



UNIVERSITI PUTRA MALAYSIA

**DETECTION AND PHYLOGENETIC PROFILING OF THE RNA2
FRAGMENTS OF NODAVIRUS ASSOCIATED WITH WHITE TAIL
DISEASE IN MALAYSIAN *Macrobrachium rosenbergii* De Man BY
RT-PCR**

TAYEBEH AZAM SAEDI

FBSB 2011 32

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By

TAYEBEH AZAM SAEDI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of Requirements for the Degree of Master of Science**

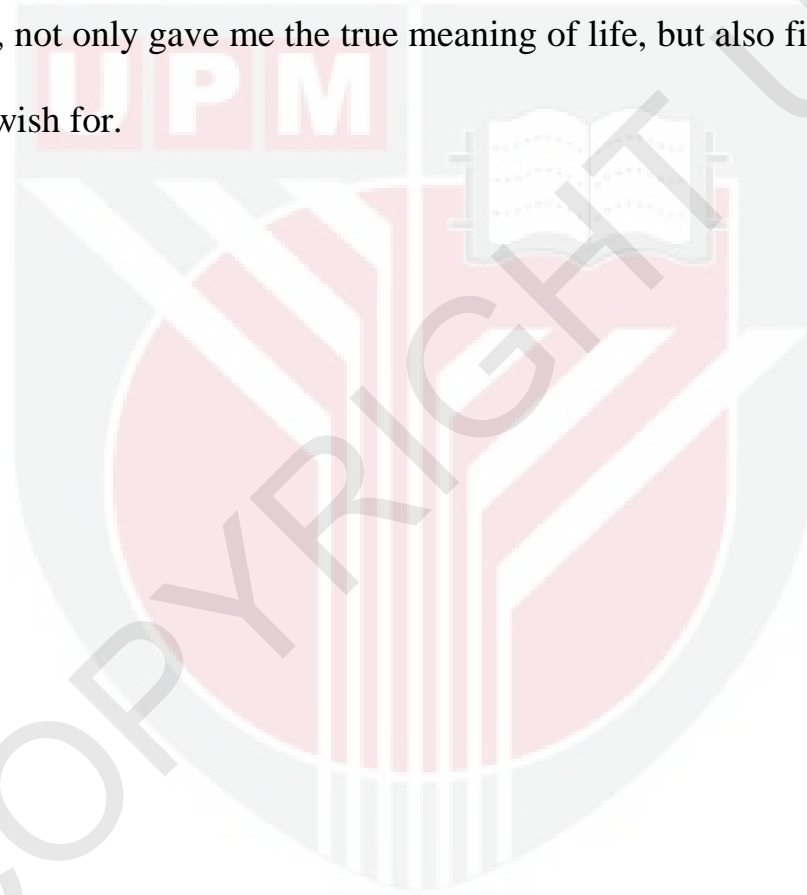
April 2011

DEDICATION

Allah you are my supreme love

Thank you for everything

This thesis is dedicated to my beloved parents who are the most special persons in my life. They, not only gave me the true meaning of life, but also fill it with all the love one can wish for.



Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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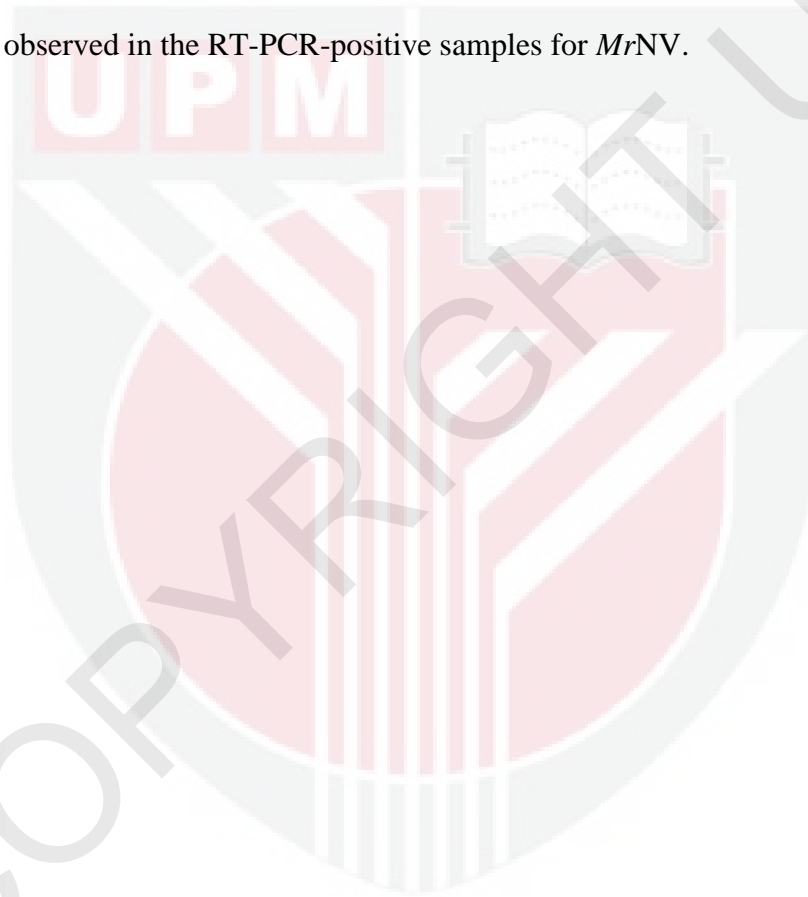
April 2011

Chair: Professor Tan Soon Guan, PhD

Faculty: Biotechnology and Biomolecular Sciences

The detection of nodavirus using RT-PCR and histopathological assay was carried out in Malaysian *Macrobrachium rosenbergii*. Nodaviruses, which were originally isolated from insects, are small non enveloped riboviruses with two single-stranded RNA genomes, RNA1 and RNA2. The 3.1 kb RNA1 gene encodes the RNA-dependent RNA polymerase and the 1.4 kb RNA2 gene encodes the coat protein. The giant fresh water prawn, *M. rosenbergii*, locally known in Malaysia as 'Udang Galah' is widely distributed in most of the tropical and subtropical regions worldwide. The popularity of this prawn has grown rapidly and its demand as food is getting progressively greater. However, wild stocks of *M. rosenbergii* have seen declines in recent years as they are threatened by over fishing and diseases. Serious outbreaks of diseases in udang galah farms in Malaysia have resulted in high mortality rates. One of the new viral diseases in *M. rosenbergii* is white tail disease (WTD). Two viruses, *M. rosenbergii* nodavirus (*MrNV*) and extra small virus (XSV) have been found to be responsible for this disease. The RT-

PCR method was used to detect the 650 bp and the 1114 bp fragments of the RNA2 of *MrNV* in infected samples. However, the presence of the XSV RNA was detected in two samples of the *MrNV*- positive samples. Partial nucleotide sequence analysis of the Malaysian isolates showed 98% nucleotide identity with the reported sequence of *MrNV* isolate from China (AY231437.2). The pathology of the disease was also investigated where progressive myofiber degeneration of the tail muscles, liquifactive myopathy, muscle fiber, fragmentation, granulation, and hemocyte infiltration were observed in the RT-PCR-positive samples for *MrNV*.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PENGESANAN DAN PROFIL FILOGENETIK FRAGMENT RNA2 VIRUS NODA BAGI PENYAKIT EKOR PUTIH *Macrobrachium rosenbergii* De Man DI MALAYSIA OLEH RT-PCR

Oleh

TAYEBEH AZAM SAEDI

April 2011

Pengerusi: Profesor Tan Soon Guan, PhD

Fakulti: Bioteknologi Dan Sains Biomolekul

Pengesanan nodavirus melalui kaedah RT-PCR dan ujian histopatologi telah dilakukan terhadap Malaysian *Macrobrachium rosenbergii*. Nodavirus yang pada asalnya diasingkan daripada serangga adalah ribovirus kecil yang tidak diliputi dan terdiri daripada dua rantai tunggal genom RNA iaitu RNA1 dan RNA2. Gen RNA1 yang bersaiz 3.1kb mengekod enzim RNA-dependent RNA polymerase manakala gen RNA2 yang bersaiz 1.4 kb pula mengekod protin kot. *M. rosenbergii* adalah sejenis udang gergasi air tawar yang juga dikenali dengan nama tempatan sebagai udang galah banyak terdapat di kebanyakan kawasan tropika dan subtropika di seluruh dunia. Kepopularan udang ini sebagai sumber makanan menjadikan permintaan terhadap udang ini semakin tinggi. Walau bagaimanapun, sumber udang *M. rosenbergii* ini semakin menurun dalam beberapa tahun terakhir ini kerana ancaman daripada penangkapan ikan yang berlebihan dan penyakit. Wabak penyakit yang serius dalam penternakan udang galah di Malaysia

mengakibatkan kadar kematian yang tinggi. Salah satu penyakit virus baru yang terdapat pada *M. rosenbergii* adalah penyakit Ekor Putih (White Tail Disease, WTD). Dua jenis virus iaitu *M. rosenbergii nodavirus* (*MrNV*) dan extra small virus (XSV) telah dikenalpasti sebagai penyebab penyakit tersebut. Kaedah RT-PCR digunakan untuk mengenalpasti kewujudan fragmen 650bp dan 1114bp pada RNA2 virus *MrNV* dalam sampel yang telah dijangkiti. Walau bagaimanapun, virus XSV RNA turut dapat dikesan pada dikesan pada dua sampel *MrNV*-sampel positif. Analisis turutan nukleotida separa sampel Malaysia menunjukkan 98% kesamaan identiti nukleotida dengan sampel *MrNV* China (AY231437.2). Patologi penyakit ini juga telah dikaji dimana degenerasi myofiber otot ekor, myopati liquifaktif, fiber otot, fragmentasi, granulasi, dan infiltrasi hemosit telah dikenal pasti melalui kaedah RT-PCR dalam *MrNV*-sampel positif.

ACKNOWLEDGEMENTS

First and foremost, I thank Allah the Most Gracious and Most Merciful, for everything.

I would like to thank Universiti Putra Malaysia (UPM) and the Ministry of Science, Technology and Innovation, Malaysia (MOSTI).

I would like to express my appreciation and gratitude to my Supervisory Committee, my supportive chairman Professor Dr. Tan Soon Guan and co-supervisors Professor. Datin Paduka Dr. Khatijah Bt Mohd Yusoff, Professor Dr. Tan Wen Siang, Associate Professor. Dr. Hassan bin Hj Mohd Daud and Dr. Subha Bhasu for guidance, suggestion and encouragement throughout this project. All their invaluable help is greatly appreciated.

My sincere thanks also goes to my labmates Dr. Hassan Moeini and Dr. Faezeh Yazdani for the valuable discussion, suggestions and help in this project. Also thanks to one of my dear lecturer, Dr Kua Beng Chu, from the Institute of Fisheries Research, Penang

I am sincerely grateful to my beloved parents for their love, endless support, encouragement, and reassurance during my study especially my dear brother, Ali mohammad, for his understanding. I love you all.

I certify that an Examination Committee has met on 22 April to conduct the final examination of Tayebeh Azam Saedi on her thesis entitled " Detection and phylogenetic profiling of the RNA2 fragments of nodavirus associated with white tail disease in Malaysian *macrobrachium*

rosenbergii De Man by RT-PCR" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



TAYEBEH AZAM SAEDI

Date: 18 April 2011



TABLE OF CONTENTS

	Page
<u>DEDICATION</u>	<u>ii</u>
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENT	vii
<u>DECLARATION</u>	<u>x</u>
<u>LIST OF TABLES</u>	<u>xii</u>
<u>LIST OF FIGURES</u>	<u>xiv</u>
<u>LIST OF ABBREVIATIONS</u>	xvi

CHAPTERS

<u>1 INTRODUCTION</u>	ERROR! BOOKMARK NOT DEFINED.
<u>1.1 Introduction</u>	Error! Bookmark not defined.
<u>1.2 Problem Statements, Objectives and Hypothesis</u>	Error! Bookmark not defined.
<u>2 LITERATURE REVIEW</u>	ERROR! BOOKMARK NOT DEFINED.
<u>2.1 <i>Macrobrachium rosenbergii</i></u>	Error! Bookmark not defined.
<u>2.1.1 Taxonomy</u>	Error!
Bookmark not defined.	
<u>2.1.2 Morphology of <i>M. rosenbergii</i></u>	Error! Bookmark not defined.
<u>2.1.3 Biological Characters of <i>M. rosenbergii</i></u>	Error! Bookmark not defined.
<u>2.2 White Tail Disease</u>	Error! Bookmark not defined.
<u>2.2.1 Viral Etiology of white Tail Disease</u>	Error! Bookmark not defined.
<u>2.2.2 Control and Prevention</u>	Error! Bookmark not defined.
<u>2.2.3 Diagnostic Tools of WTD</u>	Error! Bookmark not defined.
<u>3 METHODOLOGY</u>	ERROR! BOOKMARK NOT DEFINED.
<u>3.1 Samples Collection</u>	Error! Bookmark not defined.
<u>3.2 Molecular Methods</u>	Error! Bookmark not defined.
<u>3.2.1 RNA extraction</u>	Error! Bookmark not defined.
<u>3.2.2 RNA Concentration and Purity</u>	Error! Bookmark not defined.
<u>3.2.3 Deoxyribonuclease (DNase) Treatment</u>	Error! Bookmark not defined.
<u>3.2.4 Primer Design</u>	Error!
Bookmark not defined.	
<u>3.2.5 Reverse Transcription-Polymerase Chain Reaction</u>	Error! Bookmark not defined.
defined.	
<u>3.2.6 Agarose Gel Electrophoresis</u>	Error! Bookmark not defined.

	3.2.7 TA Cloning of the Purified RT-PCR Products	Error! Bookmark not defined.
	3.3 Phylogenetic Analysis	Error! Bookmark not defined.
	3.4 Statistical Analysis	Error! Bookmark not defined.
	3.4.1 Analysis of Molecular Variance (AMOVA)	Error! Bookmark not defined.
	3.5 Histopathological Study	Error! Bookmark not defined.
	3.5.1 Sample Preservation	Error! Bookmark not defined.
	3.5.2 Dehydration and clearing process	Error! Bookmark not defined.
	3.5.3 Embedding Process	Error! Bookmark not defined.
	3.5.4 Sectioning by Microtome	Error! Bookmark not defined.
	3.5.5 Hematoxylin and Eosin Staining	Error! Bookmark not defined.
4	<u>RESULTS</u>	ERROR! BOOKMARK NOT DEFINED.
	4.1 RNA Isolation	Error! Bookmark not defined.
	4.2 Amplification of <i>M_rNV</i> RNA 2 and XSV RNA by RT-PCR	Error! Bookmark not defined.
	4.3 TA-cloning of the Amplified Fragments	Error! Bookmark not defined.
	4.4 PCR analysis of the recombinant plasmids	Error! Bookmark not defined.
	4.5 Sequence Analysis and Variations	Error! Bookmark not defined.
	4.6 Phylogenetic Relationships Between the Samples	Error! Bookmark not defined.
	4.7 Haplotype Distribution and Population Structure	Error! Bookmark not defined.
	4.8 Analysis of Molecular Variance	Error! Bookmark not defined.
	4.9 Pathological Changes of WTD	Error! Bookmark not defined.
	4.9.1 Histopathological Examination	Error! Bookmark not defined.
5	<u>DISCUSSION</u>	ERROR! BOOKMARK NOT DEFINED.
6	<u>CONCLUSION AND FUTURE RECOMMENDATION</u>	ERROR! BOOKMARK NOT DEFINED.
	REFERENCES	ERROR!
	BOOKMARK NOT DEFINED.	
	<u>APPENDICS</u>	ERROR!
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	<u>BIODATA OF STUDENT</u>	ERROR!
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