STUDIES ON PATHOGENIC BACTERIA AND FUNGUS OF SHRIMP, *PENAEUS MONODON* CULTURED IN SEMI-INTENSIVE PONDS IN THE COASTAL AREA OF THANJAVUR DISTRICT, TAMIL NADU, INDIA

THESIS SUBMITTED TO
BHARATHIDASAN UNIVERSITY, TIRUCHIRAPPALLI
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN ZOOLOGY

By
R. RAVICHELVAN

Under the guidance of
Dr. A. AMSATH, Associate Professor

POST-GRADUATE AND RESEARCH DEPARTMENT OF ZOOLOGY
KHADIR MOHIDEEN COLLEGE
ADIRAMPATTINAM – 614 701

JUNE 2011

DEDICATED TO
MY FAMILY MEMBERS

Dr. A. AMSATH, M. Sc., M. Phil., Ph. D.,
Associate Professor and Research Advisor
P.G. and Research Department of Zoology,
Khadir Mohideen College,
Adirampattinam – 614 701.
Tamil Nadu, India

Date:……

CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON PATHOGENIC BACTERIA AND FUNGUS OF SHRIMP, *PENAEUS MONODON* CULTURED IN SEMI-INTENSIVE PONDS IN THE COASTAL AREA OF THANJAVUR DISTRICT, TAMIL NADU, INDIA**" submitted to Bharathidasan University, Tiruchirappalli, for the aaward of the degree of **DOCTOR OF PHILOSOPHY IN ZOOLOGY,** embodies the result of the bonafide research work carried out during the year 2008-2011 by **Mr. R. RAVICHELVAN,** under my guidance and supervision in the P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam, Thanjavur District, Tamil Nadu, India.

I further certify that no part of this thesis has been submitted anywhere else for the award of any degree, diploma, associateship, fellowship or other similar titles to any candidate.

DECLARATION

I do hereby declare that this thesis work has been originally carried out during the year 2008-2011 by me under the guidance and supervision of **Dr. A. AMSATH, M.Sc., M.Phil., Ph.D.,** Research Advisor and Associate Professor, P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam, affiliated to Bharathidasan University, Tiruchirapalli – 620 024 and this work has not been submitted elsewhere for any other degree.

Station : Adirampattinam (R. RAVICHELVAN)

Date : Signature of the Candidate

ACKNOWLEDGEMENTS

First and foremost, I find great pleasure in expressing my deep sense of gratitude and heartful thanks to my research advisor **Dr. A. AMSATH,** Associate Professor, P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Thanjavur District,Tamil Nadu, for his valuable guidance, sincere advise, enthusiasm and unfailing interest throughout the period of research programme.

I express my sincere thanks to Honourable Janab **Dr. S. MOHAMED ASLAM**, Secretary and Correspondent, **Dr. A. MOHAMED ABDUL KHADER,** Principal, Khadir Mohideen College, Adirampattinam-614 701, for providing the necessary facilities for my research programme in the college of excellence.

I am indebted and owe my due respects to **Dr. P. KUMARASAMY,** Associate Professor, Head of the Department, Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, for kind support, keen interest and encouragement.

Words are not adequate to express my gratitude to **Dr. G. SRIDHARAN,** Doctoral Committee Member, Associate Professor, P.G. and Research Department of Zoology, Rajah Serfoji Govt. College, Thanjavur, who has put me in a right path by offering constant help, constructive criticisms and encouragements in every phase of the work till bringing out this thesis.

I am very grateful thanks to **Dr. S.S. RAJENDRAN,** Associate Professor, Head of the Department, Department of Zoology, Rajah Serfoji Govt. College, Thanjavur, for his valuable suggestions.

I have great pleasure in expressing my heartfelt thanks to **Dr.A.PANNEERSELVAM,** Associate Professor, Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur district, for his moral support, keen interest and offering constant help, constructive criticisms and encouragement in every phase of the work till bringing out this volume.

I would like to express my thanks to **Dr. S. RAVEENDRAN,** Associate Professor and **Dr. K. MUTHUKUMARAVEL,** Assistant Professor, **Dr. O. SATHICK,** Assistant Professor, **Mrs. V. GANAPRIYA,** Assistant Professor and **Dr. A. MAHARAJAN,** Assistant Professor, P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, for their moral support and encouragement.

I appreciate the kind gestures **Prof. R. RAVICHANDRAN** and **Prof. D. MADHAVAN,** Department of Zoology, Rajah Serfoji Govt. College, Thanjavur, for their help in various ways during my study period and field trip.

I record my gratefulness to **Mr. M. PRABAKARAN,** Chairman, **Mr.V. THENNARASU,** Manager, **Mr. R. MANIKANDAN** and **Mr. R. KARTHICK,** Sri Gowri Biotech Research Institute, Thanjavur, for their help in every possible ways.

My sincere thanks to **Mrs. K. UMAMAKESHWARI, Mr. M. BHARATHIDASAN, Miss. S. SUBHA,** Sri Gowri Biotech Research Institute, Thanjavur, for their help in identification of bacteria and fungal organisms.

I record my gratefulness to **Prof. A. MUTHUSELVAM,** Muthaiah Research Foundation, Thanjavur, for rendering the help in analysis of biochemical test.

I wish to thank my wife **Mrs. S. SAKTHI,** my daughters **R. RASIKA** and **R. SARIKA** for their help during the course of my study period.

I highly indebted to my god mother, father, brothers, father-in-law, mother-inlaw, brother-in-law and my sister-in-law for their constant encouragement supports during my research programme.

I wish to record my heartful thanks **to M/s. NAGU COMPUTERS,** Thanjavur, for their neatly typing work regarding the manuscript in a splendid manner.

R. RAVICHELVAN

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

APPENDIX

(Media Composition and indicators)

Gram Stain

Crystal Violet

Solution – A

Crystal violet (90% dye content)	-	2.0 gm
Ethyl alcohol	-	20.0 ml

Solution – B

Ammonium oxalate	-	0.8 gm
Distilled water	-	80.0 ml

Solution A and B were mixed.

Gram's Iodine

Iodine	-	1.0 gm
Potassium iodide	-	2.0 gm
Distilled water	-	300.0 ml

Iodine and potassium iodide were dissolved in distilled water.

Ethyl alcohol (95%)

Ethyl alcohol (100%)	-	95.0 ml
Distilled water	-	5.0 ml

Safranin

Safranin (2.5 g in 95% ethyl alcohol)	-	100 ml
Distilled water	-	100 ml

Tryptone broth

Tryptone	-	10 gm
Sodium chloride	-	5 gm
Calcium chloride	-	10 ml
Distilled water	-	1000 ml
pH	-	7.1

Kovac reagent

P-dimethyl amino benzaldehyde	-	5.0 gm
Amyl alcohol	-	75.0 ml
Conc. HCl	-	25.0 ml

Methyl Red – Voges Proskauer (MR-VP) broth

Peptone	-	5.0 gm
Dipotassium hydrogen phosphate	-	5.0 gm
Glucose (10%)	-	50.0 ml
Distilled water	-	1000 ml

Methyl red indicator

Methyl red	-	0.1 gm
Ethanol	-	300 ml
Distilled water	-	200 ml

Voges Proskauer Reagent – I

Alpha naphthol	-	5.0 gm
Ethyl alcohol	-	95.0 ml

Dissolved alpha napthol in the ethyl alcohol with constant stirring.

Voges Proskauer Reagent – II

Potassium hydroxide	-	40.0 gm

Distilled water	-	100 ml

Simmon Citrate Agar

Magnesium sulphate	-	0.2 gm
Sodium Chloride	-	5.0 gm
Ammonium dihydrogen phosphate	-	1.0 gm
Dipotassium hydrogen phosphate	-	1.0 gm
Sodium citrate	-	2.0 gm
Bromothymol blue	-	0.08 gm
Agar	-	20.0 gm
Distilled water	-	1000 ml
pH	-	6.6

Fermentation of carbohydrate

Trypticase / Peptone	-	10.0 gm
Carbohydrate	-	5.0 gm
Sodium chloride	-	5.0 gm
Phenol red	-	0.018 gm
Distilled water	-	1000 ml
pH	-	7.3

A specific carbohydrate was added.

Catalase

Hydrogen peroxide solution

Hydrogen peroxide	-	3 ml

Made upto 100 ml with distilled water.

Oxidase

N, N, N, N Tetra methyl P-phenyl

diamine dichloride	-	1.0 gm

Distilled water	-	100 ml

Tetramethyl-para-phenylenediamine dichloride

P-phenylenediamine dichloride	-	5.0 gm
Distilled water	-	50 ml

Urea agar

Peptone	-	1.00 gm
Sodium chloride	-	5.00 gm
Potassium monohydrogen dihydrogen phosphate	-	2.00 gm
Glucose	-	1.00 gm
Phenol red (0.02% solution)	-	6.00 ml
Urea (20% aqueous solution)	-	100 ml
Distilled water	-	1000 ml
pH	-	6.8

Phenol red

Phenol red	-	0.2 gm
Ethyl alcohol (95%)	-	500 ml
Distilled water	-	500 ml

LIST OF ABBREVIATIONS

kg	-	kilogram
ha	-	hectare
Rs.	-	Rupees
°C	-	Degree celcius
ppt	-	parts per thousand
WSSV	-	white spot syndrome virus
Fig.	-	Figure
cm	-	centimeter
sq.m.	-	square meter
sp.	-	species
g	-	gram
conc.	-	concentrated
N/L	-	Normality per litre
ml	-	milli litre
mts	-	minutes
vol.	-	volume
lit	-	litre
mg	-	milli gram
nm	-	nano meter
OD	-	Optical Density
%	-	Percentage
ver	-	Version
PDA	-	Potato Dextrose Agar medium
hrs	-	hours
MR	-	Methyl red
VP	-	Voges Proskauer
TSI	-	Triple Sugar Iron
mm	-	millimeter
µl	-	micro litre
µm	-	micro mole
µ	-	micron

Chapter 1

INTRODUCTION

Chapter 2

REVIEW OF LITERATURE

Chapter 3

MATERIALS AND METHODS

Chapter 4

RESULTS

Chapter 5

DISCUSSION

Chapter 6

SUMMARY

Chapter 7

REFERENCES

Appendix

LIST OF PAPER PUBLICATIONS

Appendix

LIST OF PAPER PRESENTATIONS

Appendix

LIST OF MEDIA COMPOSITION & INDICATORS