Biochemical and Molecular Characterization of Pathogenic Free-Living Amoebae in the Aquatic Environment

A thesis submitted for the degree of Ph.D. in Zoology

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Abstract

Name: Wafaa Mohamed Abd-Allah Hikal

Title: "Biochemical and molecular characterization of pathogenic free-living amoebae in the aquatic environment"

Degree: Ph.D. (Zoology)

Objectives: Occurrence of heat-tolerant free-living amoebae in the Nile, tap and swimming-pool waters in the Nile Delta region, Egypt. Morpho-physiological, biochemical and molecular characterization of the isolated strains of free-living amoebae.

Materials & Methods: Cultivation of free-living amoebae on non-nutrient agar. Identification of the isolated strains based on the morphology of cyst and trophozoite forms as well as temperature and osmo-tolerance assays. DNA extraction and PCR amplification of the isolated amoebae using genus specific primer pairs. Biochemical characterization of the isolated amoeba strains using quantitative and qualitative (SDS-PAGE) assays as well as qualitative determination of proteolytic activity in zymograph analysis.

Results: Potentially pathogenic free-living amoebae were isolated from all of the examined water sources. Molecularly, detection of *Acanthamoeba* and *Naegleria* were confirmed, while amoebae of the genus *Balamuthia* were not detected. Colorimetric assays showed protease activity in heat-tolerant isolates of *Acanthamoeba* and *Naegleria*. All pathogenic isolates of free-living amoebae exhibited higher protease activity than did

the non-pathogenic ones. The zymographic protease assays showed various banding patterns for different strains of *Acanthamoeba* and *Naegleria*.

Conclusion: The incidence and prevalence of the pathogenic free-living amoebae in the aquatic environment using parasitological and molecular diagnostic tools will provide baseline data against which the risk factors associated with waterborne transmission can be identified.

Keywords: Free-living amoebae, heat-tolerance, proteases, PCR, drinking water sources.

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Dedication

I dedicate this work to my father

and soul of my mother

With gratitude to my husband Dr.

Hussein Abdel basit Said Al-Ahl

and my lovely daughter

Rama

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List of abbreviations

A	Acrylamide
APS	Ammonium per sulphate
bp	Base pair
BLAST	Basic local alignment search tool
BisA	Bisacrylamide (N,N'-Methylenebisacrylamide)
BSA	Bovine serum albumin
Cm	Centemeter
CNS	Central nervous system
cDNA	Complementary DNA
C	Control
CTAB	Hexadecyl trimethyl-ammonium bromide
°C	Degree celsius
dNTPs	Deoxy nucleotide triphosphates
DNA	Deoxy ribonucleic acid
DF3	Diagnostic fragment 3
DEPC	Diethylpyrocarbonate
DMSO	Dimethyl sulfoxide
dH_2O	Distilled water
ddH_2O	Double distilled water
E. aerogenes	*Enterobacter aerogenes*
E. coli	*Escherichia coli*
EB	Ethidium bromide
EDTA	Ethylene diamine tetra acetic acid

Fig.	Figure
FLA	Free-living amoebae
G.S	Glucose salt
g/gm	gram
GAE	Granulomatous amoebic encephalitis
hr	Hour
hrs	Hours
pH	Hydrogen ion concentration
ITS	Internal transcribed spacer
kDa	Kilo dalton
LSD	Least significant differences
L	Liter
M/Mr	Marker
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromole
mg/ml	Milligram/ml
mg/l	Milligram / liter
ml	Milliliter
mM	Millimole
min.	Minute
(Mt) DNA	Mitochondrial DNA
M	Mole

nm	Nanometer
NN	Non-nutrient
NNE	Non-nutrient agar+ *E. coli*
No.	Number
OD	Optical density
PAS	Page's amoeba saline
PBS	Phosphate buffer saline
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
RAPD	Randomly amplified polymorphic DNA
RFLPs	Restriction fragment length polymorphism
rpm	Revolution per minute
RNA	Ribonucleic acid
sec	Second
Sr RNA	Small ribosomal subunit RNA
SSU r DNA	Small subunit ribosomal RNA gene
SDS-PAGE	Sodium dodecylsulphate-polyacrilamide gel electrophoresis
Spp.	Species
Sp.	Species
TEMED	N, N, N`, N`-tetramethylethylendiamine
TAE	Tris -acetate- EDTA
Tris-base	2-Amino-2-(hydroxymethyl)- 1,3-propanediol

Tris-HCl	2-Amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride
V/V	Volume/Volume
W/V	Weight/Volume