UNIVERSITI PUTRA MALAYSIA

Macrobrachium rosenbergii De Man NODAVIRUS
RECOMBINANT CAPSID PROTEIN PRODUCTION
AND ITS INTERACTIONS WITH RNA

GOH ZEE HONG

FBSB 2015 5
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

*Macrobrachium rosenbergii* De Man NODAVIRUS RECOMBINANT CAPSID PROTEIN PRODUCTION AND ITS INTERACTIONS WITH RNA

By

GOH ZEE HONG

May 2015

Chairman : Professor Tan Wen Siang, PhD
Faculty : Biotechnology and Biomolecular Sciences

*Macrobrachium rosenbergii* nodavirus (*MrNv*) is a causative agent of white tail disease (WTD) causing nearly 100% mortality in post-larvae of giant freshwater prawns. In 2012 the major farming areas in Malaysia were found to be infected by *MrNv* but till now information on the Malaysian isolate *MrNv* is still unavailable in the NCBI database and studies of this newly emerged member of the *Nodaviridae* family were not in depth. Studies on the other members of the *Nodaviridae* family revealed that the RNA binding site is important for virus assembly and removal of this region inhibits the virus maturation. Therefore, researches were conducted to identify the Malaysian *MrNv* capsid sequence, structural morphologies, nucleic acid contents and the RNA binding site of this virus. In this study, *MrNv* was isolated from infected prawns obtained from a prawn farm in Negeri Sembilan, Malaysia. Prawn muscle tissues were screened with PCR to detect *MrNv*. The nucleotide sequence of the *MrNv* capsid gene isolated from a Malaysian isolate was sequenced and later compared with those available in the NCBI database. Phylogenetic analysis of *MrNv* capsid protein revealed that the Malaysian isolate was closely related to the Chinese isolates. The coding region of *MrNv* capsid protein was cloned into pTrcHis2-TOPO expression vector and introduced into *Escherichia coli* TOP10 cells. The recombinant capsid protein of *MrNv* containing a His-tag was purified by using immobilized metal affinity chromatography (IMAC). The purified capsid protein was analysed using transmission electron microscopy (TEM), dynamic light scattering (DLS) and sucrose density gradient ultracentrifugation, which revealed the formation of virus-like particles (VLPs) of about 30±3 nm in diameter. RNA molecules were found to be encapsidated inside the cavity of *MrNv* VLPs which suggested that VLPs resembled the native virus. Amino acid sequence analysis of the *MrNv* capsid protein revealed that 8 out of 10 amino acids located at residues 20th to 29th are positively-charged suggesting RNA binding region is located in this region. Deletion mutagenesis and amino acid substitutions of the positively-charged amino acids located at the N-terminal end of the *MrNv* capsid protein were performed to determine the RNA binding region. A total of seven mutants were created with different deletion and point mutations starting from residues 1 to 29 of the N-terminal end of the *MrNv* capsid protein. All the mutants were shown to assemble into VLPs ranging from 18 to 34 nm in diameter. Mutants
with the positively-charged amino acids deleted, namely 29ΔMrNvc and 20-29ΔMrNvc did not contain RNA molecules in their VLPs. A point mutation mutant, namely K20R21R22K23R24A, showed a significantly lower amount of RNA molecules compared with that of mutant R26R27R29A, suggesting that the five positively-charged amino acids residues at positions 20 to 24 play an important role in RNA binding. This study showed the positively-charged amino acids at positions 20 to 29 of the capsid protein are the RNA binding site of MrNv. In conclusion, the nucleotide sequence of Malaysian MrNv capsid gene was determined and showed high similarity with the Chinese isolates. The recombinant MrNv capsid protein produced in bacteria was able to assemble into VLPs which resembled the native virus. The RNA binding site of the capsid protein was identified and located at position 20th to 29th. Removal of this region did not affect virus assembly suggesting that the presence of the assembly domain. This information is useful for the development of a vaccine against MrNv and its structural analysis.
PENGHASILAN *Macrobrachium rosenbergii* De Man NODAVIRUS PROTEIN
KAPSID REKOMBINAN DAN INTERAKSINYA DENGAN RNA

Oleh

GOH ZEE HONG

Mei 2015

Pengerusi : Profesor Tan Wen Siang, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

*Macrobrachium rosenbergii* nodavirus (*Mr*Nv) adalah agen penyebab penyakit ekor putih (WTD) dan ia menyebabkan hampir 100% kematian di pos-larva udang galah. Pada tahun 2012, udang galah di kawasan perternakan utama di Malaysia telah dijangkiti *Mr*Nv tetapi sehingga kini tiada maklumat yang boleh didapati mengenai *Mr*Nv Malaysia di pangkalan data NCBI dan kajian terhadap virus yang baru muncul ini masih tidak mendalam. Kajian terhadap ahli keluarga *Nodaviridae* yang lain mendedahkan kepentingan tapak pengikatan RNA dalam perkumpulan virus dan penyingkiran tapak ini akan menghalang penyatuan virus. Oleh itu, penyelidikan telah dilakukan untuk mengenalpasti urutan nukleotida kapsid *Mr*Nv Malaysia, ciri-ciri dan tapak pengikatan RNA virus ini. Dalam kajian ini, *Mr*Nv telah diasingkan daripada udang terjangkit virus yang diperolehi daripada ladang udang di Negeri Sembilan, Malaysia. Tisu otot udang telah dianalisis dengan PCR untuk mengesan *Mr*Nv. Urutan nukleotida *Mr*Nv capsid gen diasingkan daripada udang galah Malaysia telah dibandingkan dengan yang terdapat di pangkalan data NCBI. Analisa filogenetik daripada *Mr*Nv capsid protein mendedahkan bahawa *Mr*Nv Malaysia berkait rapat dengan *Mr*Nv China. DNA yang mengekodkan *Mr*Nv capsid protein telah diklon ke vektor pTrcHis2-TOPO dan diperkenalkan ke dalam sel-sel *Escherichia coli* TOP10. Protein kapsid rekombinan daripada *Mr*Nv mengandungi His-tag telah ditulenkan dengan menggunakan kromatografi logam kekal afiniti (IMAC). Protein kapsid ditulenkan dan dianalisa dengan menggunakan mikroskop elektron transmisi (TEM), penyerakan cahaya dinamik (DLS) dan sukarosa kecerunan ketumpatan ultracentrifugan. Diameter partikel menyerupai virus (VLPs) adalah kira-kira 30 ± 3 nm. Molekul RNA telah dikesan di dalam ruang dalam VLPs mencadangkan VLPs menyerupai virus asli. Analisis jujukan asid amino protein *Mr*Nv capsid mendedahkan bahawa 8 daripada 10 asid amino terletak di kedudukan ke-20 hingga 29 adalah bercas positif mencadangkan bahawa RNA mengikat di bahagian ini. Mutagenesis penghapusan bes dan penggantian asid amino pada asid amino yang bercas positif yang terletak di hujung-N protein *Mr*Nv kapsid telah dijalankan untuk menentukan kawasan ini mengikat RNA. Sebanyak 7 mutan telah dihasilkan dengan penghapusan dan titik mutasi yang berbeza bermula dari kedudukan 1 hingga 29 pada hujung-N protein *Mr*Nv kapsid. Semua mutan telah ditunjuk membentuk VLP antara...
ACKNOWLEDGEMENTS

I would like to thank many people who contributed to the work presented in this thesis. I would not have successfully completed my study without the guidance of my supervisory committee, help from my fellow colleagues and support from my family and girlfriend.

I would like to express my deepest gratitude to my main supervisor, Prof. Dr. Tan Wen Siang for his guidance and rational thinking which make this study more complete and presentable. His patience and strict guidance ensured my English writing and work was done in a correct way. I am so thankful because he gave me a lot of freedom and this trained me to be more independent and to think logically. I learned many skills under his guidance. I also appreciated the work done by my supervisory committee members, Prof. Dr. Tan Soon Guan and Assoc. Prof. Dr. Subha Bhassu who always gave me moral support whenever I failed in my experiments or the publishers rejected my research papers for publication. Thanks for their knowledge, encouragement and enthusiasm, without them I would not have completed my PhD study.

I would like to thanks my seniors, Dr. Lee Khai Wooi, Dr. Yap Wei Boon, Tang Kah Fai and Yong Chean Yeah for teaching me and giving me guidance when I first entered the lab. Without their help, I would not have learnt my skills in protein work, molecular work, protein purification and also dynamic light scattering.

Thirdly, I would like to thank my fellow colleagues in Lab 134. We always have a good time in the lab sharing our thoughts to help one another solving problems and troubleshooting experimental failures. Frankly speaking, we had limited equipment in the lab but we managed to arrange the usage time properly without disturbing one another timetable. I felt happy with them because whenever you are hungry or tired after working long hours, they were there supporting me by buying me some food and snacks so that I can continue my journey.

I would like to thank many of my UPM friends, Sien Yei, Sze Yen, Woo Kiat, Yee Wei and others for having shared joyful moments and experiences so that I would not feel bored for the study period. Big thanks go to my good friends, Dr. Gouk Shiou Wah and others by giving me moral support whenever I have problems with my study. We gathered whenever we have spare time and sent our regards to one another. I really appreciate their generosity and understanding.

Lastly, I would like to thank my relatives for giving me support in this long journey. No other word can be used to describe my gratitude to both of my dearest parents who have been patiently waiting for me to complete my study. Sorry for the long waiting period. Dad and mom, I am ready to work and support the family and both of you can start your retirement life. Last but not least, I would like to thank Li Teng. Your presence makes my life happy. We encouraged each other and discussed our study rationally to ensure we do our things correctly. I felt lovely before but now I have found a clear path for the future with your presence.
I certify that a Thesis Examination Committee has met on 6 May 2015 to conduct the final examination of Goh Zee Hong on his thesis entitled "Macrobrachium rosenbergii De Man Nodavirus Recombinant Capsid Protein Production and Its Interactions with RNA" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Noorjahan Banu binti Mohammed Alitheen, PhD**  
Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Seow Heng Fong, PhD**  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Siti Suri binti Arshad, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Shin-Ichiro Suye, PhD**  
Professor  
University of Fukui  
Japan  
(External Examiner)

---

ZULKARNAIN ZAINAL, PhD  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 17 June 2015
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Tan Wen Siang, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Tan Soon Guan, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Subha Bhassu, PhD**
Associate Professor
Faculty of Sciences
University Malaya
(Member)

---

**BUJANG BIN KIM HUAT, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
Declaration by Graduate Student

I hereby confirm that:
• this thesis is my original work;
• quotations, illustrations and citations have been duly referenced;
• this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
• intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
• written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
• there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________ Date: __________________

Name and Matric No.: GOH ZEE HONG GS26076
Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: Prof. Dr. Tan Wen Siang
Name of Chairman of Supervisory Committee: Prof. Dr. Tan Soon Guan

Signature: Assoc. Prof. Dr. Subha Bhassu
Name of Chairman of Supervisory Committee:
TABLE OF CONTENTS

ABSTRACT i
ABSTRAK iii
ACKNOWLEDGEMENTS v
APPROVAL vi
DECLARATION viii
LIST OF TABLES xiv
LIST OF FIGURES xv
LIST OF APPENDICES xvii
LIST OF ABBREVIATIONS xviii

CHAPTER
1 INTRODUCTION 1

2 LITERATURE REVIEW
2.1 Giant Freshwater Prawn (*Macrobrachium rosenbergii*) 5
2.1.1 Growing of Giant Freshwater Prawn 6
2.1.2 Geographical Distribution and Prawn Industry of Giant Freshwater Prawn 6
2.1.2.1 Geographical Distribution of Giant Freshwater Prawn in Malaysia 7
2.1.3 Immune Response in Giant Freshwater Prawn 8
2.1.4 History of White Tail Disease (WTD) Infecting Giant Freshwater Prawn 9

2.2 *Nodaviridae* 10
2.2.1 Members of *Nodaviridae* 11
2.2.1.1 *Macrobrachium rosenbergii* Nodavirus (*MrNv*) 12
2.2.1.2 *MrNv* Genome and Protein 12
2.2.1.3 *MrNv* Life Cycle 13
2.2.1.4 Detection of *MrNv* 14

2.3 Sequence Alignment and Phylogenetic Study of Virus 15
2.3.1 Sequence Alignment and Phylogenetic Study of *Nodaviridae* 16

2.4 Virus-Like Particles, a Non-Infectious Recombinant Protein Resemble Virus with Multiple Functions 18
2.4.1 Invention of Recombinant DNA Technology and the Discovery of VLPs 19
2.4.2 The Uses of VLPs 19

2.5 Characterisation of VLPs 27
2.5.1 Sucrose Density Gradient Ultracentrifugation 29
2.5.2 Dynamic Light Scattering (DLS) 31
2.5.3 Transmission Electron Microscopy (TEM) 33
2.5.4 Mutagenesis 37

2.6 Virus Assembly 38
2.6.1 Protein-Protein Interaction in Virus Assembly 38
2.6.2 RNA-Protein Interaction in Virus Assembly 39
2.6.3 RNA Binding Region 40
2.7 Concluding Remarks 42

3 GENERAL MATERIALS AND METHODS
3.1 RNA Extraction Using Phenol Chloroform Method 44
3.2 Quantification of Nucleic Acid Concentration and Purity 44
  3.2.1 Quantification Using Hitachi U2900 Spectrophotometer 44
  3.2.2 Quantification Using Implen Nanophotometer® P300 45
3.3 Reverse Transcription Polymerase Chain Reaction (RT-PCR) 45
3.4 Extraction and Purification of DNA from Agarose Gels 45
3.5 Competent Cell Preparation 46
3.6 DNA Cloning 46
3.7 PCR Screening of Bacteria Cells Colonies 47
3.8 Extraction of Plasmid Using Miniprep Alkaline Lysis Solution Method 47
3.9 Restriction Endonuclease Digestion 48
3.10 The Bradford Assay 48
3.11 Sucrose Density Gradient Ultracentrifugation Analysis 49
3.12 Dynamic Light Scattering (DLS) Analysis 49
3.13 Transmission Electron Microscopic Analysis 49
3.14 Sodium Dodecyl Sulfate Polyacrylamic Gel Electrophoresis (SDS-PAGE) 49
3.15 Western Blotting 50

4 PHYLOGENETICS OF *Macrobrachium rosenbergii* NODAVIRUS ISOLATED FROM MALAYSIA AND WORLDWIDE
4.1 Introduction 52
4.2 Materials and Methods
  4.2.1 Sample Collection and Processing 53
  4.2.2 Total RNA Extraction 53
  4.2.3 Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Cloning of *capsid* gene 53
  4.2.4 Plasmid Extraction Using Alkaline Lysis Solution 54
  4.2.5 DNA Sequencing and Sequence Analysis 54
4.3 Results
  4.3.1 RNA Extraction 54
  4.3.2 RT-PCR of *MrNv* *capsid* gene 55
  4.3.3 Nucleotide Sequence Analysis of the *MrNv* *capsid* gene of Malaysian Isolate 56
  4.3.4 Sequence Alignment of *MrNv* Capsid Protein 57
  4.3.5 Phylogenetic and Distance Matrix Studies of *MrNv* Isolated from Malaysia 60
4.4 Discussion 63
4.5 Conclusion

5 VIRUS-LIKE PARTICLES OF Macrobrachium rosenbergii NODAVIRUS PRODUCED IN BACTERIA
5.1 Introduction
5.2 Materials and Methods
  5.2.1 Extraction of Total RNA
  5.2.2 RT-PCR and Cloning
  5.2.3 Plasmid Extraction Using Alkaline Lysis Method
  5.2.4 Restriction Endonuclease Digestion of Recombinant Plasmids
  5.2.5 Expression of MrNv Capsid Protein
  5.2.6 Analysis of Protein Solubility and Optimization of Protein Expression
  5.2.7 Optimisation of Protein Purification Using IMAC
  5.2.8 Sucrose Density Gradient Ultracentrifugation
  5.2.9 Light Scattering Analysis of MrNv Recombinant Capsid Protein
  5.2.10 Transmission Electron Microscopy
  5.2.11 Analysis of Nucleic Acid Content in VLPs
5.3 Results
  5.3.1 Cloning and Expression of MrNv Capsid Protein
  5.3.2 Time Course, Solubility and Localisation Analyse
  5.3.3 Purification of MrNv Capsid Protein Using IMAC
  5.3.4 Purified MrNv Capsid Protein Assembled Into VLPs
  5.3.5 Nucleic Acids in VLPs
5.4 Discussion
5.5 Conclusion

6 RNA BINDING REGION OF Macrobrachium rosenbergii NODAVIRUS
6.1 Introduction
6.2 Materials and Methods
  6.2.1 Plasmid Extraction Using the Alkaline Lysis Method
  6.2.2 Construction of N-terminal Deletion, Internal Deletion and Multiple Point Mutants
  6.2.3 Protein Expression and Purification
  6.2.4 Sucrose Density Gradient Ultracentrifugation
  6.2.5 Dynamic Light Scattering Analysis of Purified Capsid Proteins
  6.2.6 Transmission Electron Microscopy (TEM)
  6.2.7 Detection of RNA Molecules that Associate with Mutated MrNv Capsid Proteins
6.2.8 Native Agarose Gel Electrophoresis (NAGE) of MrNv VLPs

6.3 Results

6.3.1 PCR Generation of Deletion and Multiple Point Mutageneses of MrNv Capsid Protein 85
6.3.2 Deletion and Multiple Point Mutageneses of MrNv Capsid Protein 88
6.3.3 Analysis of Recombinant Encoding Mutant MrNv Capsid Protein 88
6.3.4 Expression of the Deleted Mutants of MrNv Capsid Protein 91
6.3.5 MrNv Capsid Mutants Formed VLPs 92
6.3.6 Extraction of RNA from the MrNv Capsid Mutants 95

6.4 Discussion 98
6.5 Conclusion 102

7 SUMMARY, GENERAL CONCLUSION AND FUTURE STUDIES

7.1 Summary 103
7.2 General Conclusion 104
7.3 Future Studies 105
7.3.1 Improvement of MrNv Capsid Protein Yield and Large Scale Purification of the MrNv Capsid Protein 106
7.3.2 MrNv Capsid Formation Domain 106
7.3.3 Drug Delivery and Gene Therapy Using Positive-Charged Region of MrNv Capsid 107
7.3.4 Structural Study of MrNv Capsid Protein 107

REFERENCES 109
APPENDICES 134
BIODATA OF STUDENT 164
LIST OF PUBLICATIONS 165
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Summary of the uses of the VLPs and their respective field of study</td>
<td>25</td>
</tr>
<tr>
<td>2.2</td>
<td>Analysis of VLPs by using sucrose density gradient ultracentrifugation and their downstream applications</td>
<td>30</td>
</tr>
<tr>
<td>2.3</td>
<td>VLPs studies by using DLS and their downstream applications</td>
<td>33</td>
</tr>
<tr>
<td>3.1</td>
<td>Primer sequences for RT-PCR</td>
<td>45</td>
</tr>
<tr>
<td>3.2</td>
<td>Recipe for the preparation of SDS-PAG (12%)</td>
<td>50</td>
</tr>
<tr>
<td>4.1</td>
<td>The number of differences of amino acid and genetic distance between MrNv capsid, Malaysian isolate and reference isolates</td>
<td>62</td>
</tr>
<tr>
<td>5.1</td>
<td>Diameter and size variation of MrNv capsid protein measured by DLS</td>
<td>75</td>
</tr>
<tr>
<td>6.1</td>
<td>Primers used in PCR for the construction of MrNv capsid mutants</td>
<td>82</td>
</tr>
<tr>
<td>6.2</td>
<td>List of MrNv capsid mutant and their diameter measurements</td>
<td>95</td>
</tr>
<tr>
<td>6.3</td>
<td>Concentration of RNA extracted from MrNv capsid mutants</td>
<td>96</td>
</tr>
<tr>
<td>6.4</td>
<td>Position of positively-charged amino acid residues of Nodaviridae capsid proteins</td>
<td>101</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The morphology of giant freshwater prawn</td>
<td>5</td>
</tr>
<tr>
<td>2.2</td>
<td>Major player of the giant freshwater prawn industry</td>
<td>7</td>
</tr>
<tr>
<td>2.3</td>
<td>Global production of giant freshwater prawn across the years</td>
<td>7</td>
</tr>
<tr>
<td>2.4</td>
<td>Major locations of wild giant freshwater prawns in Peninsular Malaysia</td>
<td>8</td>
</tr>
<tr>
<td>2.5</td>
<td><em>Nodaviridae</em> viral and genome structure</td>
<td>12</td>
</tr>
<tr>
<td>2.6</td>
<td>Replication of an alphanodavirus genome in a host cytoplasm</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>Phylogenetic tree analysis of <em>Nodaviridae</em> family</td>
<td>18</td>
</tr>
<tr>
<td>2.8</td>
<td>Principle of sucrose density gradient ultracentrifugation</td>
<td>30</td>
</tr>
<tr>
<td>2.9</td>
<td>The principle of dynamic light scattering</td>
<td>32</td>
</tr>
<tr>
<td>2.10</td>
<td>Principle of transmission electron microscopy</td>
<td>35</td>
</tr>
<tr>
<td>2.11</td>
<td>Protein-RNA interaction involves in particles formation</td>
<td>41</td>
</tr>
<tr>
<td>4.1</td>
<td>RNA extracted from muscle cell of prawn samples suspected of white tail disease</td>
<td>55</td>
</tr>
<tr>
<td>4.2</td>
<td>RT-PCR of <em>MrNv</em> capsid gene from infected prawn</td>
<td>56</td>
</tr>
<tr>
<td>4.3</td>
<td>The full coding sequence of <em>MrNv</em> capsid protein of Malaysian isolate</td>
<td>57</td>
</tr>
<tr>
<td>4.4</td>
<td>Amino acid sequence alignment of <em>MrNv</em> isolates using MEGA 6 software</td>
<td>59</td>
</tr>
<tr>
<td>4.5</td>
<td>Phylogenetic study of <em>MrNV</em> capsid gene and protein of Malaysian isolate with other reference isolates</td>
<td>60</td>
</tr>
<tr>
<td>5.1</td>
<td>SDS-PAGE and Western blot analysis of the recombinant <em>MrNv</em> capsid protein</td>
<td>70</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.2</td>
<td>Time course study on the expression of the recombinant MrNv capsid protein by <em>E. coli</em> cells</td>
<td>71</td>
</tr>
<tr>
<td>5.3</td>
<td>Solubility analysis of MrNv capsid protein expressed in <em>E. coli</em> TOP10 strain</td>
<td>72</td>
</tr>
<tr>
<td>5.4</td>
<td>Purification of the recombinant MrNv capsid protein using IMAC</td>
<td>73</td>
</tr>
<tr>
<td>5.5</td>
<td>Sedimentation profile of MrNv capsid protein in 8-40% sucrose gradient</td>
<td>74</td>
</tr>
<tr>
<td>5.6</td>
<td>Transmission electron microscopic analysis of MrNv VLPs produced in <em>E. coli</em></td>
<td>75</td>
</tr>
<tr>
<td>5.7</td>
<td>Agarose gel electrophoresis of the nucleic acid extracted from MrNv VLPs</td>
<td>76</td>
</tr>
<tr>
<td>6.1</td>
<td>Primary structures of the deletion and multiple point mutants of MrNv capsid protein</td>
<td>80</td>
</tr>
<tr>
<td>6.2</td>
<td>Mutants MrNv capsid generated from pTrcHis2-TARNA2 using PCR</td>
<td>86</td>
</tr>
<tr>
<td>6.3</td>
<td>Mutants MrNv capsid gene inserted into pTrcHis2-TOPO vector screened with PCR and restriction endonuclease digestion</td>
<td>89</td>
</tr>
<tr>
<td>6.4</td>
<td>SDS-PAGE and Western blot analysis of the N-terminal deletion, internal deletion and multiple point mutants of MrNv capsid proteins</td>
<td>91</td>
</tr>
<tr>
<td>6.5</td>
<td>Sedimentation profiles of the mutated MrNv capsid proteins in 8-40% sucrose gradient</td>
<td>93</td>
</tr>
<tr>
<td>6.6</td>
<td>Transmission electron microscopic analysis showed the size and morphologies of the MrNv capsid mutants</td>
<td>94</td>
</tr>
<tr>
<td>6.7</td>
<td>Agarose gel electrophoresis of the RNA extracted from the mutated MrNv capsid proteins</td>
<td>97</td>
</tr>
<tr>
<td>6.8</td>
<td>Native agarose gel electrophoresis of VLPs formed by MrNv capsid mutants</td>
<td>98</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>The vector map of pGEM-T vector</td>
<td>134</td>
</tr>
<tr>
<td>B</td>
<td>The vector map of pTrcHis2-TOPO expression vector</td>
<td>136</td>
</tr>
<tr>
<td>C</td>
<td>The chromatogram of the sequencing result of MrNv capsid, Malaysian isolate</td>
<td>138</td>
</tr>
<tr>
<td>D</td>
<td>Analysis of MrNv capsid coding region isolated at different geographical areas using MEGA 6</td>
<td>150</td>
</tr>
<tr>
<td>E</td>
<td>The chromatograms of wild-type Malaysian MrNvc and mutants</td>
<td>151</td>
</tr>
<tr>
<td>F</td>
<td>Protein sequence for wild-type and mutants MrNv capsid protein and their respective isoelectric point and molecular weight</td>
<td>156</td>
</tr>
<tr>
<td>G</td>
<td>Dynamic Light Scattering results for all capsid mutants</td>
<td>159</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

Δ  deletion
6× His  six residues of histidine
a  centrifugal force
A_260  Optical Density at absorbance 260 nm
A_280  Optical Density at Absorbance 280 nm
AAV2  adeno-associated virus serotype 2
AHNV  Atlantic halibut *Hippoglossus hippoglossus* nodavirus
AK-1  Arginine kinase-1
AMV  alfalfa mosaic virus
AMV  avian myeloblastosis virus
ANTXR2  Anthrax toxin receptor 2
APC  antigen-presenting cell
APS  ammonium persulfate
ATP  adenosine triphosphate
Au  gold
BBV  Black beetle virus
BCIP  5-bromo-4-chloro-3’-indolyphosphate p-toluidine-salt
BFNNV  barfin flounder nervous necrosis virus
BMV  Brome Mosaic virus
bp  base pair
Ca^{2+}  calcium ion
CaCl_2  calcium chloride
CaCO_3  Calcium carbonate
Casp3c  Caspase 3c
CBB G-250  \textit{coomassie brilliant blue G-250}
CBB R-250  Coomassie brilliant blue R-250
CCMV  Cowpea chlorotic mottle virions
cDNA  complementary deoxyribonucleic acid
CHIKV  Chikungunya virus
CMV  cucumber mosaic virus
CoV  coronavirus
CPV  canine parvovirus
cryo-EM  cryo-electron microscopy
CTL  cytotoxic T lymphocytes
Cu  copper
D  diffusion coefficient
ddH$_2$O  double distilled water
DENV-2  Dengue virus 2
DGNNV  dragon grouper nervous necrosis virus
DIG  digoxigenin
DLS  dynamic light scattering
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
dNTPs  deoxynucleotide triphosphates
DOF  Department of Fisheries
DOX  doxorubicin
dsDNA  double-stranded DNA
dsRNA  double-stranded RNA
DTT  Dithiothreitol
ECV  endosomal carrier vesicles
EDTA  ethylenediaminetetraacetic acid
EGFP  Enhance green fluorescence protein
EM  electron microscope
env  envelope protein
FAO  Food and Agriculture Organisation of the United Nations
FFRC  Freshwater Fisheries Research Centre
fg  femto-gram
FHV  Flock House virus
g  gravity force
GnRH  gonadotropin releasing hormone
GP  glycoprotein
h  hour
HBcAg  Hepatitis B core antigen
HBsAg  hepatitis B surface antigen
HBV  hepatitis B virus
HCl  Hydrochloric acid
HCV  hepatitis C virus
HDAg  hepatitis delta antigen
HEV  hepatitis E virus
HIV  human immunodeficiency virus
HPV  human papillomavirus
Hz  Hertz
ICM-MS  Intact cell MALDI-TOF mass spectrometry
ICTV  International Committee on Taxonomy of Viruses
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>immunoglobulin type G</td>
</tr>
<tr>
<td>IHHNV</td>
<td>infectious hematopoietic and hypodermal necrosis virus</td>
</tr>
<tr>
<td>IMAC</td>
<td>immobilized metal affinity chromatography</td>
</tr>
<tr>
<td>IMN</td>
<td>idiopathic muscle necrosis</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl β-D-1-thiogalactopyranoside</td>
</tr>
<tr>
<td>IRDR-1</td>
<td>interferon related developmental regulator-1</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal titration calorimetry</td>
</tr>
<tr>
<td>JE</td>
<td>Japanese Encephalitis</td>
</tr>
<tr>
<td>JGMV</td>
<td>Johnsongrass mosaic virus</td>
</tr>
<tr>
<td>k</td>
<td>Boltzmann’s constant</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase pair</td>
</tr>
<tr>
<td>kcal/mol</td>
<td>kilocalorie per mole</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Dalton</td>
</tr>
<tr>
<td>kg m⁻¹ s⁻¹</td>
<td>kilogram per meter per second</td>
</tr>
<tr>
<td>KLH</td>
<td>Keyhole limpet hemocyanin</td>
</tr>
<tr>
<td>LA</td>
<td>lactobionic acid</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertani</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>M1</td>
<td>matrix protein</td>
</tr>
<tr>
<td>mA</td>
<td>milli Ampere</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption/ionization-time of flight mass spectrometer</td>
</tr>
<tr>
<td>MALS</td>
<td>multi-angle light scattering</td>
</tr>
</tbody>
</table>
MCS  multiple cloning site
µg/ml  microgram per mililitre
MgSO₄  magnesium sulphate
MEGA  Molecular Evolutionary Genetics Analysis software
miRNA  micro-RNA
µm  micro meter
µM  micro Molar
mM  milli Molar
MMV  *Macrobrachium* muscle virus
MoNv  Mosinovirus
MRFV  Maze rayado fino virus
*M*  *Macrobrachium rosenbergii*
*Mrn*  *Macrobrachium rosenbergii* nodavirus
*Mrnvc*  *Macrobrachium rosenbergii* nodavirus capsid
mt  metric ton
mW  mili Watt
n  number
N  Normality
 يون  viscosity of the solvent
NaCl  sodium chloride
NAGE  native agarose gel electrophoresis
NaOH  sodium hydroxide
NaPi  sodium phosphate
NBM  nucleotide binding motif
NBT  nitro-blue tetrazolium chloride
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDV</td>
<td>Newcastle disease virus</td>
</tr>
<tr>
<td>NFκBΙ-α</td>
<td>NF kappa B inhibitor alpha</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>ammonium sulphate</td>
</tr>
<tr>
<td>NiV</td>
<td>Nipah virus</td>
</tr>
<tr>
<td>nm</td>
<td>nano meter</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NNV</td>
<td>nervous necrosis virus</td>
</tr>
<tr>
<td>NoV</td>
<td>Nodamura virus</td>
</tr>
<tr>
<td>NP</td>
<td>nucleoprotein</td>
</tr>
<tr>
<td>NS4B</td>
<td>non-structural protein 4B</td>
</tr>
<tr>
<td>nt</td>
<td>nucleotide</td>
</tr>
<tr>
<td>OD₅₉₅</td>
<td>Optical Density at absorbance 595 nm</td>
</tr>
<tr>
<td>OD₆₀₀</td>
<td>Optical Density at absorbance 600 nm</td>
</tr>
<tr>
<td>OGGNNV</td>
<td>orange-spotted grouper nervous necrosis virus</td>
</tr>
<tr>
<td>OR</td>
<td>origin of replication</td>
</tr>
<tr>
<td>PaV</td>
<td>Pariacoto virus</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>pg</td>
<td>pico-gram</td>
</tr>
<tr>
<td>PL</td>
<td>post-larvae</td>
</tr>
<tr>
<td>PMSF</td>
<td>phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td>Prdx</td>
<td>peroxiredoxin</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene fluoride</td>
</tr>
<tr>
<td>q-RT-PCR</td>
<td>real-time reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>RBSDV</td>
<td><em>rice black-streaked dwarf virus</em></td>
</tr>
<tr>
<td>RdRp</td>
<td>RNA dependent RNA polymerase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>RGNNV</td>
<td>red-spotted grouper nervous necrosis virus</td>
</tr>
<tr>
<td>Rh</td>
<td>hydrodynamic radius</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA interference</td>
</tr>
<tr>
<td>RNA/µg</td>
<td>RNA per microgram</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>rpm</td>
<td>revolution per minute</td>
</tr>
<tr>
<td>RT-LAMP</td>
<td>reverse transcription loop mediated isothermal amplification</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>s</td>
<td>sedimentation coefficient</td>
</tr>
<tr>
<td>S</td>
<td>Svedberg unit</td>
</tr>
<tr>
<td>SARS</td>
<td>severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SB</td>
<td>sodium boric acid base buffer</td>
</tr>
<tr>
<td>scFv11</td>
<td>anti-West Nile virus E antibody 11</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>sg</td>
<td>sungai</td>
</tr>
<tr>
<td>siRNA</td>
<td>short interfering RNA</td>
</tr>
<tr>
<td>SJNNV</td>
<td>striped jack nervous necrosis virus</td>
</tr>
<tr>
<td>SOC</td>
<td>Super Optimal Broth</td>
</tr>
<tr>
<td>SOCS1</td>
<td>cytokine signalling 1</td>
</tr>
<tr>
<td>SSN-1</td>
<td>snakehead-fish cell line</td>
</tr>
<tr>
<td>STNV</td>
<td>satellite tobacco necrosis virus</td>
</tr>
<tr>
<td>S-ELISA</td>
<td>Sandwich enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>s/n/c</td>
<td>substitution per nucleotide per cell infection</td>
</tr>
<tr>
<td>T</td>
<td>triangulation number</td>
</tr>
<tr>
<td>T</td>
<td>temperature in Kelvin (K)</td>
</tr>
<tr>
<td>TA</td>
<td>Thymine Adenine cloning</td>
</tr>
<tr>
<td>TAE</td>
<td>tris acetic acid EDTA</td>
</tr>
<tr>
<td>TBS</td>
<td>tris base saline</td>
</tr>
<tr>
<td>TBST</td>
<td>Tris base saline Tween 20</td>
</tr>
<tr>
<td>TBSV</td>
<td>tomato bushy stunt virus</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>TEMED</td>
<td>N,N,N’,N’-Tetramethylethylenediamine</td>
</tr>
<tr>
<td>Tfl</td>
<td><em>Thermus flavus</em></td>
</tr>
<tr>
<td>TGNNV</td>
<td>tiger puffer nervous necrosis virus</td>
</tr>
<tr>
<td>TLR4</td>
<td>toll-like receptor 4</td>
</tr>
<tr>
<td>TN</td>
<td>Tris-NaCl</td>
</tr>
<tr>
<td>TYMV</td>
<td>turnip-yellow-mosaic virus</td>
</tr>
<tr>
<td>U</td>
<td>unit</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet light</td>
</tr>
<tr>
<td>V</td>
<td>voltage</td>
</tr>
<tr>
<td>VLPs</td>
<td>virus-like particles</td>
</tr>
<tr>
<td>Vt</td>
<td>velocity terminus</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>WhNV</td>
<td>Wuhan nodavirus</td>
</tr>
<tr>
<td>WSSV</td>
<td>white spot syndrome virus</td>
</tr>
<tr>
<td>WTD</td>
<td>white tail disease</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>XSV</td>
<td>extra small virus</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

Giant freshwater prawn, scientifically known as *Macrobrachium rosenbergii* (Mr) and locally known as ‘Udang Galah’ in Malay, is an important aquaculture for human consumption. It is the largest known caridean shrimp, and can grow until 32 cm (male) and 25 cm (female) in length (Hung, 2013). Mr farming can be easily found in Malaysia, making the country one of the major players in this industry. In recent years, due to the high demand, the production and price of this prawn have increased dramatically. Based on the report of the Department of Fisheries (DOF) of Malaysia, the price for giant freshwater prawn was between RM 25 to RM 40 per kilogram in 2003. The price for the prawn increased to RM 45 per kilogram in 2011 (Sahidin and Mustapha, 2011, October).

Currently, a few types of diseases are infecting Mr, which cause high mortality, even though this species can be considered a moderately disease-resistant species (Ravi et al., 2009). Based on the Food and Agriculture Organisation of The United Nations (FAO) report (FAO, 2015), there are more than 10 types of diseases which can attack the prawn species. These diseases cause immense economic loss in prawn farming due to the high mortality rates.

White tail disease (WTD) is one of the diseases attacking this moderately disease-resistant species. The clinical signs of WTD infection are lethargy, anorexia and opaqueness of abdominal region of the prawn (Sudhakaran et al., 2007). Upon the infection of the WTD, the tail of the prawn will appear milky. After a few days of infection, the milky appearance will spread toward both the posterior and anterior parts, causing the degeneration of the telson region (Hameed et al., 2004a). Discolorations also will start to appear from the telson region toward the cephalothorax. The milky appearance of the muscle is due to the hyaline necrosis of the muscle fibres (Widada et al., 2003). WTD causes high mortality among the prawns, especially in larvae and post-larvae stages. Hameed et al. (2004b) demonstrated that this disease can cause 100% mortality in larvae and post-larvae stages after 2 to 3 days post-infection. The mortality was found to be lower in adult prawns due to a better immune system.

WTD was first reported in French West Indies in 1995 (Arcier et al., 1999). Within 10 years, the disease has spread to different countries all over the world including India (Hameed et al., 2004a), China (Qian et al., 2003), Thailand (Yoganandhan et al., 2006), Australia (Owens et al., 2009), Taiwan (Wang et al., 2008a) and most recently Malaysia (Saedi et al., 2012). An immersion challenge by using the virus isolated from the WTD infected prawn successfully caused similar appearances on healthy prawns suggesting that the virus might be the main causative agent. This was later identified as a nodavirus-like particle and was named as *Macrobrachium rosenbergii* nodavirus (*Mr*Nv). There is another type of virus known as extra small virus (XSV) which is associated with the *Mr*Nv (Qian et al., 2003). Both *Mr*Nv and XSV are icosahedral in structure. *Mr*Nv is about 26 nm in diameter while XSV is about 15 nm in diameter (Ravi et al., 2009). Both viruses are non-enveloped (Tripathy et al., 2006) and located at the cytoplasm of the infected prawn cells. *Mr*Nv and XSV have positive-sense, single-stranded RNA molecules. *Mr*Nv genome is composed of two linear positive-
sense RNAs which are encapsidated by the capsid assembled from CP-43 (Bonami et al., 2005). The genome of MrNv only codes for 3 proteins, RNA1, which codes for RNA dependent RNA polymerase (RdRp), is only 2.9 kb in length. RNA2, which codes for the capsid protein, has a genome of 1.3 kb in length. As for RNA3, which codes for protein B-2, researchers have yet to understand its function. For XSV, the genome consists of one linear positive sense, single-stranded RNA which has 796 nucleotides that codes for one capsid protein; CP-17 (Widada and Bonami, 2004). Yoganandhan et al. (2006) showed that not every post-larvae that are infected by MrNv contain XSV. Therefore, the relationship between MrNv and XSV is still unclear. This also suggests that WTD can be due to the infection of a prawn by MrNv alone.

This newly emerged MrNv poses many challenges to virologists. The genotypes of this virus share little similarity among the members in Nodaviridae family. Hence, this virus was grouped into a new sub-genus known as Gammanodavirus (NaveenKumar et al., 2013). The genome of MrNv isolated from different countries showed differences in nucleotide sequence as analysed with the Molecular Evolutionary Genetics Analysis software (MEGA) (Saedi et al., 2012). MrNv has been detected in Malaysian prawn farms since 2012, but the problems are Malaysian MrNv capsid sequence has yet to be identified and the relationship between Malaysian isolate with the other available isolates is still unclear. Thus far, the analyses done on this newly emerged member of Nodaviridae family were not in depth. The important domain and characteristics for the virus are still not available and have thus delayed the progress to develop a vaccine to encounter the virus outbreak. Therefore, the objectives for the first study will be the nucleotide sequence of MrNv capsid isolated from Malaysia prawns will be analysed and the sequence of the MrNv capsid, Malaysian isolate will be compared with other isolates to identify the nucleotide differences and the relationship of the Malaysian isolate with others.

MrNv can be transmitted horizontally or vertically (Ravi et al., 2009) either by sharing virus-polluted water sources from native host to non-native host (Sudhakaran et al., 2006) or from parental prawns to offsprings (Hameed et al., 2004b). Transmission of this virus from a marine shrimp (Penaeus sp.) to a freshwater prawn (Sudhakaran et al., 2006) further validates the horizontal transmission theory. A high mutation rate in RNA viruses due to the lack of a proof-reading RNA polymerase (Elena and Sanjuán, 2005) preliminarily poses a threat to the aquatic invertebrate where the MrNv was found to cause mortality in post-larvae in Penaeus vannamei (Senapin et al., 2012), suggesting that the virus mutation might transmit to other prawn species and pose a threat to them in future. MrNv outbreaks in many countries since the last decade suggest that the virus can be transmitted easily and the mutations occurring in the virus RNA genome may start to cross infect Penaeus vannamei, endangering other aquatic crustaceans. Currently there is still no vaccine to prevent nor a drug to treat the virus infection. Early screening and better water management schemes are the only solutions to prevent the virus outbreak.

To counter the virus outbreak and contain its spreading, knowledge on the virus life cycle and virus morphologies is needed. Introduction of virus genome into an expression system ensures the production of virus protein throughout the virus analysis. The use of bacterial expression host in virus study can be observed since decades ago such as the expression of coat protein of potyvirus, Johnsongrass mosaic
virus (JGMV) (Jagadish et al., 1991), hepatitis C virus core antigen (Lorenzo et al., 2001) and VP2 protein of canine parvovirus (CPV) (Xu et al., 2014). These recombinant proteins expressed by bacteria hosts retain the native virus properties and were shown to form virus-like particles (VLPs). These recombinant VLPs were successfully used to understand the virus assembly, genome encapsidation (Jagadish et al., 1991), virus aggregation (Lorenzo et al., 2001) and vaccine production (Xu et al., 2014). Extra small virus (XSV) which associates with the infection of MrNv has been produced in Escherichia coli and studied. The XSV capsid gene has been successfully cloned into an expression vector and introduced into a bacterial host (Wang et al., 2008). This study showed that the recombinant capsid protein of XSV can be expressed in a bacterial cell and was able to retain the capsid forming properties when analysed under a transmission electron microscope (TEM). The bacteria expression system has been widely used in many virus studies and the recombinant virus capsid genes produced by bacteria hosts were able to form into VLPs that resemble the native virus. MrNv has a low incidence rate in Malaysia and this problem has hindered the process to gain information regarding this virus using the native virus. Thus, the objectives for this part of study are to introduce the MrNv capsid gene into a bacteria expression system to ensure large production of virus protein to advance the study for better understanding of MrNv in morphology, assembly and genome encapsidation. Following that, the Malaysian MrNv capsid gene will be cloned into an expression vector and introduced into bacteria cells to ensure the production of the MrNv capsid. This recombinant MrNv capsid protein will then be studied and used as a model to understand the characteristics of the native virus.

Studies on the assembly of Brome mosaic virus (BMV) and Flock House virus (FHV), both of which are positive sense RNA viruses grouped under the families of Bromoviridae and Nodaviridae respectively, indicating that there are at least two important interactions for the capsid formation: a weaker protein-protein interaction and a stronger RNA-protein interaction (Marshall and Schneemann, 2001; Kaper, 1975). The modelling of RNA-protein interaction of Pariacoto virus (PaV) has been discussed by Harvey et al. (2009) and the process of capsid formation has also been predicted by Devkota et al. (2009). These studies suggest that these non-enveloped, icosahedral RNA viruses tend to form particles after a certain concentration of RNA has been neutralised by the positively-charged amino acids. This reaction favours the condensation of RNA-protein complex and pulls the capsid protein subunits together. Interactions between protein-protein subunits take part after the condensation of the complexes and further strengthen the capsid formation (Devkota et al., 2009). This suggests that disruption of the RNA-protein interaction might alter or halt the viral assembly. Marshall and Scheenth (2001) showed that RNA-protein interaction is important for recognising and packaging of viral genome in FHV. Nevertheless, the RNA-protein interaction site for MrNv, a newly emerged member of Nodaviridae family, has not yet been fully understood and this give rise to the research problem for the last part of this study. MrNv, which significantly shows differences in terms of nucleotide sequence with other nodaviruses, suggesting that it is of interest to locate the RNA binding site of MrNv capsid protein. Removal of the RNA interaction sites might affect the virus morphology and hence halt the virus assembly.

Amino acid sequence analysis of the first 50 amino acid residues of MrNv capsid available in the Genbank revealed that 11 residues of these 50 amino acids are positively-charged and 8 out of 10 amino acids located at positions 20th to 29th of the
N-terminal end are rich in positively-charged amino acids. The positively-charged amino acids are found to accumulate at this position but their functions are yet to be understood. This suggests that this rich positively-charged amino acid region of the MrNv capsid might be the RNA interaction site. Thus, the objective for the last part of this study is to determine the RNA binding site for this virus. Deletion or point mutation of the positively-charged amino acids located at residue 20\textsuperscript{th} to 29\textsuperscript{th} might result in the absence of RNA molecules in the mature virus cavity. Therefore, a study will be carried out to identify the RNA binding site of this virus via deletion and amino acid substitution mutageneses.

From the hypotheses and research questions mentioned above, the objectives of this study were:

1. To determine the nucleotide sequence of the capsid gene of *Macrobrachium rosenbergii* nodavirus (MrNv) isolated from Malaysia

2. To compare the capsid gene of Malaysian MrNv with other isolates

3. To produce the MrNv capsid protein in bacteria system via recombinant DNA technology

4. To characterise the recombinant MrNv capsid protein

5. To identify the RNA binding site of MrNv capsid protein by deletion and point mutageneses
REFERENCES


121


