IDENTIFICATION AND RECOMBINANT EXPRESSION OF A 30 KDA OUTER ENVELOPE LIPOPROTEIN OF *BRACHYSPIRA HYODYSENTERIAE*

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Introduction

Swine dysentery (SD) is a contagious mucohaemorrhagic diarrhoeal disease of pigs, characterised by extensive inflammation and necrosis of the epithelial surface of the large intestine (1). Economic losses due to SD result mainly from growth retardation, costs of medication, and mortality (2). To date, no completely sensitive and specific assays are available for the detection of antibodies against the aetiological agent, *Brachyspira hyodysenteriae*. Recently, we identified and cloned a 30 kDa outer membrane lipoprotein (BmpB) which is specific to *B. hyodysenteriae* and is recognised by experimentally and naturally infected pigs. We propose the use of recombinant BmpB as an antigen for future development of an improved serological test for SD diagnosis.

Materials and Methods

A λ bacteriophage genomic library prepared from *B. hyodysenteriae* strain P18A was screened with monoclonal antibody (mAb) BJL/SH1 using the plaque-lift method. Positive clones were converted to phagemid form by co-infection of *Escherichia coli* cells with a helper phage. The phagemid insert was sequenced and analysed for potential open reading frames (ORF). Potential ORFs were analysed for typical physical characteristics, and compared with sequences in GenBank and SWISS-PROT.

Expression of the phagemid insert was induced and whole cell extracts of the *E. coli* were separated by SDS-PAGE and blotted with mAb BJL/SH1. Mice and pigs were immunised with formalinised whole *E. coli* cells containing the phagemid. Serum taken from the animals were subjected to Western blot with OMP extracts of *Brachyspira* spp.. Whole extracts of *E. coli* cells containing the phagemid were also used in Western blots with sera from healthy pigs and pigs naturally infected with *B. hyodysenteriae*.

The deduced ORF from the phagemid was amplified and cloned into the *Xho*1/*Eco*R1 site of the expression vector pTrcHis. Expression of the pTrcHis fusion-protein, designated BmpB, was induced with IPTG, and the recombinant protein purified using nickel affinity columns. The purified fusion-protein was Western blotted with serum from healthy (n=7) and naturally infected (n=13) pigs.

Results

Plaque lifts with mAb BJL/SH1 produced five phagemid clones all expressing a 29 kDa protein. Sequence analysis revealed a 816 bp ORF (designated ORF1) with an ATG start codon, putative –10 (TATAAT) and –35 (TTGAAA) promotor regions, ribosome binding site (AGGAG) and a TAA stop codon. Analysis of the amino acid sequence showed the presence of a 19 aa lipoprotein precursor signal peptide at the N-terminal of the 29.7 kDa predicted polypeptide. According to the Nakai and Kanehisa (3) expert system, ORF1 encodes a lipoprotein which is possibly localised on the outer membrane of *B. hyodysenteriae*. Results from the comparison of ORF1 with GenBank and SWISS-PROT databases are shown in Table 1.

Sera from mice and pigs immunised with *E. coli* cells containing phagemid recognised a 30 kDa protein in OMP extracts of *B. hyodysenteriae*, *Brachyspira murdochii* and *Brachyspira pilosicoli*. Sera from pigs with natural SD recognised a range of proteins in the whole cell extract of *E. coli* containing phagemid. Twelve of the thirteen serum samples from these pigs reacted with a protein of similar size to the 30 kDa protein that reacted with mAb BJL/SH1. The sera from the healthy pigs did not show reactivity with proteins of this size.

Expression of BmpB in pTrcHis resulted in a 34 kDa fusion-protein reacting with mAb BJL/SH1. Twelve of the thirteen serum samples from pigs naturally infected with *B. hyodysenteriae* reacted with BmpB. Serum from the seven healthy pigs tested reacted weakly with BmpB.

Table 1. Results of GenBank and SWISS-PROT sequence comparisons for ORF1.

Bacterial species	Sequence type	Sequence name	Homology
Pasteurella haemolytica	nucleotide	plp3	54% in 735bp
P. haemolytica	amino acid	Plp1	34% in 242 aa
P. haemolytica	amino acid	Plp2	37% in 250 aa
P. haemolytica	amino acid	Plp3	39% in 272 aa
E. coli	amino acid	RcsF	40% in 267 aa
E. coli	amino acid	Lipoprotein-28	36% in 263 aa

Discussion

The gene encoding a 30 kDa protein of *B. hyodysenteriae* was identified and designated *bmp*B. The cross-reactivity of serum from animals immunised with *B. innocens* and *B. pilosicoli* was not entirely unexpected. Structural proteins in related organisms are likely to share epitopes which would stimulate cross-reacting antibodies when they are used as immunogens. The fact that mAb BJL/SH1 did not react with *Brachyspira* spp. other than *B. hyodysenteriae* confirms that the epitope reacting with this mAb was unique to *B. hyodysenteriae* (4). Sera from pigs with SD also reacted with whole cell extracts of *E. coli* expressing the recombinant BmpB, confirming the *in vivo* expression of the gene.

Unfortunately weak reactions also were obtained when the recombinant BmpB fusion protein was tested with sera from seven healthy pigs. It is possible that these cross-reactions may be low affinity non-specific antibody binding and may be removed with modifications to reaction conditions. Currently, we are mapping the mAb BJL/SH1 epitope on BmpB in order to use this peptide sequence as an antigen for future development of a serological test for SD.

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