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Veterinary Microbiology 113 (2006) 109–116

veterinary microbiology

www.elsevier.com/locate/vetmic

Short communication

SmpB: A novel outer membrane protein present in some *Brachyspira hyodysenteriae* strains

James Holden, George Moutafis, Taghrid Istivan, Peter J. Coloe, Peter M. Smooker *

School of Applied Sciences, PO Box 71, RMIT University, Bundoora 3083, Australia

Received 21 August 2005; received in revised form 28 September 2005; accepted 11 October 2005

Abstract

A novel outer membrane protein-encoding gene was identified in *Brachyspira hyodysenteriae*. The predicted protein, SmpB, was encoded by a gene that contains regions of identity with that encoding the previously identified lipoprotein SmpA. However, the majority of the reading frame encoding SmpA and SmpB share no detectable similarity. Analysis of several strains revealed that *B. hyodysenteriae* harbours either *smpA* or the newly identified gene *smpB*, but not both. *smpB* encodes for a slightly larger protein than *smpA*, 17.6 and 16.8 kDa, respectively. The predicted proteins share an identical leader sequence and the first 10 amino acids of the mature protein, however, the remainder of the predicted protein sequence shows no similarity. It is hypothesised that *smpA* and *smpB* are present on the same area of the chromosome. The proteins are antigenically unique, as antisera raised against a strain of *B. hyodysenteriae* that expresses SmpA cannot detect SmpB and vice versa. Although the presence of an identical leader peptide suggests identical localisation of SmpA and SmpB, it is not known if the two predicted proteins share similar function.
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Keywords: *Brachyspira hyodysenteriae*; Spirochetes; Outer membrane; SmpB

1. Introduction

Brachyspira hyodysenteriae is a Gram-negative, anaerobic spirochete and is the etiological agent of swine dysentery. The disease affects pigs in the post-weaning period and produces a mucohemorrhagic diarrhoea that results in rapid weight loss, and is characterised by inflammation, oedema and necrosis of the large intestine (Smith et al., 1990; ter Huurne and Gaastra, 1995).

Previous characterisation of protein components within the outer membrane of *B. hyodysenteriae* revealed the existence of a 16 kDa immunodominant lipoprotein, SmpA (Thomas et al., 1992). The gene encoding this protein was isolated and characterised by sequence analysis (Thomas and Sellwood, 1993). The N-terminal sequence of the predicted protein contains a putative acylation and signal peptidase II

* Corresponding author. Tel.: +61 3 99257129; fax: +61 3 99257110.
E-mail address: peter.smooker@rmit.edu.au (P.M. Smooker).

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doi:10.1016/j.vetmic.2005.10.025

cleavage site that produces a mature 16.1 kDa lipoprotein. SmpA is a membrane-associated lipoprotein that localises to the outer surface of the spirochete. Expression of *smpA* is environmentally regulated, with decreased expression observed in swine post infection (Sellwood et al., 1995). Monoclonal antibodies generated against SmpA agglutinate and prevent the growth of *B. hyodysenteriae* in vitro (Thomas and Sellwood, 1992). This may indicate the potential of SmpA as a vaccine candidate, although as yet no in vivo investigation of this potential has been reported. The function of SmpA remains unknown.

In previous reports, using a DNA probe encompassing the entire reading frame identified by Thomas and Sellwood (1993), strong hybridisation to isolated genomic DNA was detected in half of the strains of *B. hyodysenteriae* examined (Turner et al., 1995). When the stringency of the DNA/DNA blot was reduced, each of the 24 strains tested had a positive signal. However, when a polymerase chain reaction (PCR) using primers designed from within the reading frame was used to detect the presence of the gene, only those in which a strong signal was achieved after high-stringency washing yielded an amplification product.

As part of a study to express and characterise recombinant *B. hyodysenteriae* proteins, *smpA* was amplified by PCR and characterised. Surprisingly, the nucleotide sequence determined from the product amplified from some strains showed only regions of similarity to that previously reported by Thomas and Sellwood (1993). The protein sequence predicted also showed only a limited region of similarity. These observations, which explain the variability of gene detection and organisation observed by Turner et al. (1995), led to the identification of a predicted novel *B. hyodysenteriae* gene, *smpB*.

2. Materials and methods

2.1. Bacterial strains

B. hyodysenteriae 5380 was isolated from swine displaying symptoms of acute swine dysentery, and was characterised as a virulent isolate (Coloe, unpublished observations). Other *B. hyodysenteriae* strains (indicated within the results) were originally isolated from various sites in Australia, Canada, United Kingdom and the United States (some listed in Sotiropoulos et al., 1994). A non-disease causing species, *B. innocens* B256, was used as a non-pathogenic control. The majority of strains were sourced from Victoria, Australia. Strains were routinely cultured for 3–4 days in an anaerobic environment on Trypticase Soy Broth (Oxoid, Australia) solidified with Bacteriological agar (Oxoid, Australia) and supplemented with 5% sheep blood and 760 mg/L spectinomycin sulphate (UpJohn, Sydney, Australia).

2.2. Chromosomal DNA isolation and manipulations

High-molecular-weight DNA was extracted and PCR DNA amplification was performed using AmpliTaq polymerase (Perkin-Elmer) in accordance with the manufacturer's recommendations. For amplification of *smpA* the primers smp1 5′-ATGGATCCGTTCTTAAATAATACCATAATC and smp2 5′-ATTAGGATCCGCCAGTCAAATAATCTTTTAAT were used. The PCR reaction mixture contained 1.5 U of AmpliTaq DNA polymerase, 50 ng of *B. hyodysenteriae* DNA, PCR primers at a final concentration of 0.2 μM, 2 mM $MgCl_2$, 0.2 mM dNTP, 1× PCR buffer (Perkin-Elmer), and H_2O up to 25 μL. The PCR was incubated in a Perkin-Elmer 2400 thermocylcer. The reaction was preheated to 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, with a final extension at 72 °C for 7 min. The PCR products were cloned into the TA cloning vector pCR2.1 (Invitrogen) according to the manufacturer's recommendations. Nucleotide sequence analysis was performed using the BigDye Terminator Sequencing Kit (Applied Biosystems), according to the manufacturer's recommendations. The nucleotide sequence obtained has been submitted to GenBank (accession no: DQ223651).

Digests of smp1/smp2 amplification products were performed on 10 μL of PCR product using 2 U of *Hin*dIII according to manufacturer's directions (Promega). Digestion products were separated by electrophoresis in a 1.5% agarose gel and visualized using ethidium bromide staining.

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