

DETECTION OF THE GENE RESPONSIBLE FOR THE  
THERMOTOLERANCE OF BEPILONCHYTRIDIA MOEBAE

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DETECTION OF THE GENE RESPONSIBLE FOR THE BIOSYNTHESIS OF  
BETA-LACTAM IN AMOEBAE

By

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PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **DETECTION OF GENE RESPONSIBLE FOR THE BIOSYNTHESIS OF BETA-LACTAM IN AMOEBAE.** oleh Chua Huey Shuan, UK 7978 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains –Sains Biologi. Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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## LIST OF ABBREVIATION

bp	Base pair
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
G+C	Guanine and Cytosine Content
Kb	Kilo Base
MgCl <sub>2</sub>	Magnesium Chloride
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
nt	Nucleotide
OD	Optical Density
TAE	Tris-Acetate-EDTA

## LIST OF APPEMDICES

- A.1 TAE buffer
- A.2 PCR buffer
- A.3 Solution (Stock 1)
- A.4 Solution (Stock 2)
- A.5 PAS Solution
- A.6 Phosphate Buffer Saline (PBS)
- B.1 non-nutrient Agar Medium
- B.2 Nutrient Agar Medium

## ABSTRACT

In medicine, penicillins and cephalosporins belong to the large and complex family of Beta-lactam antibiotics. Recent developments in biotechnology, various microbes including free-living amoebae have been screened and used as sources for anti-microbe compounds. To ease the microbe screening for the presence of these compounds, molecular screening for the gene responsible for the production anti-microbe compounds should be conducted. Therefore, in this study, gradient PCR technique was used to screen for the presence of the  $\beta$ -lactam biosynthesis gene *isopenicillin N* synthase (*ipnA*) from the genomic DNA of ten samples of amoeba isolates. Two heterologous forward (IPNS- F1 and IPNS- F2) and reverse (IPNS- R1 and IPNS- R2) primers were designed based on the cDNA of four different fungus species. All the sample of amoebae isolates were screened with the different four primer combinations, which were IPNS- F1/IPNS- R1, IPNS-F1/IPNS- R2, IPNS-F2/IPNS- R2 and IPNS- F2/IPNS- R1. Results showed that, twelve of the putative bands were produced by four different primer combinations. Nine out of the twelve putative specific bands were larger than the expected size, which ranging from about 400 bp to 1500 bp while three of the samples produced putative specific bands, which were smaller than the expected size, of about 400 bp. The results obtained from this study indicated that the seven isolates of free-living amoebae might contain the  $\beta$ -lactam biosynthesis gene *isopenicillin N* synthase (*ipnA*).

## Penyaringan gen yang terlibat dalam biosintesis Beta-lactam dalam Amoeba.

### ABSTRAK

Dalam ilmu perubatan, penicillin dan cephalosporins adalah berada di bawah famili antibiotik Beta-lactam. Perkembangan terbaru dalam bidang bioteknologi telah membolehkan pelbagai mikrob termasuklah amoeba dikaji dan digunakan sebagai sumber bahan anti-mikrob. Untuk memudahkan pengesanan kehadiran bahan tersebut, penyaringan molekul terhadap gen yang berupaya menghasilkan bahan anti-mikrob perlu dijalankan. Oleh itu, teknik 'gradient PCR' telah digunakan dalam kajian ini untuk menyaring kehadiran gene yang terlibat dalam biosintesis Beta-lactam, *isopenicillin N synthase (ipnA)* dari 10 sampel genomik DNA amoeba. Dua pencetus heterologus hadapan (IPNS- F1 dan IPNS- F2) dan berbalik (IPNS- R1 dan IPNS- R2) telah direka berdasarkan cDNA dari empat spesies fungus yang berlainan. Semuan genomik DNA sample amoeba disaring dengan empat kombinasi pencetus iaitu, IPNS- F1/IPNS- R1, IPNS-F1/IPNS- R2, IPNS- F2/IPNS- R2 dan IPNS- F2/IPNS- R1. Keputusan yang diperolehi memnunjukkan 12 jalur spesifik putative telah berjaya dihasilkan. Sembilan daripadanya mempunyai saiz yang daripada jangkaan, iaitu dari ~400 bp hingga ~1500 bp. Manakala tiga sampel lain menghasilkan jalur spesifik putatif yang bersaiz lebih kecil dari saiz jangkaan., iaitu ~400 bp. Keputusan ini, membuktikan bahawa tujuh sampel amoeba kemungkinan besar mengandungi gen biosintesis Beta-lactam, *isopenicillin N synthase (ipnA)*.