

ANTIBIOTIC EFFECTS OF *Acetabularia* sp. III. HUMAN
BLASTOCYSTICIDIN (HBF-7) CELL CULTURE

HEINZ BOGNER

UNIVERSITY OF CALIFORNIA, BERKELEY

UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

1965

2079

1100036840

LP 47 FST 1 2005



1100036840
Cytopathic effects of acaninamoeba spp. in human breast
adenocarcinoma (mcf-7) cell culture / Yew Foo On.



PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100036840		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

CYTOPATHIC EFFECTS OF *Acanthamoeba* spp IN
HUMAN BREAST ADENOCARCINOMA (MCF-7) CELL CULTURE

By

Yew Foo On

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Yew, F.O. 2005. Cytopathic effects of *Acanthamoeba* spp in human breast adenocarcinoma (MCF-7) cell culture. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 90p.

No part of this report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.



JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:
Cytopathic Effect of *Acanthamoeba* spp In Human Breast Adenocarcinoma
(MCF-7) cell culture

oleh Yew Foo On, no. matrik: UK6591 telah
diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan
kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan
memperolehi Ijazah Bachelor Sains (Sains Biologi), Fakulti Sains dan
Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**

Cop Rasmi:

Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 4/4/2005

DR. MD. MAHFIZUL HOQUE

Penyelia Kedua (jika ada)

LECTURER

Nama: Department of Biological Science

Faculty of Science & Technology

University College of Science and Technology Malaysia

21030 Kuala Terengganu.

Tarikh: April 4, 2005

Ketua Jabatan Sains Biologi

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**

Cop Rasmi:

Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 4/4/2005

ACKNOWLEDGEMENT

First of all, I wish to thank Assoc. Prof. Dr. Nakisah Mat Amin and Dr. Mahfuzul Hoque for their instruction in protozoa's and cell cultures techniques and also for their unconditional guidance and support.

Secondly, I would like to acknowledge the assistance of Miss Norhaszalina and Miss Mashitoh from UPM for helping me in cell culture technique. Not forgetting Prof. Abdul Manaf Ali and also Assoc. Prof. Dr. Abdul Rahman Omar from UPM for their guidance and advices in cell culture techniques.

Thirdly, heartfelt thanks to my parents and sister for their support and loving encouragement. Thank you so much.

Fourthly, I also would like to extend my warmest appreciation to Microbiology's Laboratory assistances Madam Mahidawati and Madam Zarina for their help, guidance and advices. I feel it is important that to acknowledge Lab Officers, Miss Norazlina and Miss Ku Naiza for making the project successful. Thank you for all the support, encouragement and sacrifices that you have made for me.

Last but not least, I wish to thank those who have been directly and indirectly helping me making this project a success. Although it is hard to finish this project, but I have learn and experienced many new things.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	viii
ABSTRACT	x
ABSTRAK	xi
CHAPTER 1 INTRODUCTION	
1.1 Introduction	1
1.2 Objectives	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Free-living amoebae	4
2.1.1 <i>Acanthamoeba</i> spp	4
2.1.2 Ecology and distribution	7
2.1.3 Life cycle	7
2.1.4 Morphology	9
2.1.5 <i>Acanthamoeba</i> spp as infectious agents	10
2.1.6 <i>Acanthamoeba</i> infections	11
2.2 Cytopathic effects of <i>Acanthamoeba</i> spp	15
CHAPTER 3 METHODOLOGY	
3.1 Amoebae	18

3.1.1	Source of Amoebae	18
3.1.2	Preparation of Polypeptone and Page's Amoeba Saline	23
3.2	Cell cultures	23
3.2.1	Source of cell line	23
3.2.2	Maintenance and Subculture of the cell line	26
3.3	<i>In vitro</i> cytopathic assay using trophozoites of <i>Acanthamoeba</i> spp	27
CHAPTER 4 RESULTS		
4.1	Cytopathic effects (CPEs) of <i>A. castellanii</i> CCAP 1501/2A to MCF-7 cell monolayer	29
4.2	Cytopathic effects (CPEs) of <i>A. polyphaga</i> CCAP 1501/3A to MCF-7 cell monolayer	41
4.3	Cytopathic effects (CPEs) of <i>Acanthamoeba</i> sp (marine isolate) to MCF-7 cell monolayer	52
4.4	Cytopathic effects (CPEs) of <i>Acanthamoeba</i> sp (from human's corneal scrapping) to MCF-7 cell monolayer	63
CHAPTER 5 DISCUSSION		74
CHAPTER 6 CONCLUSION		80
REFERENCES		81
CURRICULUM VITAE		90

LIST OF TABLES

TABLE NUMBER		PAGE
4.1	Degree of cytopathic effects (CPE) of <i>A. castellanii</i> 1501/2A to MCF-7 cell monolayer	29
4.2	Degree of cytopathic effects (CPE) of <i>A. polyphaga</i> 1501/3A to MCF-7 cell monolayer	41
4.3	Degree of cytopathic effects (CPE) of <i>Acanthamoeba sp</i> (marine isolate) to MCF-7 cell monolayer	52
4.4	Degree of cytopathic effects (CPE) of <i>Acanthamoeba sp</i> (human's corneal scrapping) to MCF-7 cell monolayer	63

LIST OF FIGURES

FIGURE NUMBER		PAGE
2.1	Scanning electron micrograph of <i>Acanthamoeba</i> trophozoites.	6
2.2	The life cycle of <i>Acanthamoeba</i> consists of the trophic or feeding stage amoeba alternating with a dormant, thick wall cysts.	8
3.1	Trophozoites of <i>A. castellanii</i> (Douglas) olkonsky 1931 CCAP 1501/2A.	19
3.2	<i>Acanthamoeba polyphaga</i> (Puschkarew) Volkonsky 1931 CCAP 1501/3A.	20
3.3	<i>Acanthamoeba sp</i> (marine isolate).	21
3.4	Trophozoites of <i>Acanthamoeba sp</i> from human's corneal scrapping.	22
3.5	Human breast adenoarcinoma (MCF-7) cell line.	24
4.1.1	For 1:1 experiment, 1.2×10^6 cell/mL <i>A.castellanii</i> was used.	31
4.1.2	It showed the 1 to 10 ameoba: target cell ratio in which 1.2×10^5 cell/mL <i>Acanthamoeba castellanii</i> was used.	34
4.1.3	Using 1 to 100 amoeba: target cell ratio.	38
4.2.1	For 1:1 experiment, 1.2×10^6 cell/mL of <i>A. polypahaga</i> was used.	42
4.2.2	For the 1:10 experiment, 1.2×10^5 cell/mL of <i>A. polyphaga</i> was used.	45
4.2.3	For 1: 100 experiment, 1.2×10^4 cell/mL of <i>A. polyphaga</i> was used.	49
4.3.1	It showed the level of CPE produced by 1 to 1 ameoba: target cell ratio.	53
4.3.2	It showed the levels of CPE produced by <i>Acanthamoeba sp</i> for 1:10 experiment.	57
4.3.3	For 1:100 experiment, 1.2×10^4 cell/mL of <i>Acanthamoeba sp</i> was used.	60

- 4.4.1 For 1:1 experiment, 1.2×10^6 cell/mL of *Acanthamoeba sp* from human corneal scrapping was used. 64
- 4.4.2 It showed the level of CPE produced by 1:10 ratio of ameba: target cell. 67
- 4.4.3 For 1 to 100 ratio test, 1.2×10^4 cell/mL of *Acanthamoeba sp* from human corneal scrapping was used. 71

LIST OF ABBREVIATIONS

CPE	cytopathic effect
MCF-7	human breast adenocarcinoma cell line designation
PAME	primary amebic meningoencephalitis
GAE	granulomatous amebic encephalitis
CNS	central nerve system
<i>A. castellanii</i>	<i>Acanthamoeba castellanii</i>
<i>A. polyphaga</i>	<i>Acanthamoeba polyphaga</i>
<i>A. hatchetti</i>	<i>Acanthamoeba hatchetti</i>
<i>A. culbertsoni</i>	<i>Acanthamoeba culbertsoni</i>
<i>A. rhyodes</i>	<i>Acanthamoeba rhyodes</i>
<i>A. griffini</i>	<i>Acanthamoeba griffini</i>
<i>A. quina</i>	<i>Acanthamoeba quina</i>
<i>A. lugdunensis</i>	<i>Acanthamoeba lugdunensis</i>
<i>A. lenticulata</i>	<i>Acanthamoeba lenticulata</i>
<i>A. astronyxis</i>	<i>Acanthamoeba astronyxis</i>
<i>N. gruberi</i>	<i>Naegleria gruberi</i>
<i>N. jadini</i>	<i>Naegleria jadini</i>
CCAP	the Culture Collection of Algae & Protozoa
HKL	Hospital Kuala Lumpur
ECACC	European Collection of Cell Cultures
PAS	Page's ameba saline

PBS	phosphate buffered saline
CGM	complete growth media
RPMI 1640	Roswell Park Memorial Institute
FBS	fetal bovine serum
NaCl	sodium chloride
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen orthophosphate
Na_2HPO_4	disodium hydrogen phosphate
NaHCO_3	sodium bicarbonate
EDTA	ethylenediaminetetracetic acid disodium salt
dH_2O	distilled water

ABSTRACT

In this investigation, the cytopathic effects (CPE) properties of axenically grown of *Acanthamoeba* spp were conducted *in vitro* on human breast adenocarcinoma, MCF-7 cell cultures. This is the first report describing the CPE of *Acanthamoeba* spp on MCF-7 cell cultures. In this study, *Acanthamoeba* trophozoites were incubated with MCF-7 cell cultures at different amoeba: target cell ratio, which were 1:1, 1:10 and 1:100. The CPE were observed at interval periods of 1h, 12h, 24h, 48h and 72h after the co-cultures incubation. The results showed that the amoebae caused destruction of the monolayer of the cell cultures at varying degrees. The time requested for amoebae to destroy the cultured cells monolayer depended on the amoeba: target-cell ratio. Common morphological features of affected cells alterations such sharpening of cell ends, elongation, packing and picnosis of the nuclei caused by the amoebae were observed.

KESAN SITOFATIK OLEH *Acanthamoeba* spp DALAM KULTUR SEL BARAH PAYU DARA (MCF-7)

ABSTRAK

Dalam kajian ini, kesan sitofatik (CPE) *Acanthamoeba* spp yang dikulturkan secara aksenik ke atas kultur sel barah payu dara dilakukan secara *in vitro*. Laporan ini merupakan pertama kali melaporkan kesan sitofatik yang disebabkan oleh *Acanthamoeba* spp dengan menggunakan kultur sel MCF-7. Dalam kajian ini, trofozoit *Acanthamoeba* dieram dengan 'monolayer' sel barah payu dara pada nisbah ameba: sel barah yang berlainan, iaitu 1:1, 1:10 dan 1: 100. Kesan sitofatik diperhatikan dalam tempoh masa 1 jam, 12 jam, 24 jam, 48 jam dan 72 jam selepas pengeraman ko-kultur. Keputusan yang diperolehi menunjukkan ameba yang digunakan dapat menyebabkan pemusnahan 'monolayer' kultur sel tersebut pada tahap yang berbeza-beza. Tempoh masa yang diperlukan oleh ameba untuk memusnahkan 'monolayer' kultur sel bergantung kepada nisbah dan kepekatan awal ameba. Secara amnya, perubahan rupabentuk kultur sel seperti ketajaman hujung sel, pemanjangan sel dan piknosis nukleus yang disebabkan oleh ameba dapat dilihat.