PROTEINASES IN PATHOGENIC
Acanthamoeba spp.

NURUL JANNAH BINTI MAT @ MOHAMAD

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

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Lihat Sebelah

PROTEINASES IN PATHOGENIC *Acanthamoeba* spp.

By

NURUL JANNAH BINTI MAT @ MOHAMAD

A PITA report submitted in partial fulfillment
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PENGAKUAN DAN PENGESAHAN LAPORAN PITA

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: PROTEINASES IN PATHOGENIC *ACANTHAMOEBA* SPP. oleh NURUL JANNAH BINTI MAT @ MOHAMAD, no matrik: UK19051 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:

.....

Penyelia Utama

Nama:

Cop Rasmi: Tarikh :.....

.....

Ketua Jabatan Sains Biologi

Nama: DR. FARIDAH BT MOHAMAD

Cop Rasmi: Ketua Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 1/7/2012

DECLARATION

I hereby declare that this PITA research report entitled Proteinases in Pathogenic *Acanthamoeba* spp. is the result of my own research except as cited in the references.

Signature :
Name : Nurul Jannah Binti Mat @ Mohamad
Matric No. : UK 19051
Date : 8 July 2012

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PROTEINASES IN PATHOGENIC *ACANTHAMOEBA* SPP.

ABSTRACT

Proteinases one of the important factors that contribute to the pathogenicity of *Acanthamoeba* but unfortunately they gained a very little attention. Therefore, this study was conducted to detect the proteinase that presence in the pathogenic *Acanthamoeba* spp. and characterized them based on their molecular weight, activities at different pH levels either with or without DTT (dithiothreitol), and the effects of inhibitors on the activity. Two species of *Acanthamoeba* were used in this study, *Acanthamoeba* sp. (HKL isolate) and *Acanthamoeba castellanii* (IMR isolate). In this study, the gelatin SDS-PAGE gels was used to detect the proteinases in the amoebae. On the gels, two bands A and B bands of proteinases were detected in *Acanthamoeba castellanii* (IMR isolate). The A band has its molecular weight was ~116.0 kDa, and was active at pH 7.0, 7.5, and 8.0 in the presence of DTT, and was inactivated at pH 5.5, 6.0, and 6.5 with DTT. The B band with a molecular weight between 66.2 and 116.0 kDa was active at all pH with or without DTT and was detected in both isolate of *Acanthamoeba*. The A band was not inhibited by any inhibitors used. The B band in *Acanthamoeba castellanii* (IMR isolate) was inhibited by antipain, elastatinal, pepstatin, and E-64 (L-trans-epoxysuccinyl-L-leucilamido [4-guanidino]-butano) suggesting that are serine, cysteine, and aspartic proteinase group. There were no inhibitory effects on this proteinase band presence in *Acanthamoeba* sp. (HKL isolate). The results show that the A band only appears in the *Acanthamoeba castellanii* (IMR isolate) at higher pH with DTT, while the B band was observed in both of *Acanthamoeba* at all pH used either with or without DTT.

PROTEINASES IN PATHOGENIC *ACANTHAMOEBA* SPP.

ABSTRAK

Proteinase merupakan salah satu faktor penting yang menyumbang kepada patogenisiti daripada *Acanthamoeba* tetapi malangnya mereka mendapat perhatian yang sangat sedikit. Oleh itu, kajian ini telah dijalankan untuk mengesan kehadiran proteinase dalam patogenik *Acanthamoeba* spp. dan mencirikan mereka berdasarkan berat molekul, aktiviti pada pH yang berbeza samada dengan atau tanpa DTT (dithiothreitol), dan kesan perencat ke atas aktiviti proteinase. Dua spesies telah digunakan dalam kajian ini iaitu *Acanthamoeba castellanii* (pencilan IMR) dan *Acanthamoeba* sp. (pencilan HKL). Dalam kajian ini, gelatin SDS-PAGE gel telah digunakan untuk mengesan kehadiran proteinase dalam amoeba. Di atas gel, dua jalur iaitu jalur A dan jalur B telah dikesan dalam *Acanthamoeba castellanii* (pencilan IMR). Jalur A telah dikesan mempunyai berat molekul ~116.0 kDa, dan aktif pada pH 7.0, 7.5, dan 8.0 dengan DTT, dan tidak aktif pada pH 5.5,6.0, dan 6.5 dengan DTT. Manakala, jalur B dengan berat molekul antara 66.2 dan 116.0 kDa adalah aktif pada semua pH dengan atau tanpa DTT, dan dikesan dalam kedua-dua pencilan *Acanthamoeba*. Jalur A tidak direncat oleh mana-mana perencat yang digunakan. Jalur B dalam *Acanthamoeba castellanii* (pencilan IMR) kda telah direncat oleh antipain, elastatinal, pepstatin, dan E-64 (L-trans-epoxysuccinyl-L-leucilamido [4-guanidino]-butano) mencadangkan bahawa ia adalah kumpulan serine, sistein, dan aspartik proteinase. Tiada sebarang kesan perencatan pada aktivit proteinase dalam *Acanthamoeba* sp. (pencilan HKL). Hasil kajian menunjukkan bahawa jalur A hanya muncul dalam *Acanthamoeba castellanii* (pencilan IMR) pada pH yang tinggi dengan DTT, manakala jalur B dilihat di dalam kedua-dua *Acanthamoeba* pada kesemua pH yang digunakan samada dengan atau tanpa DTT.