

## Molecular Characterization of Newcastle Disease Virus

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### Introduction

NDV is a major pathogen in poultry. Control of the virus is mainly through vaccination. Any effort to develop an effective vaccine against the disease would require a detailed understanding of their molecular biology and mechanism of infection. The glycoprotein genes, haemagglutinin-neuraminidase (HN) and fusion (F) are immunogenic and are involved in viral pathogenesis. In addition, the large (L) polymerase protein as well as the phosphoprotein (P) and nucleoprotein (NP) have been shown to be involved in viral transcription. Their molecular characterization and interactions would benefit in the development of a subunit vaccine against the virus. This project has been extended from phase I of RM7. We have successfully cloned the HN and F genes into baculovirus vector and transfected into insect tissue cultures. There was expression of the recombinant proteins which are potential candidates for subunit vaccine development. This project was undertaken to continue such studies. Specific regions of the NDV strain AF2240 genome were amplified and sequenced before being studied in detail. In addition, various peptides from biopanning experiments against the virus will be used to develop a diagnostic assay for NDV and its potential as anti-viral peptide was evaluated.

### Materials and Methods

Viruses in the study included several NDV field isolates and reference strains, including the strain AF2240. The viruses were grown, purified and their genomic RNAs were extracted. Primers were constructed to amplify specific regions in the genome by RT-PCR. The amplified products were analysed by restriction enzyme analysis and sequencing. The NP, P, M, F, HN and parts of the L genes of NDV strain AF2240 were cloned into *Escherichia coli* and sequenced. Diagnostic kits for NDV identification were developed using (i) RT nested PCR-ELISA assay and (ii) a recombinant phage which was isolated from biopanning experiments. The HN and F genes were subcloned into the Baculovirus expression system. All of the expressed gene products were then studied in detail. Specific peptide sequences which bind to NDV were determined through biopanning with a phage display library. Various chimaeras and mutants are currently being constructed and their biological functions are determined.

### Results and Discussion

**Diagnosis of NDV.** Various NDV isolates could be distinguished by sequence analysis of the cleavage site of the F protein gene. A nested RT-PCR ELISA diagnostic kit was developed for the determination and identification of NDV. This kit is more sensitive and specific than the current serological tests. We have filed a patent in Malaysia. The NDV kit has been included as a finalist in the FEER-HP "Young Inventors Awards" and won a Silver Medal in the Expo Science & Technology 2001 organised by the Ministry of Science, Technology and the Environment of Malaysia and a consolation prize in the UPM Inventors and Innovations Award 2000 competition.

**Sequence determinations of the various genes of NDV and their expressions.** The sequences of all the genes of velogenic-viscerotropic NDV strain AF2240, except the L gene, have been completely determined and each given EMBL/GenBank database accession numbers. The HN, F, NP, P gene sequences have been published and the remaining gene sequences are in the process of being published. The heat stability of the HN protein was studied. The NP and P gene sequences have been filed for patents in Malaysia and US. The NP protein was expressed in *E. coli* as ring and herringbone-like structures. These structures were shown to be able to carry extra peptide fragments at the C-terminal end and can act as antigenic carriers. This has been filed for patent.

**Cloning and expression of HN and F genes of NDV.** The recombinant HN protein has been shown to be immunogenic. The HN genes of V4(UPM), V4(QUE) and AF2240 have also been cloned into Baculovirus and the expressed recombinant proteins were studied for the heat stability. In addition, the HN and F genes have also been cloned into *Pichia pastoris* and eukaryotic expression vectors for the development of alternative recombinant vaccines. Some positive results have been obtained for these recombinant proteins expressed as DNA vaccines. The expression of these proteins in *E. coli* are being studied in detail.

**Biopanning of NDV proteins through use of a phage display library.** Various kinds of tests have been developed to distinguish the different strains of NDV. Unfortunately, these tests are often laboratory specific, expensive or tedious and they were not able to distinguish between the vaccine strains (mesogenic and lentogenic strains) and the field isolates (velogenic strains) which are the etiologic agents for the disease. We have developed a novel peptide (Malaysian Patent Pending PI 20013687)

that can distinguish between vaccinated chickens and those that were infected with the field isolates of NDV. It was found that this form of NDV typing was not previously reported, and furthermore it is the first invention that can distinguish the velogenic from the mesogenic strains. This invention is therefore useful as a routine diagnostic test to locate the source of an epidemic. In addition, this peptide is able to inhibit the replication of the virus and may be used as an antiviral drug. In addition, two anti-NDV peptides have been constructed and shown to inhibit NDV replication.

*NDV proteins interactions* Work on the protein-protein interactions of the recombinant proteins are being carried out to determine the mechanism(s) of virus-cell interactions. Chimaeras comprising various NDV protein segments with the NP protein have been constructed and their immunogenicity tested. These results are published and a patent has been filed in Malaysia and the US.

### Conclusions

Diagnostic test for NDV has been developed. The complete sequences of the NP, P, M, F and HN genes of NDV strain AF2240 were determined and given EMBL/GenBank database accession numbers. The F and HN genes of NDV strain AF2240 were cloned and expressed in the Baculovirus, *E. coli*, yeast and other expression systems. Anti-NDV peptides have been developed. The receptor and protein-protein interactions of the NDV proteins are being studied in detail. The NP protein can be expressed as a ring structure in *E. coli* and may be suitable as a carrier in future drug delivery system.

### Benefits from the study

Development of diagnostic kits and subunit vaccines for NDV; patents for the PCR-ELISA kit and NP and P gene sequences; and training of molecular biologists.

### Patent(s), if applicable

Nucleotide sequences of the nucleocapsid (NP) and phosphoprotein (P) genes of a Malaysian velogenic Newcastle disease virus strain AF2240 and the production of the NP and P proteins in *Escherichia coli* (Malaysian Patent Pending PI 20004837). US Patent being filed (App. No. 09/970,851)

Detection of Newcastle disease virus (Malaysian Patent Pending: PI 20005526)

Peptides that inhibit the propagation of Newcastle disease virus (Malaysian Patent Pending PI 20013687)

Nucleocapsid protein of Newcastle disease virus as a carrier for immunogens (Malaysian Patent Pending: PI20021709)

### Stage of Commercialization, if applicable:

Nil

### Project Publications in Refereed Journals

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#### Graduate Research

	Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
14	Kho Chiew Ling	Nucleocapsid (NP) and Phospho-(P) proteins of Newcastle disease virus: Identification of regions on NP that form particles and interact with P	Molecular Biology	PhD	2003
14	Omeima Salih	Sequencing, cloning and expression of the Newcastle disease virus fusion protein gene of strain AF2240	Molecular Biology	PhD	1999
15	Alan Ong Han Kiat	Cloning and expression of the haemagglutinin-neuraminidase (HN) gene from Newcastle disease virus (NDV) strain AF2240 in Baculovirus (AcNPV)	Molecular Biology	PhD	1999
15	Wong Sing	Cloning and expression of the genes encoding the envelope proteins of Newcastle disease virus	Molecular Biology	PhD	2003

15	Priadarishini Ramanujam	Peptide ligands that interact with Newcastle disease virus: selection, characterization and applications	Molecular Biology	PhD	2003
15	Loke Fung Chui	Towards the development of DNA vaccine against Newcastle disease virus	Molecular Biology	PhD	2002
15	Eni Kusumaningtyas	Sequence determination of the large L protein gene of Newcastle disease virus strain AF2240	Molecular Biology	MS	2003
213	Tang Kiong Yik	Thermostability of the recombinant haemagglutinin-neuraminidase glycoprotein of Newcastle disease virus	Molecular Biology	MS	2003
214.	Amir Rabu	Nucleocapsid protein of Newcastle disease virus as an antigenic carrier	Molecular Biology	MS	2002
215.	Chang Li Yen	Cloning and expression of the xylanase gene from <i>Bacillus coagulans</i> and the M gene of Newcastle disease virus in <i>Lactococcus lactis</i>	Molecular Biology	MS	2001
216.	Kho Chiew Ling	Development of an RT Nested PCR-ELISA diagnostic test for the detection of NDV	Molecular Biology	MS	1999
217	Siti Fatimah Putery Jemain	Sequence determination of the matrix gene in NDV strain AF2240	Molecular Biology	MS	1998

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