengklonan dan penjujukan Gen AHTM daripada SPN1 *Acanthamoeba* telah diaplikasikan. Fragmen putatif AHTM5 dipilih untuk amplifikasi dan diklonkan ke dalam pGEM-T Easy Vector. Koloni rekombinan putatif dipilih untuk ektrask plasmid dan kehadiran DNA dikenalpasti dengan menggunakan teknik PCR. Jujukan nukleotid yang lengkap mengandungi 243bp manakala penggabungan asid amino daripadanya adalah 81. Jujukan pAHTM5 yang telah diterjemahkan menunjukkan 81%-85% persamaan positif dan identiti sebanyak 64%-67% kepada protein anthranilat fosforibosiltransferase di dalam Bank Gen pada organisma yang berbeza iaitu *Burkholderia cenocepacia AU 1054, Streptomyces coelicolor A3 (2), Ralstonia eutropha H16, Ralstonia eutropha JMP134* and *Pseudomonas aeruginosa PAO1*.