CLONING AND SEQUENCING OF PUTATIVE
BETA-LACTAM GENES FROM AMOEBA
(*Acanthamoeba* sp.)

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Lihat sebelah

CLONING AND SEQUENCING OF PUTATIVE BETA-LACTAM GENE FROM AMOEBA (*Acanthamoeba* spp.)

By

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LIST OF ABBREVIATIONS

~	Approximately
bp	Basepair
DNA	Deoxyribonucleic Acid
dNTP	Aeoxynucleotide Triphosphate
g	Gram
L	Liter
LB	Lurie Bertani
Kb	Kilo Base
$MgCl_2$	Magnesium Chloride
mL	Mililiter
NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
µg	Microgram
OD	Optical Density
U	Unit

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ABSTRACT

Acanthamoeba spp. has been known as sources for anti-microbe compounds. Antibiotic penicillin and cephalosporin under the group of beta-lactam antibiotics are important as antibacterial treatment for bacterial infection. Previous study had successfully identity twelve putative fragments with PCR technique. The putative fragments consist beta-lactam gene were cloned and sequenced. Two selected putative fragments, Spn1-1 (1500bp) and Spn5-1 (900bp) were re-amplified and cloned into the pGEM-T vector. The putative recombinant colonies were screened by using colony PCR technique. The positive recombinant colonies were selected for plasmid extraction and further confirm the presence of the inserted DNA with PCR technique. The complete nucleotide sequence of clone pSpn1-1 consists of 1494bp while the deduced amino acids from the complete nucleotide are 498 amino acids. The translated acid amino for clone pSpn1-1 shows 39 – 43% positive similarity and 23 - 28% identity to different protein sequence from different species in the Gene Bank database. The high potential proteins obtained were hypothetical protein, ubiquitin specific protease, ubiquitin carboxyl-terminal hydrolase and ubiquitin specific peptidase from different species. The complete nucleotide sequence of clone pSpn5-1 consists of 783bp while the deduced amino acids from the complete nucleotide are 261 amino acids. The translated acid amino clone pSpn5-1 shows 41 – 63% positive similarity and 34 - 44% identity to hypothetical protein from different species in the Gene Bank database.

PENGKLONAN DAN PENJUJUKAN PUTATIF BETA-LACTAM GEN DARI AMOEBA (*Acanthamoeba* spp.)

ABSTRAK

Acanthamoeba spp. telah dikenalpasti sebagai sumber bahan anti-mikrob. Antibiotik penicillin dan cephalosporins di bawah kumpulan beta-lactam antibiotik adalah penting untuk rawatan antibakteria. Kajian lepas telah berjaya mengenal pasti dua belas fragmen putatif dengan menggunakan teknik PCR. Fragmen putatif yang mengandungi beta-lactam gen telah diklonkan dan dijujukkan. Dua fragmen putatif Spn1-1 (1500bp) dan Spn5-1 (900bp) yang dipilih telah diamplifikasi dan diklonkan ke dalam vector pGEM-T. Koloni rekombinan yang putatif telah dipencilkan melalui teknik koloni PCR. Koloni rekombinan yang positif telah dipilih untuk pengekstrakkan plasmid dan kehadiran DNA dikenalpasti dengan menggunakan teknik PCR. Jujukan nukleotid yang lengkap untuk klon Spn1-1 mengandungi 1494bp dan gabungan 498 asid amino. Asid amino yang diterjemaah untuk klon pSpn1-1 menunjukkan 39 – 43% persamaan dan 23 – 28% identiti kepada jujukan protein dalam pengkalan data Bank Gen. Protein yang telah diperolehi seperti protein hipotetikal, protease spesifik ubiquitin, carboksil-terminal hidrolase ubiquitin dan peptidase spesifik ubiquitin dari spesis yang berlainan. Jujukan nukleotid yang lengkap untuk klon Spn5-1 mengandungi 783bp dan gabungan 261 asid amino. Asid amino yang diterjemaah untuk klon pSpn5-1 menunjukkan 41 – 63% persamaan dan 34 - 44% identiti kepada protein hipotetikal dalam pengkalan data Bank Gen.