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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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.

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Yong Song

ABSTRACT

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.*

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.

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mining, and help in using various computer algorithms and in coordination of the selection of suitable candidate genes for further analysis.

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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.

The patented genes are six (NAV-H7, NAV-H8, NAV-H12, NAV-H17, NAV-H34, NAV-H42) for *B. hyodysenteriae*, PCT Patent No. 2005-903317 and two (NAV-P2 and NAV-P10) for *B. pilosicoli*, PCT Patent No. 2005-902418.

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.

Song, Y. and Hampson, D. J. (2006) Reverse vaccinology: application of functional genomics to *B. hyodysenteriae* and *B. pilosicoli* (Poster). *16th Annual Queenstown Molecular Biology Meeting*. Queenstown, New Zealand.

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ABBREVIATIONS AND SYMBOLS LIST

amino acid	aa
ammonium persulphate	APS
alkaline phosphatase	AP
Australian Genome Research Facility	AGRF
base pair	bp
bovine serum albumin	BSA
Center for Comparative Genomics	CCG
centimeter	cm
comparative genomic hybridization	CGH
concentration unit	CU
confidence intervals	CI
Degree Celsius	°C
deoxyribonucleic acid	DNA
distilled H_2O	dH_2O
Escherichia coli	*E. coli*
enzyme-linked immunosorbent assay	ELISA
fluorescence-activated cell sorter	FACS
genetic horizontal transfer	GHT
immobilized-metal affinity chromatography	IMAC
immunoglobulin	Ig
intramuscular	im
leukocyte migration inhibition agarose test	LMAT
interleukin	IL

kilobase/s	Kb
kilodalton	kDa
kilogram/s	kg
lipopolysaccharide	LPS
Luria Broth	LB
megabases/s	Mb
melting point	Tm
meter/s	m
micro	m
microgram/s	mg
micrometer/s	mm
micromolar	μM
millilitre/s	ml
millimolar	mM
minute/s	m
NADH oxidase	*Nox*
nanogram/s	ng
nanometer/s	nm
deoxyribonucleotide triphosphate	dNTP
open reading frame	ORF
optical density	OD
outer membrane proteins	OMPs
pathogenicity islands	PAIS

percent	%
percentage guanosine and cytosine	% G+C
periplasmic flagella	FP
phosphate buffered saline	PBS
plaque forming unit	pfu
polymerase chain reaction	PCR
reverse transcription	RT
revolution per minute	rpm
ribonucleic acid	RNA
second/s	s
sodium dodecyl sulphate	SDS
SDS-polyacrylamide gel electrophoresis	SDS-PAGE
swine dysentery	SD
tris-buffered saline	TBS
trypticase soy broth	TSB
tumor necrosis factor	TNF
ultra violet	UV
unit/s	u
volts	v
volume : volume ratio	v/v
weight : volume ratio	w/v