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

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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

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

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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* (*Mr*NV) 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 *Mr*NV in infected samples. However, the presence of the XSV RNA was detected in two samples of the *Mr*NV- positive samples. Partial nucleotide sequence analysis of the Malaysian isolates showed 98% nucleotide identity with the reported sequence of *Mr*NV 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 *Mr*NV.

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

PENGESANAN DAN PROFIL FILOGENETIK FRAGMEN 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 histopatalogi 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 kebayakan 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* (*Mr*NV) 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 *Mr*NV dalam sampel yang telah dijangkiti. Walau bagaimanapun, virus XSV RNA turut dapat dikesan pada dikesan pada dua sampel *Mr*NV-sampel positif. Analisis turutan nukleotida separa sampel Malaysia menunjukkan 98% kesamaan identiti nukleotida dengan sampel *Mr*NV China(AY231437.2). Patologi penyakit ini juga telah dikaji dimana degenerasi myofiber otot ekor, myopati liquifaktif, fiber otot, fragmentasi, granulasi, dan infilterasi hemosit telah dikenal pasti melalui kaedah RT-PCR dalam *Mr*NV-sampel positif.

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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.

Members of the Thesis Examination Committee were as follows:

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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.

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TAYEBEH AZAM SAEDI

Date: 18 April 2011

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