Molecular Characterization of Newcastle Disease Virus

Khatijah Yusoff, Aini Ideris, Abdul Manaf Ali, Abdullah Sipat, Wen Siang Tan, Abdul Rahman Omar

Department of Biochemistry and Microbiology,
Faculty Science and Environmental Studies
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

Telephone Number of Corresponding Author: 03-89466692
E-mail of Corresponding Author: khatijah@fsas.upm.edu.my

Key words: Newcastle disease virus, molecular biology, diagnostics, vaccines, biotechnology

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 (P) 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 peptides 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 then 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. Ion 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 PendingPI 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 PendingPI 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

Ong, H.K.A., Ali, A.M., Omar, A.R., Tan, W.S. and Yusoff, K. 1999. N-linked glycosylated HN protein of NDV strain AF2240 expressed in Baculovirus-infected Sf9 cells. Journal of Biochemistry, Molecular Biology and Biophysics 3: 147-151.

Ong, H.K.A., Ali, A,M., Omar, A.R. and Yusoff, K. 1999. Cloning and the steady-state expression of the HN gene from Newcastle disease virus strain AF2240 in Sf9 insect cells. *Cytotechnology* 32: 243-251.

Kho, C.L., Mohd. Azmi, M.L., Arshad, S.S. and Yusoff, K. 2000. Performance of an RT nested PCR ELISA for detection of Newcastle disease virus. Journal of Virological Methods 86: 71-83.

Salih, O., Omar, A.R. Ali, A.M. and Yusoff, K. 2000. Nucleotide sequence analysis of the F protein gene of a Malaysian velogenic NDV strain AF2240. Journal of Biochemistry, Molecular Biology and Biophysics 4: 51-57.

Tang, Y.K., Ong, H.K.A., Tan W.S. and Yusoff, K. 2000. Heat stability assay on the haemagglutinin (HA) and neuraminidase (NA) activities of Newcastle disease virus (NDV) strains AF2240, V4UPM and V4QUE. Malaysian Journal of Biochemistry and Molecular Biology 5: 79. (Abstract only)

Salih, O., Omar, A.R. Ali, A.M. and Yusoff, K. 2001. Analysis of a recombinant Baculovirus expressing the fusion glycoprotein gene of the Malaysian velogenic-viscerotropic Newcastle disease virus strain AF2240. Journal of Biochemistry Molecular Biology and Biophysics 5: 67-74.

Kho, C.L., Tan, W.S. and Yusoff, K. 2001. Sequence analysis of the nucleoprotein of a Malaysian heat resistant NDV straincomparison with other members of *Paramyxoviridae*. Journal of Biochemistry Molecular Biology and Biophysics 5463-471.

Yusoff, K. and Tan, W.S. 2001. NDV: macromoleculaes and opportunities Avian Pathology 30: 439-455.

Kho, C.L., Tan, W.S. and Yusoff, K. 2001. Production of the nucleocapsid protein of Newcastle disease virus in *Escherichia coli* and its assembly into ring- and nucleocapsid-like particles. Journal of Microbiology 39: 293-299.

Kho, C.L., Tan, W.S. and Yusoff, K. 2002. Cloning and expression of the phosphoprotein gene of Newcastle disease virus in *Escherichia coli*. Journal of Molecular Biology, Biochemistry and Biophysics 6117-121.

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 2002. Novel peptides that inhibit the replication of Newcastle disease virus. Archives of Virology 147: 981-993.

Rabu, A., Tan, W.S., Kho, C.L. Omar, A.R. and Yusoff, K. 2002. Potential of the nucleocapsid protein of Newcastle disease virus as a carrier for antigenic polypeptides. Acta Virologica 46: 211-217.

Kho, C.L., Tan, W.S., Tey, B.T. and Yusoff, K. 2003. Identification of nucleocapsid protein self-assembly domain of Newcastle disease virus. Journal of General Virology 842163-2168.

Loke, C.F., Raha, A.R., Omar, A.R. and Yusoff, K. 2003. Protection against Newcastle disease virus by DNA vaccines. Vaccine (submitted)

Kho, C.L., Tan, W.S., Tey, B.T. and Yusoff, K. 2003. NDV nucleocapsid: self assembly and length determination domains. Archives of Virology (revised).

Loke, C.F., Raha, A.R., Omar, A.R. and Yusoff, K. 2003. Enhanced expression of F and HN Proteins of NDV in a mammalian expression system by alteration of their *Kozak* Sequences. Plasmid (submitted)

Loke, C.F., Raha, A.R., Omar, A.R. and Yusoff, K. 2003. Immunogenicity of chickens immunized with plasmids encoding the F glycoproteins of Newcastle disease virus. Immunopathology (submitted)

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 2003. NDV-phage interaction: pathotyping and affinity constants. Biotechniques (submitted)

Project Publications in Conference Proceedings

Ong, H.K.A., Ali, A.M., Omar, A.R., Ideris, A., Poh, Y.M. and Yusoff, K. 1999. Electron microscopy and immunological studies of the recombinant baculovirus expressing the HN gene of NDV strain Af2240. In: Proceedings of the 11th National Biotechnology Seminar: 107-109.

Chang, L.Y., Raha, A.R., Sipat, A.B. and Yusoff, K. 1999. Cloning of a thermostable xylanase gene from *Bacillus coagulans* ST-6 and an M gene from Newcastle disease virus into Lactococcal expression vector pMG236e. In: Proceedings of the 11th National Biotechnology Seminar: 289- 290.

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 1999. A heptapeptide containing four hydroxyl groups interacts with Newcastle disease virus. In9th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology. pp. 20.

Wong, S.K., Tan, W.S., Tan, C.S. and Yusoff, K. 1999. Cloning of the envelope glycoprotein genes (HN and F) of Newcastle disease virus strain AF2240 into *Pichia pastoris*. In: 9th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology. pp. 45.

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 1999. Selection of peptide ligands that interact with Newcastle disease virus. In: 3rd. UNESCO National Workshop on Promotion of Microbiology in Malaysia. pp. 38.

Tan, W.S., Aziz, H.A., Tamanujam, P. and Yusoff, K. 1999. Determination of the binding affinity of a fusion phage that interacts with Newcastle disease virus. In: 22nd Microbiology Symposium, pp 102.

Wong S.K., Tan W.S., Tan, C.S. and Yusoff, K. 2000. *In vitro* transcription and translation of truncated hemagglutinin-neuraminidase (HN) gene from a local Newcastle disease virus (NDV) isolate. In: 10th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 42.

Kho, C.L., Tam, W.S. and Yusoff, K. 2000. Cloning and expression of NP protein of Newcastle disease virus strain AF2240 in *Escherichia coli*. In: 10th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 42.

Loke, C.F., Raha, A.R., Omar, A.R. and Yusoff, K. 2000. Cloning and expression of the fusion (F) and haemagglutinin-neurminidase (HN) genes from Newcastle disease virus (NDV) strain AF2240 in a mammalian expression system. In: 10th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 43.

Rabu, A., Kho, C.L., Tan, W.S. and Yusoff, K. 2000. Fusion of the immunogenic peptides of haemagglutinin-neuraminidase (HN) glycoprotein to the nucleocapsid (NP) protein of NDV. In: 10th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 43.

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 2000. Biophysical properties of phages that interact with Newcastle disease virus. In: 10th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 44.

Tang, Y.K., Ong, H.K.A., Tan, W.S. and Yusoff, K. 2000. Heat stability assay on the haemagglutinin (HA) and neuraminidase (NA) activities of Newcastle disease virus (NDV) strains AF2240, V4UPM and V4QUE. In: Proceedings of Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology 25: 34 .

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 2000. Inhibition of Newcastle disease virus propagation with synthetic peptides. In: Proceedings of the 12th National Biotechnology Seminar: 23-24.

Rabu, A., Kho, C.L., Tan, W.S. and Yusoff, K. 2000. *Construction of chimeric proteins of Newcastle disease virus (NDV).* In: Proceedings of the 12th National Biotechnology Seminar: 52.

Wong, S.K., Tan, W.S., Tan, C.S. and Yusoff, K. 2000. Salt-induced expression of the extracellular domains of the envelope proteins of Newcastle disease virus in *E. coli.* In: Proceedings of the 12th National Biotechnology Seminar53-54.

Kho, C.L., Tan, W.S. and Yusoff, K. 2000. Cloning and expression of the phosphoprotein (P) of Newcastle disease virus strain AF2240 in *Escherichia coli* system. In: Proceedings of the 3rd. Symposium of the Malaysian Society for Microbiology79-80.

Chiew Ling Kho, Wen Siang Tan and Khatijah Yusoff 2001. Newcastle disease virus nucleocapsid protein expressed in *Escherichia coli* assembles into ring-like and herringbone-like structures. In: 11th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 34.

Ramanujam, P., Tan, W.S., Nathan, S. & Yusoff, K. 2001. Identification of the binding site of a synthetic peptide that interacts with Newcastle disease virus. In: 11th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 38.

Tang, Y.K., Ong, H.K. A., Tan, W.S. and Yusoff, K. 2001. Cloning and expression of the haemagglutinin-neuraminidase (HN) gene of avirulent Newcastle disease virus strains V4QUE and V4UPM in baculovirus expression system. In: 11th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 43.

Wong, S.K., Tan, W.S., Tan, C.S., Omar, A.R. and Yusoff, K. 2001. Immunogenic properties of the recombinant HN and F proteins of Newcastle disease virus produced in *Escherichia coli.* In: 11th Scientific Meeting of Malaysian Society for Molecular Biology and Biotechnology: 43.

Rabu, A., Tan, W.S., Kho, C.L., Omar, A.R. and Yusoff, K. 2001. *Immunogenic properties of the nucleocapsid (NP) fusion proteins of* Newcastle disease virus. In: Proceedings of the 3rd National Biotechnology Seminar: 47-48.

Kho, C.L., Tan, W.S. and Yusoff, K. 2001. Mutagenesis of the nucleocapsid protein of Newcastle disease virus. In: Proceedings of the 3rd National Biotechnology Seminar: 51-53.

Ramanujam, P., Tan, W.S., Nathan, S. and Yusoff, K. 2001. Detection of Newcastle disease virus pathotypes with a recombinant phage. In: Proceedings of the 3rd National Biotechnology Seminar: 70-71.

Tan, S.W., Aini, I., Omar, A.R., Yusoff, K. and Tan, W.S. 2001. Phylogenetic analysis of recently isolated Newcastle disease virus (NDV). In: Proceedings of the 3rd National Biotechnology Seminar: 79-81.

Wong, S.K., Tan, W.S., Tan, C.S., Omar, A.R. and Yusoff, K. 2001. Cloning of the genes encoding envelope glycoproteins of Newcastle disease virus (NDV) strain AF2240 into yeast expression vector and bacterial secretion vector. Proceedings of the 3rd National Biotechnology Seminar: 83-85.

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 Newcastyle disease virus (NDV) strain AF2240 in Baculovirus (AcNPV)	Molecular Biology	PhD	1999
15	Wong Sing ing	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 Chui Fung	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 Yik Kiong	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 *Bacillus coagulans* and the M gene of Newcastle disease virus in *Lactococcus lactis*	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 bt Jemain	Sequence determination of the matrix gene in NDV strain AF2240	Molecular Biology	MS	1998

IRPA Project number: 01-02-04-0107
UPM Research Cluster: BAB
(An update of the abstract published in UPM Research Report 1997-2000.)