IDENTIFICATION OF PEPTIDES FROM A PHAGE DISPLAY LIBRARY
FOR DIFFERENTIATING NEWCASTLE DISEASE VIRUS PATHOTYPES

By

LEE THONG CHUAN

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

March 2006
Newcastle disease virus (NDV) strains can be classified as virulent or avirulent based upon the severity of the disease. Biopanning experiments were performed using a disulfide constrained phage display heptapeptide library against three pathotypes of NDV strains: velogenic (highly virulent), mesogenic (moderately virulent) and lentogenic (avirulent). A phage clone bearing the peptide sequence SWGEYDM was isolated and shown to be able to differentiate virulent from avirulent NDV strains. This phage clone was employed as a capturing reagent in a dot-blot assay to detect virulent NDV strains in allantoic fluid of embryonated chicken eggs. The performance of the dot blot assay was compared with that of mean death time (MDT) in embryonated chicken eggs and the reverse transcription-polymerase chain reaction (RT-PCR) methods. The dot blot was shown to be specific for virulent NDV strains and able to differentiate between the virulent and avirulent NDV strains.
ACKNOWLEDGEMENTS

I would like to thank all those who have taken upon themselves, as a personal goal or as a matter of duty, to provide nourishment and guidance academically or otherwise, that ultimately has led to this thesis.

Associate Professor Dr. Tan Wen Siang who, through his own example, has inspired and directed me to achieve the highest level of science possible, and who has provided me with the tools for successful competition in science.

Professor Datin Dr. Khatijah Yusoff who, has inspired me to be creative and innovative, provided me with the critical and enlightening comments.

Associate Professor Dr. Sheila Nathan who, has given an excellent guidance and supervision, and provided me with a concrete and informational advises in this study.

Thank you for all of your kindness and patience during the duration of the work and thesis writing.

Vun Khai Yan, who has helped me in the nucleotide sequencing of partial F gene and mean death time.

iv
Dr. Kho Chiew Ling, who has been supportive and provided me advises in Newcastle disease virus and molecular biology techniques.

Dr. Majid Eshagi, a postdoctoral fellow, who has been a good friend and mentor in our group, and who has kindly trained me to be a skilled scientist.

Dr. Priadarishni Ramanujam, who has shared with me the knowledge of phage display.

Dr. Abdul Rahman Omar, who has provided some of the viruses used in this study.

I would also like to thank to all members of the virology group for their sharing of scientific knowledge and for the productive working conditions, Lee Lian Kiat, Chia Suet Lin, Firoozeh Jahansari, Iswan Budi, Lalita Ambigai, Mokrish, Ong Swee Tin, Raha Raus, Shaherny Zaid, Siti Salwa Hasmoni, Mohamed Rajik, Tan Geok Hun, Tang Kah Fai, Tang Kie Hie, Taznim Begam, and Zulkefley Othman.

Personal thanks to Noor Suhana Adzahar, and my family.

I duly acknowledge the financial support, IRPA grant 01-02-04-003-BTK/ER/006 for supporting this study and the National Science Foundation (NSF) scholarship from the Ministry of Science, Technology and Innovation, Malaysia (MOSTI).
I certify that an Examination Committee has met on 29 March 2006 to conduct the final examination of Lee Thong Chuan on his Master of Science thesis entitled “Identification of Peptides from a Phage Display Library for Differentiating Newcastle Disease Virus Pathotypes” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Suhaimi Mustafa, PhD**
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

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

**Abdul Rahman Omar, PhD**
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Wan Kiew Lian, PhD**
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

---

**HASANA MOHD GHAZALI, PhD**
Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia.

Date:
This thesis 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 the Supervisory Committee are as follows:

**TAN WEN SIANG, PhD**  
Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**KHATIJAH YUSOFF, PhD**  
Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**SHEILA NATHAN, PhD**  
Associate Professor  
Faculty of Science and Technology  
Universiti Kebangsaan Malaysia  
(Member)

---

**AINI IDERIS, PhD**  
Professor / Dean,  
School of Graduate Studies,  
Universiti Putra Malaysia.

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LEE THONG CHUAN

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>v</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xiv</td>
</tr>
</tbody>
</table>

## CHAPTER

1. INTRODUCTION

2. LITERATURE REVIEW
   2.1 Newcastle disease
   2.1.1 Occurrence of Newcastle disease
   2.1.2 Transmission and clinical symptoms of Newcastle disease
   2.2 Newcastle disease virus
   2.3 Molecular basis of pathogenicity
   2.4 Diagnosis of Newcastle disease virus
      2.4.1 Conventional diagnosis of Newcastle disease virus
      2.4.2 Advances in diagnosis of Newcastle disease virus
   2.5 Filamentous phage display
   2.6 Filamentous phage
      2.6.1 Life cycle
   2.7 Types of filamentous phage display systems
   2.8 Biopanning
   2.9 Phage display of peptides

3. MATERIALS AND METHODS
   3.1 Materials
   3.2 Propagation and purification of NDV
      3.2.1 Propagation and harvesting of NDV
      3.2.2 Purification of NDV
      3.2.3 Haemagglutination (HA) assay
      3.2.4 The Bradford assay
      3.2.5 SDS-PAGE
   3.3 Assessment of NDV virulence
      3.3.1 Mean death time
      3.3.2 Nucleotide sequencing of F protein cleavage site

Deleted: 5  Deleted: 6  Deleted: 8  Deleted: 9  Deleted: 29
3.4 Biopanning with NDV
3.4.1 Direct biopanning
3.4.2 Subtractive biopanning
3.4.3 Amplification of phage eluate
3.5 General phage methods
3.5.1 Phage titration
3.5.2 Small scale propagation and partial purification of phage
3.5.3 Quantitation of phage concentration
3.5.4 Large scale propagation and purification of phage
3.6 Characterization of peptide insert
3.6.1 Phage ssDNA extraction and purification
3.6.2 Nucleotide sequencing phage insert
3.6.3 Binding study of phage clone to NDV
3.7 Differentiation of NDV pathotype by various assays
3.7.1 Indirect phage ELISA
3.7.2 Phage capturing dot blot

4 RESULTS
4.1 Propagation and purification of NDV
4.2 Assessment of NDV virulence
4.2.1 Mean death time
4.2.2 Nucleotide sequencing of F protein cleavage site
4.3 Biopanning with NDV
4.4 Characterization of peptide insert
4.5 Differentiation of NDV pathotypes by various assays

5 DISCUSSION
5.1 Assessment of NDV virulence
5.2 Biopanning with NDV
5.3 Characterization of peptide insert
5.4 Differentiation of NDV pathotypes by various assays

6 CONCLUSION

REFERENCES
BIODATA OF THE AUTHOR