

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



**POST-GRADUATE AND RESEARCH DEPARTMENT OF ZOOLOGY  
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**JUNE 2011**



**DEDICATED TO  
MY FAMILY MEMBERS**

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## **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 award 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.

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## 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 naphthol 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

# INTRODUCTION

## REVIEW OF LITERATURE

## MATERIALS AND METHODS



## RESULTS

## DISCUSSION



## SUMMARY

## REFERENCES

## Appendix

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## LIST OF PAPER PUBLICATIONS

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### LIST OF MEDIA COMPOSITION & INDICATORS