PRODUCTION OF A RECOMBINANT VP1 PROTEIN OF THE CHICKEN ANAEMIAVIRUS FOR THE DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY

By

ELHAM OMER MAHGOUB

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree Master of Science

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NOVEMBER 2004

Chairman: Professor Dr. Mohd Azmi Mohd Lila, Ph.D.

Faculty: Veterinary Medicine

VP1 gene is an important gene responsible for the expression of a protein that may facilitate the diagnosis of chicken anaemia virus (CAV) infection. The VP1 gene was cloned and expressed in prokaryotic expression vector pRSET–B. The VP1 protein expression in *E. coli* was achieved as a fusion protein containing an immunogenic epitope. The protein expressed was detected by anti-VP1 monoclonal antibody with an apparent molecular weight of 50 kDa. Batch fermentation was used to scale up production of the protein in *E. coli*. The recombinant VP1 protein was successfully expressed during high cell density culture. The use of tangential flow filtration (TFF) filtration step for dialysis and desalting increased both the specific activity and the final yield of the purified fraction. The protein expressed has been tested as an antigen for detection of antibody to CAV in

infected chicken. An enzyme-linked immunosorbent assay (ELISA) applied for the detection of serum antibody to CAV. This test depends on the availability of CAV polyclonal antibodies present in convalescent chicken serum to react with the VP1 antigen adsorbed to the ELISA plate. When serum samples from infected chicken were tested, the sensitivity of the assay was found to be greater than 93.3%. Furthermore, the ELISA test requires serum samples to be diluted at 1:100 compared to 1:50 for commercial ELISA, an increase in the potential of non-specific background that could be contributed by proteins available in serum samples. In conclusion the indirect ELISA approach using VP1 fusion protein has many advantages. The results of the present study can be used as a basis for the development of a reliable ELISA assay for detection of CAV.

Abstrak tesis yang dikemukakan senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGGUNAAN ASSAI IMUNOERAP TERANGKAI ENZIM (ELISA) KHUSUS VP1 UNTUK PENGESANAN ANTIBODI TERHADAP VIRUS ANEMIA AYAM

Oleh

ELHAM OMER MAHGOUB

NOVEMBER 2004

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Gen VP1 merupakan gen penting dalam penyataan protein yang mungkin boleh memudahkan diagnosis jangkitan virus anemia ayam (CAV). Gen VP1 telah diklon dan dinyatakan dalam vektor penyataan prokariot pRSET-B. Penyataan protein VP1 dalam *E. coli* ini diwujudkan sebagai protein penyatuan yang mengandungi epitop imunogenik. Protein yang dinyatakan itu dikesan melalui antibodi monoklon anti-VP1 yang berat molekul nyatanya 50 kDa. Penapaian kelompok diguna untuk menaikkan skala penghasilan protein dalam *E. coli*. Protein VP1 rekombinan telah berjaya dinyatakan dalam kultur ketumpatan sel tinggi. Penggunaan langkah penurasan aliran tangen (TFF) untuk dialisis dan penyahgaraman telah meningkatkan kedua-dua, aktiviti khusus dan hasil muktamad pecahan tertulen. Protein ternyata ini diuji sebagai antigen untuk pengesanan antibodi terhadap CAV dalam ayam terjangkit. Assai imunoerap terangkai enzim (ELISA) telah diguna dalam pengesanan antibodi serum terhadap CAV. Ujian ini bergantung kepada kebolehdapatan antibodi poliklon CAV dalam serum ayam sedang

pulih untuk bertindak dengan antigen VP1 yang terjerap pada plat ELISA. Apabila serum daripada ayam terjangkit diuji, kepekaan assai didapati lebih daripada 93%. Tambahan pula ujian ELISA memerlukan sampel serum yang dilarut pada 1:100 berbanding 1:50 untuk ELISA komersil, suatu peningkatan dalam potensi latar belakang bukan khusus yang boleh disumbang oleh protein yang ada dalam sampel serum. Kesimpulannya ialah, pendekatan ELISA tak langsung mengguna protein penyatuan VP1 mempunyai banyak kelebihan. Hasil daripada kajian ini boleh diguna sebagai asas kepada perkembangan suatu assai ELISA yang boleh dipercayai untuk pengesanan CAV.

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I certify that an Examination Committee met on **24/1/2005** to conduct the final examination of **Elham Omer Mahgoub** on her **Master of Science** thesis entitled "**Production of a Recombinant VP1 Protein of the Chicken Anaemia Virus for The Development of an Enzyme-Linked Immunosorbent Assay** " in accordance with Universiti Pertanian Malaysia (High Degree) Act 1980 and Universiti Pertanian Malaysia (High Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for the degree of Master of Science**.** The members of the Supervisory Committee are as follows:

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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.

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ELHAM OMER MAHGOUB

Date:

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