Expression and purification of recombinant PIII protein of M13 bacteriophage and its applications in competitive ELISA for detection of avian influenza virus infections.

Principal Supervisor: Dr Farhid Hemmatzadeh
Email: farhid.hemmatzadeh@adelaide.edu.au
Phone: 8303 7723

Highly pathogenic avian influenza (AI) is a major respiratory disease that affects both animal and human. In both human and animal cases vaccination is the first line of defence for protecting against avian influenza in endemic areas. Commonly available serological diagnostic tests cannot differentiate vaccinated birds from naturally infected birds. Differentiation of vaccinated from infected animals (DIVA), is currently advocated as a means to achieve full control of H5N1. Several DIVA strategies have been attempted and one of the most feasible approaches is based on use of the matrix 2 protein (M2e) of avian influenza virus in a serological test.

The external domain of matrix protein (M2e) of avian influenza virus is a small protein that is produced in large quantities in infected cells but is not packed in killed vaccines. Hence humans, or animals, vaccinated with conventional inactivated influenza vaccines are not expected to have antibodies to the M2e but infected humans or animals are produced significant amounts of anti-M2e antibodies.

A DIVA ELISA test was used to measure serological responses to the M2e protein of avian influenza virus. Alternative DIVA strategy is under the investigation to improve the sensitivity and the specificity of the DIVA ELISA test. This test is based on a competitive ELISA using recombinant antibodies, has expressed in bacteriophages which specifically target the highly conserved region of viral M2e antigen.

Detection of antibodies to the bacteriphage proteins in a competitive ELISA system indirectly shows the titer of anti M2e antibodies.   Currently available anti-bacteriophage antibodies are not reliable enough for applying in a competitive ELISA. These antibodies cannot recognise native form of bacteriophage antigens therefore they cannot react with the bacteriophages in ELISA systems. Those antibodies just react with denatured bacteriophage proteins in SDS-PAGE followed by Western blotting tests.

The research project will be focused on production of recombinant bacteriophage antigens and its applications for production of mono-specific or monoclonal antibodies. Furthermore these antibodies will be used in competitive ELISA systems for differentiation of infected and vaccinated animal in DIVA-ELISA tests.

As a summary, at the first step PIII gene of M13 bacteriophage will be amplified in PCR using specific primers for expression. The PCR product of PIII gene will be clone in to a plasmid as an expression vector ant the vector will be transfected into competent E.coli cells then expressed to produce pacteriophage protein. The protein will be purified in affinity chromatography columns and after initial evaluations will be used for immunization of rabbits to produce mono-specific antibodies.