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Identification of venvironmental isolates of acanthamoeba base
on morphological and PCR techniques / by Sivanesan
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IDENTIFICATION OF ENVIRONMENTAL ISOLATES OF *ACANTHAMOEBA*
BASED ON MORPHOLOGICAL AND PCR TECHNIQUES

By

Sivanesan Chandarasegran

A PITA report submitted in partial fulfillment
of the requirements for the award of the degree of
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DEPARTMENT OF BIOLOGICAL SCIENCES
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**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
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DECLARATION

I hereby declare that this PITA research report entitled **IDENTIFICATION OF ENVIRONMENTAL ISOLATES OF ACANTHAMOEBA BASED ON MORPHOLOGICAL AND PCR TECHNIQUES** is the result of my own research except as cited in the references.

Signature : 
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IDENTIFICATION OF ENVIRONMENTAL ISOLATES OF *ACANTHAMOEBA* BASED ON MORPHOLOGICAL AND PCR TECHNIQUES

ABSTRACT

Acanthamoeba spp. are free-living amoebae that are widely distributed in the environment. *Acanthamoeba* spp. can also act as pathogens living in their host, and as free-living organisms in nature. In this study, water samples were collected at several locations around Universiti Malaysia Terengganu (UMT) to detect the presence of *Acanthamoeba*. These amoebae were identified by morphological and PCR techniques. Only *Acanthamoeba* were focused in this study. The microscopic observation of the cysts and trophozoites of *Acanthamoeba* was determined by following Page's description. To confirm the genus, primer pair JDP1 and JDP2 was used to amplify nine region df3 of *Acanthamoeba* spp. which is highly specific to amplify the *Acanthamoeba* sp gene. The presence of 5 Bands after the PCR reaction in the gel indicates that the isolates are from genus *Acanthamoeba*. The bands were sent for sequencing analysis and the results showed that these isolates could be further confirmed up to the species level.

IDENTIFIKASI PENCILAN *ACANTHAMOEBA* DARI PERSEKITARAN BERDASARKAN TEKNIK MORFOLOGI DAN PCR

ABSTRAK

Acanthamoeba spp. merupakan amoeba hidup bebas yang boleh dijumpai dalam alam persekitaran yang luas. Ia juga boleh bertindak sebagai pathogen yang hidup dalam perumah ataupun organism hidup bebas di alam persekitaran. Dalam kajian ini, sampel air telah dikumpulkan dari beberapa lokasi di sekeliling Universiti Malaysia Terengganu (UMT) untuk mengkaji kewujudan *Acanthamoeba*. Amoeba ini telah dikaji menggunakan teknik morfologi dan PCR. Hanya *Acanthamoeba* telah difokuskan dalam kajian ini. Pemerhatian menggunakan mikroskop pada sista dan trophozoit dilakukan berdasarkan deskripsi Page. Untuk mengenalpasti genus, pasangan primer JDP1 dan JDP2 digunakan untuk mengamplifikasi lingkungan *df3* species *Acanthamoeba* yang khusus dalam gen species *Acanthamoeba*. Kehadiran 5 band selepas proses PCR dan gel electrophoresis menunjukkan bahawa pencilan yang dikenalpasti adalah daripada genus *Acanthamoeba*. Band tersebut dihantar untuk analisis sekuens dan keputusan menunjukkan bahawa pencilan boleh dikenalpasti sampai tahap species.