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Detection of the gene responsible for the biosynthesis of tetracyclines in amoebae / Mah Hoong Ting.

Lihat sebelah

DETECTION OF THE GENE RESPONSIBLE FOR THE BIOSYNTHESIS OF TETRACYCLINES IN AMOEBAE

By

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Research Report submitted in partial fulfillment of
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: DETECTION OF THE GENE RESPONSIBLE FOR THE BIOSYNTHESIS OF TETRACYCLINES IN AMOEBAE oleh Mah Hoong Ting, no. Matrik: UK 7813 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

~	Approximately
bp	Basepair
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTP	Aeoxynucleotide 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
ng	Nanogram
NCBI	National Centre for Biotechnology Information
nt	Nucleotide
OD	Optical Density
TAE	Tris-Acetate-EDTA
U	Unit

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ABSTRACT

Tetracyclines are belongs to a group of antibiotics which have the antimicrobial and pharmacokinetic properties. Anhydrotetracycline oxygenase (or anhydrotetracycline monooxygenase or AHTM) is the enzyme involve in the penultimate reaction of the tetracycline biosynthesis pathway of *Streptomyces* sp. Free-living amoebae are believed to possess various antimicrobial peptides and proteins to combat bacterial growth inside their phagosomes. Therefore in this study, the Polymerase Chain Reaction (PCR) method was employed to screen for the possible presence of tetracycline biosynthetic gene (AHTM gene) as observed in *Streptomyces* from ten samples of amoeba isolates. The primers (AHTM-F1, AHTM-F2, AHTM-R1 and AHTM-R2) for temperatures gradient PCR reaction were designed based on the conserve region of AHTM gene from different species of bacteria. Four different primer combinations were tested in order to get the desired gene. Six putative specific bands were successfully obtained from five different amoeba isolates for two primer combinations (AHTM-F1+ AHTM-R2 and AHTM-F2+ AHTM-R1). These bands were labeled as AHTM-1 (~500 bp), AHTM-2 (~450 bp), AHTM-3 (~600 bp), AHTM-4 (~450 bp), AHTM-5 (~300 bp), and AHTM-6 (~300 bp), respectively. Different bands obtained from different amoeba isolates indicating that the uncertainty of the presence of the AHTM gene in the free-living amoebae. Further study such as cloning and DNA sequencing could be carried out to determine the six putative specific bands and search for the homology in the gene bank database.

PENGESANAN GEN BERTANGGUNGJAWAB TERHADAP BIOSINTESIS TETRASIKLIN DALAM AMEBA

ABSTRAK

Tetrasiklin digolong dalam kumpulan antibiotik yang mempunyai ciri-ciri antimikrobial dan farmakokinetik. "Anhydrotetracycline oxygenase" (atau "anhydrotetracycline monooxygenase" atau AHTM) adalah enzim yang memangkin tindak balas tahap akhir dalam laluan tindak balas biosintesis tetrasiklin bagi *Streptomyces* sp. Ameba hidup bebas dipercayai mempunyai pelbagai jenis protein dan peptida antimikrobial yang mampu menghalang pertumbuhan bakteria dalam vakuol fagostik. Teknik PCR telah digunakan untuk mengesan kemungkinan kehadiran gen biosintesis tetrasiklin daripada sepuluh sampel ameba yang seperti dijumpai dalam *Streptomyces*. Pencetus-pencetus yang digunakan dalam tindak balas PCR yang berkecerunan suhu berjaya direka dengan berpandukan kawasan terabadi gen AHTM daripada pelbagai jenis bacteria. Empat pasangan pencetus dikaji dan sebanyak enam jalur spesifik putatif berjaya dihasilkan daripada lima sample ameba dengan dua pasangan pencetus (AHTM-F1+AHTM-R2 dan AHTM-F2+AHTM-R1). Serpihan-serpihan tersebut dinamakan AHTM-1 (~500 bp), AHTM-2 (~450 bp), AHTM-3 (~600 bp), AHTM-4 (~450 bp), AHTM-5 (~300 bp), and AHTM-6 (~300 bp). Jalur berlainan yang didapati menunjukkan kemungkinan kehadiran gene AHTM dalam amoeba hidup bebas. Kajian selanjut seperti pengklonan dan penjujukan serpihan DNA boleh dijalankan untuk menentukan keenam-enam spesifik produk putatif PCR tersebut dan pencarian homologi dalam pengkalan data Bank Gen.