Application of a functional genomics approach to the identification of vaccine subunits and diagnostic antigens for use in the control of swine dysentery

by

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This thesis is submitted for the degree of Doctor of Philosophy of Murdoch University

2007
Dedicated to my wife: Lulu Yin,

my daughters: Anya Song and Enya Song,

my parents: Guanghua Song and Xiaoyang Xu
DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

..........................
Yong Song
The intestinal spirochaete *Brachyspira hyodysenteriae* is the causative agent of swine dysentery (SD), a diarrhoeal disease of pigs which has significant economic impact worldwide. Controlling SD remains problematic, particularly as there is no effective vaccine and there are few definitive diagnostic methods available. In this study, a partial genomic sequence of *B. hyodysenteriae* was screened *in silico*. A total of 19 putative open reading frames (ORFs) encoding outer-membrane proteins then were selected and these were subjected to a laboratory screening process. To select potential universal vaccines, a preliminary study was conducted using PCR to determine the distribution of the putative genes in 23 strains of *B. hyodysenteriae*. A total of 17 of the 19 ORFs were considered to be suitable for further testing as they were found to be present in the majority of strains investigated. After molecular cloning and protein expression and purification, of 19 cloned candidate molecules derived from 17 genes (one large gene was divided into two parts encoding N and C terminal proteins, respectively), 14 were expressed in *E. coli* and the recombinant proteins were successfully produced. A variety of sera from pigs naturally and experimentally infected with *B. hyodysenteriae* were tested for reactivity with the 14 recombinant proteins in an immunoblotting assay. Seven molecules from six genes reacted strongly with the tested sera, and therefore were selected and used to immunize mice. All these proteins generated a specific antibody response. Post-immunization sera raised against each recombinant protein had the capacity to agglutinate *B. hyodysenteriae* cells, and also recognized the cognate proteins of *B. hyodysenteriae* in cell extracts. Further sequencing analysis demonstrated that these molecules were highly conserved in the genomes of different *B.
*B. hyodysenteriae* strains. Therefore, from the genomic-based study, the products of six genes were identified as promising candidates for vaccines or as diagnostic targets.

Four genes were expressed on a large scale, the products (NAV-H7, NAV-H17 C-terminal, NAV-H34 and NAV-H42) were combined into one vaccine, and then this preparation was used to immunise pigs that subsequently were challenged with *B. hyodysenteriae*. These antigens generate systemic and colonic antibody responses, and vaccination tended both to delay the onset of clinical signs and attenuate lesion development. Hence these recombinant proteins showed promise as components for further SD vaccines.

Recombinant proteins from the selected genes also were used as antigens in class-specific ELISAs used as serological assays for SD. Three antigens (NAV-H8, NAV-H42 and Bhlp29.7) were selected as good indicators of seroconversion in IgM ELISAs, and these were evaluated further using a large range of serum samples. The NAV-H8 IgM ELISA using a cut-off value 2.5 times the mean value of all negative pigs could be used as a herd test for SD, and both the NAV-H8 and NAV-H42 IgM ELISAs had potential for detecting exposure to *B. hyodysenteriae* at the pig level.
ACKNOWLEDGEMENTS

I would firstly like to express my gratitude to my principal supervisor, Professor David Hampson, for giving me this opportunity to undertake these studies and for his invaluable guidance, endless patience, support, and encouragement. As a supervisor of a student originating from non-English country, he spent considerable time and efforts in improving my English communication skills, which will certainly have great impact on my future research career.

My deep thanks go to Dr Tom La, whose excellent expertise, patience, kindness and friendship help me get through all the difficult stages of the project. The experimental components could have not been completed smoothly without his input and help. During the vaccine and diagnostic study, Tom generously provided the recombinant Bhlp29.7 as a control, which enriched the content of the research. I would also like to thank Dr Nyree Phillips for her assistance in bacterial culture, assistance with the pig trial and support in routine laboratory work. Also thanks to the other team members, Dr Kirsty Townsend, Abdolreza Movahedi and Arif Munshi for giving me advice, sharing experience and knowledge.

The vaccine project involved substantial bioinformatic analysis. I am very grateful to the members in bioinformatics side, my associate supervisor, Professor Matthew Bellgard (Center for Comparative Genomics, Murdoch University) for providing a training platform and helpful advice in bioinformatics during my first year of PhD training, and to Dr David Dunn and Mr Yair Motro for their great efforts in performing genomic
mining, and help in using various computer algorithms and in coordination of the selection of suitable candidate genes for further analysis.

Many people in the State Agricultural Biotechnology Centre where I conducted most of my experiments should be acknowledged. My thanks are due to Dr David Berryman for his generous advice and assistance in using various laboratory equipments, Frances Brigg for running the sequencing, and Dr Rongchang Yang for being such a good friend.

This study was funded by Australian Research Council and Novartis Animal Vaccines (NAV) as the industry partner. Dr Ian Thompson from NAV was particularly supportive throughout the project.

Finally, I owe particular thanks to my parents and my dearest wife, Lulu Yin, and my lovely daughter, Anya Song. They always support me and are a source of pride for me over many years. Their faith, sustenance, understanding and companionship were the sources of my strength to pursue this dream.
ACHIEVEMENTS ASSOCIATED WITH THIS STUDY

Some of the genes identified during my PhD study have been granted provisional patents, and currently they are being applied for full patent protection. Two papers have been prepared, but only can be submitted once full patents are obtained.


Publications prepared:
Identification and recombinant expression of candidate components for a swine dysentery vaccine.

Comparative evaluation of the usefulness of *B. hyodysenteriae* surface-associated antigens for serodiagnosis of swine dysentery.

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### ABBREVIATIONS AND SYMBOLS LIST (Continued)

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