Distribution of the *smpA* gene from *Serpulina hyodysenteriae* among intestinal spirochaetes

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Forty intestinal spirochaete strains were investigated for nucleotide sequences related to the *smpA* locus from *Serpulina hyodysenteriae* by Southern hybridization of chromosomal DNA using the *smpA* locus from *S. hyodysenteriae* strain P18A as a probe and by PCR using primers internal to the *smpA* gene. The intensity of the hybridization signal at high stringency and positive PCR results suggested that 12 *S. hyodysenteriae* strains possessed a similar nucleotide sequence. PCR was negative for another 12 *S. hyodysenteriae* strains and the hybridization signal obtained from 11 of these was weak and one was negative. All *S. hyodysenteriae* strains hybridized under low stringency conditions. These results indicated that there is variation among the *smpA* loci of *S. hyodysenteriae* strains. Among seven strains of *S. innocens*, and the proposed species '*S. intermedius*' and '*S. murdochii*', hybridization was weak and no PCR products were obtained, suggesting that these species have sequences related to, but divergent from, the *smpA* sequences of strains of *S. hyodysenteriae*. Both gene probe hybridization and PCR analysis of nine strains of the proposed new genus '*Anguillina*', including isolates from pigs and humans, gave negative results.

Keywords: swine dysentery, *Serpulina hyodysenteriae*, *smpA* gene, intestinal spirochaetes, PCR

INTRODUCTION

Serpulina hyodysenteriae, an intestinal spirochaete, is the causative agent of swine dysentery (Taylor & Alexander, 1971; Harris *et al.*, 1972). However, the severe clinical signs of the disease are manifest only when other bacterial species are present (Lysons *et al.*, 1978). Spirochaetes have also been isolated from pigs with mild diarrhoea (Hudson *et al.*, 1976; Kinyon *et al.*, 1977; Lemcke & Burrows, 1979; Taylor *et al.*, 1980) and from healthy pigs showing no clinical signs of disease (Kinyon & Harris, 1979; Lemcke & Burrows, 1979). Some of these spirochaetes are non-pathogenic and therefore have been called *S. innocens* (Kinyon & Harris, 1979). More recently, other researchers have observed in pigs intestinal disorders associated with spirochaetes other than *S. hyodysenteriae* and *S. innocens* (Taylor *et al.*, 1980; Andrews & Hoffman, 1982; Binek & Szykiewicz, 1984; Jacques *et al.*, 1989). This condition has been called 'spirochaetal diarrhoea' or 'intestinal spirochaetosis' (Taylor *et al.*, 1980).

A comprehensive classification and study of the diversity of the intestinal spirochaetes has recently been performed. These investigations identified a number of groups of

spirochaetes distinct from *S. hyodysenteriae* and *S. innocens*. It was proposed that these additional groups be called '*S. intermedius*', '*S. murdochii*' and '*Anguillina coli*' (Lee *et al.*, 1993a, b, c; Lee & Hampson, 1994).

Anaerobic spirochaetes also have been isolated from the colon of humans with diarrhoea (Sanna *et al.*, 1982, 1984; Ruane *et al.*, 1989; Barrett, 1990; Lee & Hampson, 1992), and 71 of these isolates have been shown to be also of the group '*Anguillina*' spp. (Lee *et al.*, 1993c; Lee & Hampson, 1994).

Investigation of the outer-membrane proteins from *S. hyodysenteriae* has identified a highly antigenic 16 kDa lipoprotein (Sellwood *et al.*, 1989; Thomas *et al.*, 1992). This lipoprotein was shown to be present on the surface of several strains of *S. hyodysenteriae*, but was not detected in *S. innocens* or '*S. intermedius*' strains when tested using a hyperimmune polyclonal serum (Sellwood *et al.*, 1989) or monoclonal antibodies (Thomas & Sellwood, 1992) raised against the SmpA antigen. The gene for this 16 kDa lipoprotein has been cloned from *S. hyodysenteriae* strain P18A and is called *smpA* (Thomas & Sellwood, 1993). Little is known about the function of the SmpA lipoprotein and its role in pathogenesis in swine dysentery.

Table 1. Southern hybridization and PCR analysis of intestinal spirochaetes using a probe and primers from the *smgA* gene of *S. hyodysenteriae* strain P18A

Spirochaete	Hybridization stringency*		PCR†	Reference
	High	Low		
<i>S. hyodysenteriae</i>				
B234	±	+	—	Kinyon & Harris (1979)
MLC52/80	+	+	+	Kent <i>et al.</i> (1989)
WA-1	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
NSW1	+	+	+	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
Q18	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
Q14	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
B78	+	+	+	Kent <i>et al.</i> (1989)
KF9	±‡	±‡	—	Kent <i>et al.</i> (1989)
A1	+	+	+	Kinyon & Harris (1979)
P18A	+	+	+	Kent <i>et al.</i> (1989)
WA-3	+	+	+	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
WA-6	+	+	+	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
B204	+	+	+	Kinyon & Harris (1979)
B169	+	+	+	Kent <i>et al.</i> (1989)
WA-15	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
Q1	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
NSW2	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
Q19	±‡	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
Vic38	±‡	±‡	+	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
SA3	—	±‡	—	Combs <i>et al.</i> (1992); Lee <i>et al.</i> (1993a)
VS1	+	+	+	Kent <i>et al.</i> (1989)
S75/1	±	+	NT	Kent <i>et al.</i> (1989)
P35/2	+	+	+	Kent <i>et al.</i> (1989)
JWPM	±	+	—	Lemcke & Bew (1984)
<i>'S. intermedius'</i>				
2818.5	—	±§	—	Lee <i>et al.</i> (1993b)
PWS/A	—	±‡	—	Hudson <i>et al.</i> (1976)
889	—	±‡	—	Lee <i>et al.</i> (1993b)
<i>S. innocens</i>				
B256	±	+	—	Kinyon & Harris (1979)
4/71	—	±	—	Hudson <i>et al.</i> (1976)
<i>'S. murdochii'</i>				
155-20	±¶	±¶	—	Lee <i>et al.</i> (1993b); Lee & Hampson (1994)
88-4609	—	±**	—	Lee <i>et al.</i> (1993b); Lee & Hampson (1994)
<i>'A. coli'</i>				
P43/6/78	—	—	—	Taylor <i>et al.</i> (1980)
3295	—	—	—	Lee <i>et al.</i> (1993b)
M1	—	—	—	Lemcke & Burrows (1979)
Kar	—	—	—	Lee <i>et al.</i> (1993c)
HRM7	—	—	—	Sanna <i>et al.</i> (1984)
Mat	—	—	—	Lee <i>et al.</i> (1993c)
2717	—	—	—	Lee <i>et al.</i> (1993b)
Jer	—	—	—	Lee <i>et al.</i> (1993c)
L72	—	—	—	Lee <i>et al.</i> (1993b)

* +, Strong hybridization; ±, weak hybridization; —, no detectable hybridization.

† +, PCR product obtained; —, no detectable PCR product; NT, not tested.

‡ Hybridization to 1.0 kbp fragment.

§ Hybridization to 1.4 kbp fragment.

|| Hybridization to 7 kbp fragment.

¶ Hybridization to fragments of 1.4 and 2.0 kbp.

** Hybridization to 2.2 kbp fragment.

The polyclonal serum (Sellwood *et al.*, 1989) and monoclonal antibody (Thomas & Sellwood, 1992) data suggest that SmpA may be specific to *S. hyodysenteriae*. However, detection of SmpA could be unreliable due to variation in levels of expression of the *smpA* gene. Therefore, we have investigated the distribution of the *smpA* gene and related sequences among *Serpulina* and other intestinal spirochaetes using nucleic acid hybridization and PCR analysis.

METHODS

Spirochaetes. All of the spirochaetes used in this study have been described elsewhere and their source is given in Table 1. Spirochaetes were grown in trypticase soy broth (TSB) containing 5% (v/v) rabbit serum as described by Kent *et al.* (1989), except for the Australian isolates, which were grown anaerobically in TSB containing 2% (v/v) foetal calf serum, 0.5% newborn calf serum and 0.002% cholesterol.

Preparation of chromosomal DNA. DNA was prepared by a modification of the method of Silhavy *et al.* (1984). Spirochaete cells were harvested from 50 ml bottle cultures and resuspended in 0.7 ml 10 mM Tris/HCl, pH 8, 1 mM EDTA. Proteinase K (0.15 ml of 1 mg ml⁻¹) in 0.5% SDS, 50 mM Tris/HCl, pH 8, 0.4 M EDTA was added and the mixture was incubated at 60 °C for 20–60 min. The mixture was then extracted with phenol/chloroform (50:50, v/v) and the nucleic acids were precipitated by addition of 0.1 vol. 3 M sodium acetate, pH 5.2 and 2 vols absolute ethanol. The spool of nucleic acid was rinsed twice in 70% (v/v) ethanol then resuspended in 0.2–1.0 ml 10 mM Tris/HCl, pH 8, 1 mM EDTA containing RNaseA (0.2 mg ml⁻¹). Chromosomal DNA was quantified visually by agarose gel electrophoresis. Restriction endonucleases *Sau3AI* and *HindIII* were obtained from Amersham and Stratagene. Electrophoresis of digested chromosomal DNA was performed using 0.8–1% (w/v) agarose and 0.04 M Tris-acetate, 1 mM EDTA electrophoresis buffer at 40–60 V for 2–4 h, and Southern transfer was performed according to standard protocols (Sambrook *et al.*, 1989).

Southern hybridization. The *smpA* probe was constructed from a region of pWT734, the plasmid containing the complete *smpA* gene from strain P18A described by Thomas & Sellwood (1993). DNA from positions 119 to 1001 bp of the DNA insert from pWT734, which includes the *smpA* open reading frame, was amplified by PCR using primers with *Bam*HI sites at their outside ends. *E. coli* XL1-Blue cells were transformed by electroporation (Dower *et al.*, 1988) with phagemid pBluescript SK(–) (Stratagene) containing the amplified fragment cloned into the *Bam*HI site using standard procedures (Sambrook *et al.*, 1989). The fragment was purified by restriction digestion of plasmid DNA followed by agarose gel electrophoresis and re-isolation of the fragment using a GENECLAN II kit (Bio 101). The purified fragment was labelled and hybridization performed using an enhanced chemiluminescence random prime labelling and detection kit and Hybond N+ membranes (Amersham) for 15–17 h with the *smpA* probe at 10–20 ng ml⁻¹. Membranes were washed under low (1 × SSC, 0.1% SDS at 55 °C) or high (0.1 × SSC, 0.1% SDS at 65 °C) stringency and chemiluminescence detection was allowed to proceed for 55–65 min.

PCR amplification of spirochaete DNA. The nucleotide sequences for primers SH1 and SH4 were determined from DNA sequences within the *smpA* open reading frame. SH1 (5'-TTTAGTAGTTGCCGCATCTG-3') was from nucleotide 145

to 164 and SH4 (5'-AATTTGATGTTCCAAGAGCTT-3') was the reverse complementary sequence of nucleotides 504–524 of the pWT734 insert (Thomas & Sellwood, 1993). Template DNA (100–200 ng) was amplified by PCR (Scharf *et al.*, 1988) using primers SH1 and SH4 (20 ng) in a reaction buffer containing MgCl₂ (2.0 mM). The PCR reaction mix was incubated for an initial denaturation period (5 min, 94 °C) followed by 35 cycles consisting of annealing (1 min, 55 °C), elongation (30 s, 72 °C) and subsequent denaturation (30 s, 94 °C). The PCR products were separated by electrophoresis in 1.5% agarose in Tris-borate buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0) at 80 V for 50 min. The gel was stained with ethidium bromide and photographed using UV transillumination.

RESULTS

Results of DNA hybridization and PCR analysis are summarized in Table 1.

Hybridization of chromosomal DNA with *smpA*

When chromosomal DNA from *S. hyodysenteriae* strains was digested with *Sau3AI* and hybridized with the *smpA* probe under high stringency conditions 12 of the 24 *S. hyodysenteriae* strains produced a strong signal (Fig. 1; lanes 2, 4, 7, 9, 10, 11, 12, 13, 14, 19, 38, 40, 42 and 43, with lanes 19 and 43 showing the same strain, and lanes 10 and 42 showing the same strain); the probe hybridized to a 1.4 kbp fragment in all these strains except strain Vic38 (lanes 19 and 43), for which hybridization was mainly to a fragment of approximately 1.0 kbp. The probe hybridized weakly to a 1.4 kbp fragment in three strains (lanes 1, 39 and 41), to a 1.0 kbp fragment in eight strains (lanes 3, 5, 6, 8, 15, 16, 17 and 18) and one strain called SA3 (lane 20) appeared negative. When hybridization was performed under low stringency conditions the *smpA* probe hybridized to a mixture of fragments in all the *S. hyodysenteriae* strains (not shown), and for strain SA3 the strongest hybridization was to a 1.0 kbp fragment. For the 12 *S. hyodysenteriae* strains that showed strong hybridization under high stringency conditions the probe hybridized at low stringency to up to five additional fragments, while the other *S. hyodysenteriae* strains showed hybridization to one or two additional fragments.

The seven spirochaetes belonging to *S. innocens* and the proposed species '*S. intermedius*' and '*S. murdochii*' failed to hybridize strongly with the *smpA* probe under high stringency (Fig. 1; lanes 21–27). *S. innocens* strain B256 (lanes 24 and 44) and '*S. murdochii*' strain 155–20 (lane 26) both gave weak signals, and under low stringency the remaining strains also showed weak hybridization to one or two fragments. For two of the '*S. intermedius*' strains (PWS/A and 889) this low stringency hybridization was to a fragment of 1.0 kbp, whilst for the other strains the fragment sizes varied.

Nine spirochaetes belonging to the proposed genus '*Anguillina*' (Fig. 1; lanes 28–36), including four isolates from humans (lanes 31, 32, 33 and 35), were negative under both high and low stringency hybridization.

Fig. 1. Chemiluminograph of chromosomal DNA from intestinal spirochaetes digested with *Sau3AI* and hybridized with the *smpA* probe under high stringency conditions. Lanes: 1, B234; 2, MLC52/80; 3, WA-1; 4, NSW1; 5, Q18; 6, Q14; 7, B78; 8, KF9; 9, A1; 10, P18A; 11, WA-3; 12, WA-6; 13, B204; 14, B169; 15, WA-15; 16, Q1; 17, NSW2; 18, Q19; 19, Vic38; 20, SA3; 21, 2818.5; 22, PWS/A; 23, 889; 24, B256; 25, 4/71; 26, 155-20; 27, 88-4609; 28, P43/6/78; 29, 3295; 30, M1; 31, Kar; 32, HRM7; 33, Mat; 34, 2717; 35, Jer; 36, L72; 37, pWT734 *HindIII*; 38, VS1; 39, S75/1; 40, P35/2; 41, JWPM; 42, P18A; 43, Vic38; 44, B256. Plasmid pWT734 contains the *smpA* gene from strain P18A and is a positive control. Every fifth track is labelled along the top. *HindIII*-digested bacteriophage lambda DNA fragment sizes are indicated.

When chromosomal DNA from spirochaetes was digested with *HindIII*, the *smpA* probe hybridized strongly to two fragments in two *S. hyodysenteriae* strains. Strain VS1 had fragments of 8 kbp and 10 kbp, which hybridized with the probe, and strain WA-3 had fragments of 1.4 kbp and 15–20 kbp (Fig. 2). Twenty-one of the remaining *S. hyodysenteriae* strains (including strain P18A, Fig. 2) showed hybridization of varying degrees to only one fragment, and chromosomal DNA from strain Vic38 did not digest with *HindIII*. Only single bands of 1.4 kbp were observed when the probe was hybridized to DNA from VS1, WA-3 and P18A digested with *Sau3AI* alone or with *Sau3AI* and *HindIII* in double restriction enzyme digests (Fig. 2).

Analysis of intestinal spirochaetes using PCR

When primers SH1 and SH4 were used for PCR, 12 of 23 *S. hyodysenteriae* strains gave yields of product of the expected size (382 bp) which could easily be observed on agarose gels, examples of which are shown in Fig. 3. All 12 strains that produced a strong signal under high stringency hybridization conditions also were positive by PCR, whereas all the other strains (except S75/1, which was not tested) were negative. The non-*S. hyodysenteriae* spirochaetes were also negative by PCR (Table 1). These included the nine strains belonging to the proposed genus '*Anguillina*'.

DISCUSSION

This investigation was undertaken in order to establish the extent of the presence of the *smpA* gene and related nucleotide sequences in intestinal spirochaetes from pigs and man, and to ascertain if there was any preferential

association of the gene with *S. hyodysenteriae* strains as previously suggested by Sellwood *et al.* (1989) and Thomas & Sellwood (1992). PCR was employed to investigate the use of primer pairs from the nucleotide sequence of the *smpA* gene for the detection of *S. hyodysenteriae* strains and to assess the suitability of PCR for identification. The Southern hybridization and PCR results suggest some variation in the *smpA* genes or related sequences in different strains of *S. hyodysenteriae*. It appears that strains MLC52/80, NSW1, B78, A1, P18A, WA-3, WA-6, B204, B169, Vic38, VS1 and P35/2 have closely related *smpA* sequences. On the other hand, the weaker hybridization obtained for strains B234, WA-1, Q18, Q14, KF9, WA-15, Q1, NSW2, Q19, SA3, S75/1 and JWPM suggests that these have sequences which are not as closely related to *smpA*. There were no obvious correlations between these differences in the sequences and the serotype or genetic grouping of the *S. hyodysenteriae* strains (Lee *et al.*, 1993a).

The presence of two bands in VS1 and WA-3 when their DNA was digested with *HindIII*, and the presence of single bands when double *HindIII/Sau3AI* restriction enzyme digests were made suggests that two separate *smpA* loci are present in these strains. The existence of two copies of the gene in strain VS1 could explain the observation that VS1 expressed higher levels of SmpA protein (16 kDa antigen) than other strains of *S. hyodysenteriae* (Sellwood *et al.*, 1989; Thomas & Sellwood, 1992). However, the observation could also be due to other reasons, such as regulation of gene expression.

The hybridization results obtained under high and low stringency conditions indicate that *S. innocens* and the proposed species '*S. intermedius*' and '*S. murdochii*' possess a sequence which shows at least some similarity to *smpA*,

Fig. 2. Chemiluminograph of chromosomal DNA from *S. hyodysenteriae* strains P18A (lanes 1, 4 and 7), WA-3 (lanes 2, 5 and 8) and VS1 (lanes 3, 6 and 9) digested with *Hind*III (lanes 1–3), *Hind*III/*Sau*3AI (lanes 4–6) and *Sau*3AI (lanes 7–9) and hybridized with the *smpA* probe under high stringency conditions.

Fig. 3. PCR amplification of *smpA* in strains of *S. hyodysenteriae*, using primers SH1 and SH4 at high stringency. Lanes: 1, pUC19 *Hpa*II size markers; 2, no DNA; 3, B234; 4, Q1; 5, KF9; 6, P35/2; 7, VS1; 8, B169; 9, B204; 10, WA-6; 11, WA-3; 12, P18A; 13, B78; 14, NSW1; 15, MLC52/80.

though probably with more variation than exists between the *S. hyodysenteriae* strains. However, within the proposed '*Anguillina*' species the *smpA* sequence was not detected, and if an analogue of the gene is present it must be very different from *smpA*. These results are in general agreement with the classification of intestinal spirochaetes by Lee *et al.* (1993a, b, c).

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REFERENCES

- Andrews, J. J. & Hoffman, L. J. (1982). A porcine colitis caused by a weakly beta-haemolytic treponeme (*Treponema innocens*?). In *Proceedings of the 25th Annual Meeting of the American Association of Veterinary Laboratory Diagnostics*, p. 395.
- Barrett, S. P. (1990). Intestinal spirochaetes in a Gulf Arab population. *Epidemiol Infect* **104**, 261–266.
- Binek, M. & Szykiewicz, Z. M. (1984). Physiological properties and classification of strains of *Treponema* sp. isolated from pigs in Poland. *Comp Immunol Microbiol Infect Dis* **7**, 141–148.
- Combs, B. G., Hampson, D. J. & Harders, S. J. (1992). Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by restriction endonuclease analysis. *Vet Microbiol* **31**, 273–285.
- Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988). High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res* **16**, 6127–6145.
- Harris, D. L., Glock, R. D., Christiansen, C. R. & Kinyon, J. M. (1972). Swine dysentery I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. *Vet Med Small Anim Clin* **67**, 61–64.
- Hudson, M. J., Alexander, T. J. L. & Lysons, R. J. (1976). Diagnosis of swine dysentery: spirochaetes which may be confused with *Treponema hyodysenteriae*. *Vet Rec* **99**, 495–500.
- Jacques, M., Girard, C., Higgins, R. & Goyette, G. (1989). Extensive colonisation of the porcine colonic epithelium by a spirochaete similar to *Treponema innocens*. *J Clin Microbiol* **27**, 1139–1141.
- Kent, K. A., Sellwood, R., Lemcke, R. M., Burrows, M. R. & Lysons, R. J. (1989). Analysis of the axial filaments of *Treponema hyodysenteriae* by SDS-PAGE and immunoblotting. *J Gen Microbiol* **135**, 1625–1632.
- Kinyon, J. M. & Harris, D. L. (1979). *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae*. *Int J Syst Bacteriol* **29**, 102–109.
- Kinyon, J. M., Harris, D. L. & Glock, R. D. (1977). Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. *Infect Immun* **15**, 638–646.
- Lee, J. I. & Hampson, D. J. (1992). Intestinal spirochaetes colonizing Aborigines from communities in the remote north of Western Australia. *Epidemiol. Infect* **109**, 133–141.
- Lee, J. I. & Hampson, D. J. (1994). Genetic characterisation of intestinal spirochaetes and their association with disease. *J Med Microbiol* **40**, 365–371.
- Lee, J. I., Hampson, D. J., Combs, B. G. & Lymbery, A. J. (1993a). Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae*, and comparison of methods for their subspecific differentiation. *Vet Microbiol* **34**, 35–46.
- Lee, J. I., Hampson, D. J., Lymbery, A. J. & Harders, S. J. (1993b). The porcine intestinal spirochaetes – identification of new genetic groups. *Vet Microbiol* **34**, 273–285.
- Lee, J. I., McLaren, A. J., Lymbery, A. J. & Hampson, D. J. (1993c). Human intestinal spirochaetes are distinct from *Serpulina hyodysenteriae*. *J Clin Microbiol* **31**, 16–21.

- Lemcke, R. M. & Bew, J. (1984). Antigenic differences among isolates of *Treponema hyodysenteriae*. In *Proceedings of the 8th International Pig Veterinary Society Congress*, Ghent, Belgium, p. 183.
- Lemcke, R. M. & Burrows, M. R. (1979). A disc growth-inhibition test for differentiating *Treponema hyodysenteriae* from other intestinal spirochaetes. *Vet Rec* **104**, 548–551.
- Lysons, R. J., Hall, G. A., Alexander, T. J. L., Bew, J. & Bland, A. P. (1978). Aetiological agents and pathogenesis of swine dysentery. In *Proceedings of the 5th International Pig Veterinary Society Congress*, Zagreb, p. M1.
- Ruane, P. J., Nakata, M. M., Reinhardt, J. F. & George, W. L. (1989). Spirochaete-like organisms in the human gastrointestinal tract. *Rev Infect Dis* **11**, 184–196.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanna, A., Dettori, G., Grillo, R., Rossi, A. & Chiarenza, D. (1982). Isolation and propagation of a strain of *Treponema* from the human digestive tract – preliminary report. *Ig Mod* **77**, 287–297.
- Sanna, A., Dettori, G., Agliano, A. M., Branca, G., Grillo, R., Leone, F., Rossi, A. & Parisi, G. (1984). Studies of treponemes isolated from human gastrointestinal tract. *Ig Mod* **81**, 959–973.
- Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.
- Sellwood, R., Kent, K. A., Burrows, M. R., Lysons, R. J. & Bland, A. P. (1989). Antibodies to a common outer envelope antigen of *Treponema hyodysenteriae* with antibacterial activity. *J Gen Microbiol* **135**, 2249–2257.
- Silhavy, T. J., Berman, M. L. & Enquist, L. W. (1984). *Experiments with Gene Fusions*, pp. 137–139. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Taylor, D. J. & Alexander, T. J. L. (1971). The production of dysentery in swine by feeding cultures containing a spirochaete. *Br Vet J* **127**, lviii–lxi.
- Taylor, D. J., Simmons, J. R. & Laird, H. M. (1980). Production of diarrhoea and dysentery in pigs by feeding pure cultures of a spirochaete differing from *Treponema hyodysenteriae*. *Vet Rec* **106**, 326–332.
- Thomas, W. & Sellwood, R. (1992). Monoclonal antibodies to a 16 kDa antigen of *Serpulina (Treponema) hyodysenteriae*. *J Med Microbiol* **37**, 214–220.
- Thomas, W. & Sellwood, R. (1993). Molecular cloning, expression, and DNA sequence analysis of the gene that encodes the 16-kilodalton outer membrane lipoprotein of *Serpulina hyodysenteriae*. *Infect Immun* **61**, 1136–1140.
- Thomas, W., Sellwood, R. & Lysons, R. J. (1992). A 16 kilodalton lipoprotein of the outer membrane of *Serpulina (Treponema) hyodysenteriae*. *Infect Immun* **60**, 3111–3116.

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