IDENTIFICATION, CLONING, SEQUENCING, EXPRESSION AND PROTECTIVE CAPACITY OF THE GENE ENCODING A FIMBRIAL PROTEIN OF *PASTEURELLA MULTOCIDA* B:2

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DOCTOR OF PHILOSOPHY

UNIVERSITI MALAYSIA TERENGGANU MALAYSIA

2009

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Science and Technology Universiti Malaysia Terengganu

June 2009

Specially dedicated to:

Abah and Ma,

ENCIK MD. YASIN MOHD SALLEH
PUAN KHADIJAH MOHAMAD

My Siblings,

MD. YUZEIRY MD. YASIN
IDA MURYANY MD. YASIN
OMAR SAUFY MD. YASIN
AHMAD SYAKIR MD. YASIN
AHMAD SYAMIL MD. YASIN
MD. MUHAIMIN MD YASIN
WAHIZATUL AFZAN AZMI
KHAIROL HAFIZ HARIS

My Little Nephews,

ERFAN KHEIRY MD. YUZEIRY
YUSUF DANIAL KHAIROL HAFIZ

My Soulmate,

MOHD. TERMIZI YUSOF

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Doctor of Philosophy

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Haemorrhagic septicaemia (HS), an economically important disease of cattle and buffaloes, is caused by *Pasteurella multocida* B:2. Vaccination against this disease is widely practiced but the protection was unclear. In order to improve the protection effectiveness of the vaccines, the causative organism has been fractionated and the various cell surface components were studied. The fimbrial proteins are particularly attractive candidate for development of recombinant vaccine because they are strong immunogens. This study was conducted to characterize and determine the antigenicity of the fimbrial protein of *Pasteurella multocida* B:2, followed by development of recombinant cell vaccine expressing the fimbrial protein. The study also was carried out on the potential and efficacy of *Pasteurella multocida* B:2 recombinant fimbrial proteins in the production of vaccine against haemorrhagic septicaemia.

The protein profile and antigenicity of selected isolates of *Pasteurella multocida* B:2 were determined. Local isolates of *Pasteurella multocida* B:2 showed the presence of 18 kDa protein, which was identified as the fimbrial protein by SDS

PAGE. Following immunoblotting using either rabbit serum raised against the 18 kDa fimbrial protein or the lung lavage fluid of goat exposed to the fimbrial protein of *Pasteurella multocida* B:2, the results revealed that both serum and lung lavage fluid recognized the 18 kDa protein band of *Pasteurella multocida* B:2. These findings suggested that the fimbrial protein was antigenic, and should be able to stimulate the production of antibodies and eventually provides protection against challenge.

Investigation at the molecular level indicates that isolation and detection of the gene encoding the 18 kDa fimbrial protein of *Pasteurella multocida* B:2 was successful as shown by the amplification of approximately 430 bp DNA product. As a result from the cloning procedure, two recombinant vectors, pET32/LIC-fimbrial and pTriEx/LIC-fimbrial plasmid were created. DNA sequencing analysis showed that the gene sequence of interest kept in frame with the vector sequence. It was confirmed that the inserted gene was the fimbrial gene of *Pasteurella multocida* B:2 and found to contain 435 bp. The gene encodes a deduced protein of 144 amino acids. Analysis of the nucleotide sequence of fimbrial gene *Pasteurella multocida* B:2 isolate PMX revealed 100% to 99% homology to the fimbrial gene of *Pasteurella multocida* type B:2 but slightly different to serotypes A:1, D:1 and F:3 of *Pasteurella multocida*.

On the road to develop recombinant cell vaccine expressing fimbrial protein, the SDS PAGE and Western immunoblotting analysis revealed that the expressed fusion protein of the pET32/LIC-fimbrial was approximately 33 kDa, which contained 17.8 kDa of tagged protein while the pTriEx/LIC-fimbrial plasmid was 21.4 kDa, containing 6.2 kDa of the tagged protein and remaining is 15.2 kDa of the fimbrial protein after detection using His.Tag or S.Tag monoclonal antibody. The results conclusively demonstrated the successful expression of *Pasteurella multocida* fimbrial gene as a fusion protein which was tested in three different *E. coli* strains.

The next study was carried out to determine the antibody of IgG and IgA levels in goats following intranasal vaccination of an inactivated recombinant vaccine expressing the antigenic fimbrial protein of *Pasteurella multocida* B:2. During the course of study, both serum and lung lavage fluid from all groups; vaccinated, control and unvaccinated were collected to evaluate the antibody levels via enzyme-linked immunosorbent assay (ELISA). Overall, it was found that goats immunized with an inactivated recombinant vaccine through intranasal route developed a strong specific and significantly higher ($P<0.05$) IgG response in both serum and lung lavage fluid as well as IgA response when compared to the control and unvaccinated groups. At the time of challenge with live *Pasteurella multocida* B:2, the IgG and IgA levels in both serum and lung lavage fluid of vaccinated goats were significantly ($P<0.05$) higher than the unvaccinated goats. Thus, the study showed that the inactivated recombinant vaccine expressing the fimbrial protein of *Pasteurella multocida* B:2 provided high percentage of protection against high dose challenge when 84% of vaccinated goats were protected as compared to control and unvaccinated groups following intratracheal challenge exposure.

Further investigation of the potential role of fimbrial protein as a protective antigen that could be included in future subunit vaccines to *Pasteurella multocida* is necessary. On the other hand, by analyzing the pattern of antibody response against this inactivated recombinant vaccine in real host, cattle must be carried out in order to understand the immunological pattern which can be subsequently improve and enhance the potentiality of the vaccine. Moreover, in the present study, considering impact of such antigen characteristics, vaccine formulations, delivery systems and adjuvants need to be verified and carefully selected to determine the best criteria to be used in preparation of an ideal recombinant vaccine.