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**Characterisation of a secreted immunogenic protein,
phase-1 flagellin (FliC) of
Salmonella enterica subspecies *enterica* Brandenburg**

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of Doctor of Philosophy in Veterinary Microbiology
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Abstract

Cell-envelope associated and secreted proteins of *Salmonella* are integral for host-pathogen interactions, and for the induction of protective immune responses. An array of exported proteins of *S. Brandenburg* was identified through constructing an expression library using alkaline phosphatase gene technology. A partial digest of *S. Brandenburg* strain S59 was cloned into the vector pJEM11, and expressed in *E. coli*. The DNA inserts from randomly selected alkaline phosphatase positive clones were sequenced, and the sequences were analysed using public databases to find the ones that may play a role in host immune cell activation. The phase-1 flagellin (*fliC*) gene identified from an alkaline phosphatase positive phenotype was chosen for further studies. The complete nucleic acid sequence of the *fliC* gene was obtained by PCR amplification. The complete ORF, part of the variable region (V456) and region IV (V4) of the *fliC* gene were cloned into the pET14b vector for the expression of N-terminal histidine-tagged fusion proteins. The proteins were purified through metal affinity chromatography, and were evaluated for their humoral immunogenic properties by Western blotting with sera collected from 81 sheep naturally infected with *S. Brandenburg*. All 81 naturally infected sheep had IgG antibodies against recombinant FliC, V456, and V4 proteins. Furthermore, Western blotting of sera from 6 SalvexinTM+B-vaccinated sheep (Trial 2004) had IgG antibodies against the 3 recombinant proteins. Whole blood cells of vaccinated sheep did not show interferon-gamma production upon stimulation with recombinant FliC and V456 proteins. Western blotting of sera from sheep vaccinated with SalvexinTM and SalvexinTM+B (Trial 1999), and those from rabbits vaccinated with *S. Brandenburg*, *S. Hindmarsh* and *S. Typhimurium* suggested that recombinant V4 contains epitopes specific for *S. Brandenburg*. Therefore, V4 was used to develop a novel indirect enzyme-linked immunosorbent assay (ELISA) for the detection of serum IgG antibodies in *S. Brandenburg* infected sheep. The ELISA showed a specificity of 100%, and a sensitivity of 93.8%. Furthermore, a new PCR assay was developed targeting *rfbJ*(B) gene in a single reaction, and genes *invA*, *fliC* and *fljB* in a multiplex reaction for the identification of *S. Brandenburg* from pure cultures. The sensitivity and specificity of the PCR assay was calculated to be 100%.

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List of Abbreviations

g	acceleration due to gravity
PhoA	alkaline phosphatase
AP	alkaline phosphatase
APS	ammonium persulfate
AFLP	amplified fragment length polymorphism
APC	antigen presenting cell
AP-PCR	arbitrarily primed-PCR
bp	base pair(s)
BLAST	basic local alignment search tool
BSA	bovine serum albumin
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BCIP/NBT	5-bromo-4-chloro-3-Indolyl phosphate/nitroblue tetrazolium
BPW	buffered peptone water
CIAP	calf intestinal alkaline phosphatase
CO ₂	carbon dioxide
Cat. No.	Catalogue number
Cat. Nos.	Catalogue numbers
CMI	cell-mediated immune
ConA	concanavalin A
CTL	cytotoxic T-cell
°C	degrees Celsius
DC	dendritic cell
dNTP	deoxynucleotide triphosphate
DNA	deoxyribonucleic acid
DAB	3,3'-Diaminobenzidine
Na ₂ HPO ₄	disodium hydrogen phosphate
ESR	Environmental Science and Research Limited
ELISA	enzyme-linked immunosorbent assay
EDTA	ethylenediamine tetraacetic acid
H	flagellar
FAFLP	fluorescent amplified-fragment length polymorphism
Kan ^r	gene conferring kanamycin resistance
GSP	general secretory pathway
HACCP	Hazard Analysis and Critical Control Point
h	hour(s)
HCl	hydrochloric acid
H ₂ S	hydrogen sulphide
ID	identity
IMAC	Immobilized metal affinity chromatography
IgA	immunoglobulin A
IgD	immunoglobulin D
IgG	immunoglobulin G
IgM	immunoglobulin M
IFN-γ	Interferon-gamma
IL	interleukin
IAC	internal amplification control
IPTG	isopropyl-β-δ-thiogalactopyranoside

kb	kilo base pair(s)
kDa	kilodalton
LPS	lipopolysaccharide
LSP	lipoprotein signal peptide
L	litre(s)
LB	Luria-Bertani
MgCl ₂	Magnesium chloride
MgSO ₄	Magnesium sulphate
MHC	major histocompatibility complex
T _m	melting temperature
MLN	mesentric lymph nodes
mRNA	messenger RNA
μg	microgram(s)
μl	microlitre(s)
μm	micrometre(s)
μM	micromolar
mg	milligram(s)
ml	millilitre(s)
mm	millilitre(s)
mM	millimolar
min	minutes
M	molar
KH ₂ PO ₄	monobasic potassium phosphate
MAb	monoclonal antibody
ng	nanogram(s)
nm	nanometre(s)
nM	nanomolar
NCBI	National Centre for Biotechnology
NK	natural killer
NKT	natural killer T
NZ	New Zealand
TEMED	N-N-N-N-tetramethylene diamine
No.	number
ORF	open reading frame
OD	optical density
OMP	outer membrane protein
<i>p</i> NPP	para-nitrophenyl phosphate
PAMP	pathogen-associated molecular patterns
PCR-SSCP	PCR-single-strand conformation polymorphism
%	percent
PBMC	peripheral blood mononuclear cells
FliC	phase-1 flagellin
FljB	phase-2 flagellin
PBS	phosphate-buffered saline
pM	picomolar
PCR	polymerase chain reaction
PMN	polymorphonuclear leukocytes
PMB	polymyxin B sulfate
PVDF	polyvinylidene difluoride

KCl	potassium chloride
PFGE	pulsed-field gel electrophoresis
RAPD-PCR	randomly amplified polymorphic DNA-PCR
RNS	reactive nitrogen species
ROS	reactive oxygen species
rep-PCR	repetitive-element PCR
rpm	revolutions per minute
RNA	ribonucleic acid
RBS	ribosomal binding site
rRNA	ribosomal RNA
SB	<i>Salmonella</i> Brandenburg
SCV	<i>Salmonella</i> containing vacuoles
SPI	<i>Salmonella</i> pathogenicity island
sec	seconds
s-IgA	secretory-IgA
SD	Shine-Dalgarno
SPase	signal peptidase
SP	signal peptide
SRP	signal recognition particle
SI	similarity
NaHCO ₃	sodium bicarbonate
Na ₂ CO ₃	sodium carbonate
NaCl	sodium chloride
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
O	somatic
spp.	species
SD(s)	standard deviation(s)
SSP	standard signal peptide
subsp.	subspecies
SOC	super optimal broth
TLR	toll-like receptor
TMH	transmembrane helix
TSI	triple sugar iron
TAE	tris-acetate-EDTA
TBS	tris-buffered saline
TTBS	tris-buffered saline (TBS) containing 0.1% Tween-20
TE	tris-HCl-EDTA
TNF- α	tumour necrosis factor-alpha
T1SS	type I secretory system
T2SS	type II secretory system
T3SS	type III secretory system
T4SS	type IV secretory system
T5SS	type V secretory system
UV	ultraviolet
Vi	virulent
V	Volts
v/v	volume/volume
w/v	weight/volume
WHO	World Health Organization

XLD xylose-lysine--deoxycholate

Amino acids

A alanine
E glutamic acid
H histidine
L leucine
P proline
S serine
V valine
C cysteine
F phenylalanine
I isoleucine
M methionine
Q glutamine
T threonine
Y tyrosine
D aspartic acid
G glycine
K lysine
N asparagine
W tryptophan

Nucleotides

A adenosine
C cytidine
G guanosine
T thymidine

Nucleotide triphosphates

ATP adenosine triphosphate
CTP cytidine triphosphate
GTP guanosine triphosphate
TTP thymidine triphosphate

Deoxyribonucleotide triphosphates

dATP deoxyadenosine triphosphate
dCTP deoxycytidine triphosphate
dGTP deoxyguanosine triphosphate
dTTP deoxythymidine triphosphate