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GUNNAPORN VEERACHATO. STUDIES ON *Escherichia coli* EXPRESSION OF CLASSICAL SWINE FEVER VIRUS GP55 AND ITS ANTIGENIC CHARACTERIZATION. THESIS ADVISORS : SAKOL PANYIM Ph.D., SUDARAT DAMRONGWATANAPOKIN Ph.D. 155 p. ISBN974-661-531-9.

Classical swine fever (CSF) is one of the most economically important swine diseases. Diagnostic tests currently used in Thailand are virus isolation and identification by fluorescence antibody test (FAT) or by immunohistochemistry staining, nucleic acid detection by reverse trancriptase polymerase chain reaction (RT-PCR) and serological test by neutralizing peroxidase-linked assay (NPLA). Most methods require special laboratory expertise and equipment which are costly and time consuming. To avoid these disadvantages, an ELISA based diagnostic method might be suitable because it is simple and less time consuming. The goal of this thesis was to characterize the antigenicity of CSF virus (CSFV)-specific recombinant protein, gp55, for the future development of an ELISA kit.

DNA sequences of the complete and truncated gp55 genes were inserted into pCYB4 and pET16b vectors. The recombinant expression system of choice was *E.coli*. After expression optimization of time, media, and temperatures, only the truncated gp55 protein expressed in pET16b vector was observed and it was called Histag-TR55 protein. The polyhistidine tag located at the N-terminus of Histag-TR55 protein allowed purification by nickel-chelate affinity chromatography. The antigenic property of that Histag-TR55 protein was characterized by Western blot with one specific pathogen free (SPF) swine serum, one mouse monoclonal anti-gp55 antibody (MAb), twelve CSFV seronegative swine sera, four swine polyclonal anti-CSFV sera. All positive and negative sera were determined by NPLA method. It was shown that all positive swine antisera (1:200 dilution), the MAb (1:10 dilution), and ten in twelve negative swine sera (1:200 dilution) could bind to the 2 μg Histag-TR55 proteins. Two negative swine sera (1:200 dilution) and the SPF swine serum (1:200 dilution) did not bind to the 2 μg Histag-TR55 protein. No binding of positive and negative swine sera could be observed when the amount of Histag-TR55 protein was diluted to 400 ng. Thus, the results imply that this recombinant protein may lack specificity.