CHARACTERISATION, RECOMBINANT EXPRESSION AND IMMUNOGENICITY OF BHLP29.7, AN OUTER MEMBRANE LIPOPROTEIN OF *BRACHYSPIRA HYODYSENTERIAE*

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DECLARATION

I hereby declare that the work presented in this thesis has been performed by myself, except where otherwise clearly stated in the text, and that it has not been previously submitted for application for a degree at any University.

Signed ……

Dedicated to my wife, Pei Ling,
and my daughter, Kaitlin

ABSTRACT

Swine dysentery (SD) is an important endemic infection in many piggeries, and control can be problematic. In this study, the gene encoding a 29.7 kDa outer membrane lipoprotein of the causative intestinal spirochaete *Brachyspira hyodysenteriae*, was identified and sequenced. An 816 bp hypothetical open reading frame (ORF) was identified, with a potential ribosome binding site, and putative –10 and –35 promoter regions upstream from the start of the ORF. The 29.7 kDa outer membrane lipoprotein was designated Bhlp29.7 and the encoding gene named *bhlp29.7*.

The amino acid sequence of Bhlp29.7 included a 19 residue hydrophobic signal peptide, incorporating a potential signal peptidase cleavage site and membrane lipoprotein lipid attachment site. *In silico* analysis of this protein together with lipidation studies further supported its probable outer membrane localisation. Comparison of the Bhlp29.7 sequence with public sequence databases showed that it had up to 40% similarity with the D-methionine substrate-binding outer membrane lipoprotein (MetQ) of a number of bacterial pathogens. The Bhlp29.7 gene was detected in all 48 strains of *B. hyodysenteriae* examined, and in *Brachyspira innocens* strain B256^{T}, but not in 10 other strains of *B. innocens* or in 42 strains of other *Brachyspira* spp. The gene was sequenced from *B. innocens* strain B256^{T} and from 11 strains of *B. hyodysenteriae*. The *B. hyodysenteriae* genes shared 97.9-100% nucleotide sequence identity and had 97.5-99.5% identity with the gene of *B. innocens* strain B256^{T}. The Bhlp29.7 gene was subsequently cloned and expressed as a histidine fusion-protein in an *Escherichia coli* expression system.

An ELISA test using recombinant his-tagged Bhlp29.7 (His_6-Bhlp29.7) as the detecting antigen was developed and evaluated. The threshold value of the test was chosen to provide a highly stringent assessment of the disease status of a herd. The sensitivity and specificity of the test was 100%. When the test was applied to sera from eight herds with suspected SD, four

gave ELISA values indicating that the herds were diseased. The remaining four herds gave ELISA values below the threshold value. These results indicated that the Bhlp29.7-ELISA was useful as an indirect test for exposure of a herd to *B. hyodysenteriae* and may be a helpful complement to current methods of SD diagnosis.

Recombinant His_6-Bhlp29.7 was evaluated as a vaccine subunit for prevention of SD. The His_6-Bhlp29.7 was shown to be immunogenic in mice following two intramuscular injections. Vaccination of mice with His_6-Bhlp29.7 provided full protection after oral challenge with *B. hyodysenteriae*. In two experiments, intramuscular and oral vaccination of pigs with the His_6-Bhlp29.7 resulted in a 50% reduction in incidence of SD compared to unvaccinated control pigs (P=0.047). This is the first subunit vaccine shown to provide pigs with protection from SD. Further work is needed to optimise delivery routes and adjuvants for commercial development of the vaccine.

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PUBLICATIONS

Articles arising from this thesis and related work:

Lee, B. J., La, T., Mikosza, A. S. and Hampson, D. J. (2000). Identification of the gene encoding BmpB, a 30 kDa outer envelope lipoprotein of *Brachyspira* (*Serpulina*) *hyodysenteriae*, and immunogenicity of recombinant BmpB in mice and pigs. *Veterinary Microbiology* **76**: 245-257.

La, T. and Hampson, D. J. (2001). Serologic detection of *Brachyspira* (*Serpulina*) *hyodysenteriae* infections. *Animal Health Research Reviews* **2**: 45-52.

La, T., Phillips, N. D., Reichel, M. P. and Hampson, D. J. (2004). Protection of pigs from swine dysentery by vaccination with recombinant BmpB, a 29.7 kDa outer-membrane lipoprotein of *Brachyspira hyodysenteriae*. *Veterinary Microbiology* **102**: 97-109.

La, T., Tan, P., Phillips, N. D. and Hampson, D. J. (2005). The distribution of *bmp*B, a gene encoding a 29.7kDa lipoprotein with homology to MetQ, in *Brachyspira hyodysenteriae* and related species. *Veterinary Microbiology* **107**: 249-256.

Hampson, D. J., La, T., Adler, B. and Trott, D. J. (2006). Proposed revisions to the nomenclature for *Brachyspira* membrane proteins and lipoproteins. *Microbiology* **152**: 1-2.

Abstracts in conference proceedings:

La T., Lee B. J., Mikosza A. S. J. and Hampson D. J. (2000). Identification and recombinant expression of a 30 kDa outer envelope lipoprotein of *Brachyspira hyodysenteriae*. In *Proceedings of the 16th International Pig Veterinary Society Congress*, Melbourne, Australia. p. 35.

La T., Phillips N. D., Reichel M. P. and Hampson D. J. (2004). Vaccination against swine dysentery using recombinant BmpB, a 30 kDa outer membrane lipoprotein of *Brachyspira hyodysenteriae*. In *Proceedings of the 18th International Pig Veterinary Society Congress*, Hamburg, Germany. p. 248.

Patents arising from the work in this thesis:

Hampson, D. J. and La, T. (2006). BmpB novel nucleotide and amino acid sequences and diagnostic and therapeutic uses thereof. Patent number US2006057151. United States of America.

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ABBREVIATIONS

The following abbreviations have been used in the thesis:

AP	Alkaline phosphatase
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CBB	Coomassie Brilliant Blue
CSPD	Chemiluminescent substrate for alkaline phosphatase development
DAB	3-3’ Diaminobenzidinetetrahydrochloride
DIG	Digoxigenin
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FCS	Foetal calf serum
FIA	Freunds’Incomplete Adjuvant
HRP	Horseradish peroxidase
IPTG	Isopropyl-β-D-thiogalactopyranoside
LB	Luria-Bertani
LPS	Lipopolysaccharide
Mab	Monoclonal antibody
MBP	Maltose-binding protein
MWCO	Molecular weight cut-off

ABBREVIATIONS (CONTINUED)

Ni-NTA	Nickel-nitrilotriacetic acid
OMP	Outer membrane protein
ORF	Open-reading frame
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween 20
PCR	Polymerase chain reaction
PMSF	Phenylmethylsulphonyl fluoride
RT	Room temperature
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TAE	Tris-acetate-ethylenediaminetetraacetic acid
TBS	Tris buffered saline
TBST	Tris buffered saline containing 0.05% (v/v) Tween 20
TE	Tris-ethylenediaminetetraacetic acid
TEMED	N,N,N',N'-Tetramethyl-ethylenediamine
TMB	Tetramethylbenzidine
TSA	Trypticase soy agar
TSB	Trypticase soy broth
UV	Ultraviolet