UNIVERSITI PUTRA MALAYSIA

SEQUENCE ANALYSIS OF RNA-DEPENDENT RNA POLYMERASE AND DEVELOPMENT OF A TAQMAN REAL-TIME RT-PCR ASSAY FOR DETECTION OF MACROBRACHIUM ROSENBERGII NODAVIRUS IN GIANT FRESHWATER PRAWNS

ZEINABSADAT MOHAMMADI

FBSB 2012 11

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By

ZEINABSADAT MOHAMMADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the requirement for the Degree of Master of Science

August 2012

Specially Dedicated:

I dedicated this thesis in honor of my best friend forever, my lovely husband, Alireza, who has always stood with me and patiently dealt with the difficulty of living far from me to help our dreams all come true.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

SEQUENCE ANALYSIS OF RNA DEPENDENT RNA POLYMERASE AND DEVELOPMENT OF A TAQMAN REAL-TIME RT-PCR ASSAY FOR DETECTION OF *MACROBRACHIUM ROSENBERGII* NODAVIRUS IN GIANT FRESHWATER PRAWNS

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Chair: Professor Tan Soon Guan, PhD

Faculty: Biotechnology and Biomolecular Sciences

The study presented in this thesis was mainly aimed to establish a novel Taq-Man based real-time RT-PCR assay for the detection of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) which is known to be implicated in the development of a fatal disease, called white tail disease (WTD) in giant freshwater prawns. A recombinant plasmid (R2-A) carrying a 650 bp fragment of the RNA2 of *Mr*NV was constructed and used in the development of the assay. The newly established TaqMan real time RT-PCR method was 10 times more sensitive than the conventional RT-PCR assay and the results obtained from the intra-assay and the inter-assay showed the reproducibility of the assay. The PCR efficiency was calculated to be 99.6%. The developed primers and the TaqMan probe were shown to be specific to *Mr*NV as

they gave negative results to other viruses: extra small virus, (XSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV) which may also cause diseases in the giant freshwater prawn. Subsequent to the promising observations and results obtained from the newly established assay based on the plasmid R2-A, positive results were also obtained when the same assay was applied on field samples of WTD infected prawns. Determination of the whole sequence of the RNA dependent RNA polymerase of RNA1 in the Malaysian nodavirus strain was the other objective of this study. To this end, the presence of the virus in some tissues such as tail, body and gill was detected by RT-PCR using specific primers. The sequences of the inserts were determined and compared with those from the GenBank. The product size of the whole sequence of RNA1 was about 3199 bp and the Malaysian strain was shown to have the highest similarity (96%) to that of AY231436.2.

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

ANALISIS URUTAN RNA DEPENDEN RNA POLIMERASE DAN PEMBANGUNAN A TAQMAN MASA NYATA ASAI RT-PCR UNTUK PENGESANAN *MACROBRACHIUM ROSENBERGII* NODAVIRUS DALAM UDANG GALAH

Oleh

ZEINABSADAT MOHAMMADI

Ogos 2012

Pengerusi: **Profesor Tan Soon Guan, PhD**

Fakulti: **Fakulti Bioteknologi & Sains Biomolekul**

Kajian yang dibentangkan di dalam tesis ini adalah bertujuan terutamanya untuk mewujudkan sebuah novel Taq-Man berasaskan asai RT-PCR masa nyata untuk mengesan *Macrobrachium rosenbergii* nodavirus (*Mr*NV) yang diketahui terbabit dalam perkembangan penyakit yang boleh membunuh, dipanggil penyakit ekor putih (WTD) pada udang galah air tawar. Plasmid rekombinan (R2-A) membawa 650 bp serpihan RNA2 dari *Mr*NV telah dibina dan digunakan dalam perkembangan asai. Kaedah RT-PCR masa nyata TaqMan yang baru diwujudkan adalah 10 kali lebih sensitif daripada asai RT-PCR konvensional dan keputusan yang diperolehi dari intra-asai dan antara-asai menunjukkan kebolehulangan asai. Kecekapan PCR yang telah dikira adalah 99.6%. Primer yang maju dan prob TaqMan didapati khusus kepada

*Mr*NV kerana mereka memberi keputusan negatif terhadap virus-virus yang lain: Virus lebih kecil, (XSV), hipoderma berjangkit dan virus nekrosis hematopoetik (IHHNV) dan virus sindrom Taura (TSV) juga boleh menyebabkan penyakit pada udang galah air tawar. Selepas beberapa pemerhatian yang menggalakkan dan keputusan yang diperolehi dari asai yang baru diwujudkan berdasarkan kepada plasmid R2-A, keputusan yang positif telah juga diperolehi apabila asai yang sama telah digunakan di dalam sampel lapangan udang yang dijangkiti WTD. Penentuan kepada seluruh urutan RNA bergantung kepada polimerase RNA ke atas RNA1 di dalam keterikan nodavirus Malaysia adalah di dalam objektif lain kajian ini. Sehingga kini, kehadiran virus di beberapa tisu seperti ekor, badan dan insang telah dikesan oleh RT-PCR menggunakan primer khusus. Urutan pada kemasukan telah ditentukan dan dibandingkan dengan mereka dari GenBank. Saiz produk pada keseluruhan urutan RNA1 adalah kira-kira 3199 bp dan terikan Malaysia telah menunjukkan persamaan yang tertinggi (96%) daripada AY231436.2.

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I certify that an Examination Committee met on [7 August 2012] to conduct the final examination of Zeinabsadat Mohammadi on her Master of Science thesis entitled "Sequence analysis of RNA dependent RNA polymerase and development of a TaqMan real time RT-PCR assay for detection of *Macrobrachium rosenbergii* Nodavirus in giant freshwater prawns". The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the 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 other institutions.

ZEINABSADAT MOHAMMADI

Date:

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