JOURNAL OF CLINICAL MICROBIOLOGY, July 1993, p. 1746–1752
0095-1137/93/071746-07$02.00/0

Vol. 31, No. 7

Characterization of Two DNA Probes Specific for *Serpulina hyodysenteriae*

CHRIS SOTIROPOULOS,* STUART CRAIG SMITH, AND PETER JOHN COLOE

Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, G.P.O. Box 2476V, Melbourne 3001, Australia

Received 30 November 1992/Accepted 1 April 1993

Two DNA probes, one 1.1- and one 0.75-kb probe, specific for *Serpulina hyodysenteriae* were isolated from a genomic library generated from virulent *S. hyodysenteriae* 5380. These probes are highly specific and react with all *S. hyodysenteriae* strains tested. Under stringent conditions, the DNA probes did not react with the nonpathogenic species *Serpulina innocens* or with other species of enteric bacteria, including *Escherichia coli*. Both probes are able to detect *S. hyodysenteriae* in colony blot hybridizations, and when applied to fecal specimens, they can detect 10^4 *S. hyodysenteriae* cells in 0.1 g of seeded fecal matter. Both probes can detect *S. hyodysenteriae* in fecal specimens from swine with clinical signs of swine dysentery after experimental challenge and from swine from a herd with an acute outbreak of swine dysentery. These probes have application as a diagnostic tool in veterinary microbiology.

Serpulina (*Treponema*) *hyodysenteriae* is the etiological agent of swine dysentery (16, 17, 29), causing mucohemorrhagic diarrhea in swine (7). The organism causes significant morbidity and up to 30% mortality in infected herds (7), resulting in significant economic loss.

The isolation and identification of *S. hyodysenteriae* are presently performed by isolating the organism from fecal specimens, cultivating it onto blood agar plates supplemented with several antibiotics (18), and incubating it anaerobically at 37°C for 3 to 5 days. The specimens are examined for the presence of beta-hemolytic spirochetes with morphology characteristic of *S. hyodysenteriae*. Identifying the isolates to species level is important because of the presence of a nonpathogenic intestinal spirochete, *Serpulina innocens*, which is morphologically similar to *S. hyodysenteriae*. Differentiation between *S. hyodysenteriae* and *S. innocens* is based on the hemolytic activities of these bacteria, with *S. hyodysenteriae* being strongly beta-hemolytic and *S. innocens* being only weakly beta-hemolytic, and differentiation is also made biochemically by using the API ZYM assay (10, 17). Direct microscopic examination of feces is of little benefit, since the two species cannot be readily distinguished morphologically (13, 16). Moreover, in swine with clinical diarrhea not related to swine dysentery, the numbers of *S. innocens* organisms present in the intestinal contents frequently increase, leading to a false diagnosis of swine dysentery (9).

The preferred method for characterizing *S. hyodysenteriae* is sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of cellular proteins and Western blotting (immunoblotting) of the isolates with *S. hyodysenteriae*-specific antisera (25, 26). However, restriction enzyme analysis (6), serotyping (2, 20), and in vitro enteropathogenicity studies have also been used (12, 15, 21, 28), but all these tests are time-consuming, labor-intensive, and expensive.

Nucleic acid techniques have been developed in recent years for the detection of pathogens in clinical microbiology (30). DNA probes have been successfully employed for the detection of spirochetes, including *Borrelia burgdorferi* (23), *Treponema pallidum* (31), and *Leptospira interrogans* (33).

Experimental challenge of swine with *S. hyodysenteriae* 5380 has reproduced classical swine dysentery with mucohemorrhagic diarrhea, and biochemical and immunological analysis confirmed the spirochete isolate to be *S. hyodysenteriae* (26). Therefore, DNA from *S. hyodysenteriae* 5380 was used in the preparation of the genomic library. The strain has recently been classified as a novel serotype by using a lipopolysaccharide-specific monoclonal antibody (1). In the present study, DNA probes specific for *S. hyodysenteriae* have been developed to provide an alternative detection system for the rapid detection of *S. hyodysenteriae*.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains used in this study are shown in Table 1. The plasmid strains used for cloning and hybridization purposes are shown in Table 2.

Culture conditions. *S. hyodysenteriae* strains and two *S. innocens* strains, 594 and 598, were grown on Oxoid Columbia agar supplemented with 5% defibrinated horse blood with 400 mg of spectinomycin sulfate per liter (UpJohn, Sydney, Australia) for 3 to 4 days at 37°C in an anaerobic environment (H_2/CO_2 ratio, 1:1). *S. innocens* B256, 8841, 8441, and 4/71 were grown on Oxoid Columbia agar supplemented with 5% defibrinated horse blood and incubated as described above. *Campylobacter* strains were grown on Oxoid Columbia agar supplemented with 5% defibrinated horse blood and Skirrow's supplement SR-69 (Oxoid, Sydney, Australia) for 48 to 72 h at 37°C in a gaseous environment of 90% N_2, 5% CO_2, and 5% O_2. All other enteric pathogens, including *Escherichia coli* and *Salmonella* spp., were grown on nutrient agar at 37°C overnight.

Chromosomal-DNA isolation. High-molecular-weight DNA was isolated by the method of Collins and Ross (5). Chromosomal DNA extracted from *S. hyodysenteriae* 5380 was analyzed by electrophoresis, and it produced a single high-molecular-weight band with no evidence of plasmids (data not shown). Purified *S. hyodysenteriae* 5380 DNA was partially digested with the restriction enzyme *Sau*3A to yield fragments with an average size of 8 to 15 kilobase pairs (kb).

* Corresponding author.

TABLE 1. Bacterial strains used in this study

Bacterial species	Strain (serotype)[a]	Country of origin	Source[b]
Serpulina hyodysenteriae	5380	Australia	A
	B78[c] (serotype 1)	United Kingdom	A
	B204[c] (serotype 2)	United Kingdom	A
	A1[c] (serotype 4)	United Kingdom	A
	933	Australia	A
	Black 2	United Kingdom	A
	B169[c] (serotype 3)	Canada	A
	B8044[c] (serotype 5)	United States	A
	B6933[c] (serotype 6)	United States	A
	9706	United Kingdom	A
	1165	Australia	A
	1415	Australia	A
	L7273	Australia	A
	B234	United States	A
	4943	Australia	A
	Yugo	Yugoslavia	A
	G strain	Mexico	A
	32468C	Australia	A
	350	Australia	A
	320-91A	Australia	A
	320-91B	Australia	A
	2612	Australia	A
	8929	Poland	A
	WA2528	Australia	A
	VDI	Australia	A
	3460	Australia	A
	924	Australia	A
	Q9374	Australia	A
	8930	Australia	A
	Will A	Australia	A
	Will B	Australia	A
	Q5392	Australia	A
	PM88	Australia	A
	Vella	Australia	A
	27164	United States	A
	600	Australia	A
	S80	United Kingdom	A
	70A	Australia	A
Serpulina innocens	B256	United States	A
	594	Australia	A
	598	Australia	A
	8841	Australia	A
	8441	Australia	A
	4/71	United Kingdom	A
Escherichia coli	DH1	Australia	A
	O41:K85ac	Australia	A
	O149:K91:K88	Australia	A
	O141:K85ab	Australia	A
	103/1-1	Australia	B
Klebsiella pneumoniae	180/2-6	Australia	B
Streptococcus faecalis		Australia	B
Streptococcus pyogenes		Australia	B
Salmonella enteritidis	340/2-1	Australia	B
Salmonella typhimurium	340/4	Australia	B
Shigella sonnei	343/7	Australia	B
Shigella flexneri	343/1	Australia	B
Proteus vulgaris	281/1-1	Australia	B
Klebsiella oxytoca	180/4	Australia	B
Yersinia enterocolitica	40:3	Australia	A
Campylobacter jejuni	NCTC 11351	Australia	A
Campylobacter coli	NCTC 11366	Australia	A
Campylobacter-like organism	0032A RMIT	Australia	A

[a] Serotypes were confirmed by using phenol-water-extracted antigens (2).
[b] A and B, Biotechnology Unit, Department of Applied Biology and Biotechnology, and microbiology strain collection, Department of Applied Biology, respectively, Royal Melbourne Institute of Technology, Melbourne 3001, Australia.
[c] *S. hyodysenteriae* serotype reference strain.

TABLE 2. Plasmids used in this study

Plasmid	Characteristics	Source
pBR322	Ap^r Tet^r	Bolivar
pGEM3Z	Ap^r LacZ′	Promega Corporation
pBT9001	Ap^r pBR322 Ω (*S. hyodysenteriae* DNA; *Sau*3A::10.2kb)	This study
pBT9002	Ap^r pGEM3Z Ω (*S. hyodysenteriae* DNA; *Hin*dIII-*Eco*RI::1.1kb)	This study
pBT9003	Ap^r pGEM3Z Ω (*S. hyodysenteriae* DNA; *Hin*dIII-*Eco*RI::0.75kb)	This study

A total of 250 μg of digested DNA was fractionated by centrifugation on a 10 to 40% linear sucrose density gradient. Fractions containing DNA 8 to 15 kb in length were pooled, dialyzed against Tris-EDTA buffer, and ethanol precipitated. The vector DNA, pBR322, was digested with *Bam*HI, which causes inactivation of the tetracycline resistance gene in pBR322. The cleaved vector was treated with calf intestinal phosphatase to prevent recircularization.

Fractionated DNA was ligated to the dephosphorylated vector at ratios of 1:4 and 1:8. The recombinant vectors were transformed into *E. coli* DH1 by the calcium chloride procedure (19). Transformants were incubated in Luria-Bertani broth for 2 h, plated directly onto Luria-Bertani plates containing 100 μg of ampicillin per ml, and incubated overnight at 37°C. Recombinant plasmids were replica plated onto tetracycline (50 μg/ml)-supplemented Luria-Bertani plates. Tetracycline-sensitive, ampicillin-resistant transformants containing *S. hyodysenteriae* DNA inserts were isolated. Four clones were selected at random and screened with radiolabeled *S. hyodysenteriae* 5380 chromosomal DNA and *S. innocens* B256 chromosomal DNA.

Plasmid DNA extraction. Recombinant plasmids were isolated by the cesium chloride density centrifugation method described by Maniatis et al. (19). Small-scale analysis was performed by the boiling method of Holmes and Quigley (8).

Preparation of radiolabeled probe. All DNA probes were radiolabeled with [α-^{32}P]dATP (Bresatec, Adelaide, Australia) by the nick translation method (22) to a specific activity of 10^6 to 10^7 cpm/μg of DNA. Unincorporated radionucleotides were removed by passage of the reaction mixture through a Sephadex G-50 column.

Dot blot of genomic DNA. Purified genomic DNA was serially diluted from 500 to 3.90 ng, boiled for 5 min, cooled on ice, and applied to positively charged nylon membrane, Hybond N (0.45 μm) (Amersham, Sydney, Australia), with a Minifold II vacuum blotter (Schleicher and Schuell, Bartelt Instruments, Sydney, Australia). The DNA on the filter was then denatured with denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 1 min, neutralized (0.5 M Tris-HCl, 1.5 M NaCl, 0.001 M EDTA [pH 7.2]) for 1 min, dried, and cross-linked to the membrane by using UV light at 312 nm for 2.5 min. The filter was then used directly in the hybridization procedure.

Colony blot of bacterial cells. Bacterial cell numbers were estimated by using a Petroff-Hausser cell-counting chamber. Cells were counted three times, and the average was determined. Pure cultures of bacteria were applied to the nylon membrane by using the Minifold II vacuum blotting apparatus, treated with denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 10 min, and neutralized (0.5 M Tris-HCl, 1.5 M NaCl, 0.001 M EDTA [pH 7.2]) for 5 min. The filter was then used directly in the hybridization procedure.

Hybridization protocol. The filters were used in hybridization experiments in the manner described by Maniatis et al. (19) with the following modifications. Prehybridization was performed for 2 to 4 h at 65°C in a solution containing 600 mM NaCl, 60 mM trisodium citrate, 50 mM KH_2PO_4, and 4 mM EDTA (pH 7.2) supplemented with 5× Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin fraction V), 0.1% SDS, 0.002% tRNA (Boehringer Mannheim, Sydney, Australia), and 100 μg of denatured salmon sperm DNA per ml. Hybridizations were performed at 65°C for 10 h with 1 μg of labeled DNA in fresh hybridization solution. The filters were washed at 65°C to remove the nonhybridized probe with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 15 min (twice), 2× SSC–0.1% SDS for 30 min, and 0.1× SSC. The filters were air dried, and hybridization with the probe was detected by autoradiography with Fuji RX film exposed for 0.5 to 1 day at −70°C with an intensifying screen.

Membrane-stripping protocol. Probes were removed from the filter by incubating the filter in 0.4 M NaOH at 45°C for 30 min. The filter was then incubated in 0.1× SSC–0.1% SDS–0.2 M Tris-HCl for 15 min at 45°C. The filter was exposed to X-ray film to confirm the removal of the probe.

Experimental challenge. Feces (5.0 g) were collected from swine after experimental challenge with 10^{10} virulent *S. hyodysenteriae* cells on two consecutive days. Fecal samples were kept at an ambient temperature during transport to the laboratory (<4 h), and they were stored at −70°C until use. For analysis, a sample (0.1 g) was removed and added to 2 ml of sterile saline. The mixture was vortexed to resuspend the fecal matter and filtered through grade 1 Whatman chromatography paper. The filtrate was placed in a microcentrifuge tube and centrifuged at 12,000 × *g* for 15 min. The pellet was resuspended in 20 μl of sterile saline and applied directly onto a nylon membrane.

Examination of feces from herds with natural infections. Porcine fecal samples from herds with histories of acute clinical dysentery were examined for the presence of *S. hyodysenteriae*. These fecal specimens had been collected from individual swine with acute clinical swine dysentery and had been stored for 2 years at −70°C prior to examination.

RESULTS

Development of the *S. hyodysenteriae* DNA-specific probes. A total of 2,000 recombinants representing *S. hyodysenteriae* inserts of 8 to 15 kb in plasmid pBR322 were generated in a genomic library in *E. coli*. Four randomly selected clones with insert sizes of 3.8, 5.3, 7.0, and 10.2 kb were selected randomly for further evaluation. The recombinant plasmids were spotted onto the nylon membrane and hybridized to radiolabeled whole chromosomal DNA of *S. hyodysenteriae* 5380, and then they were rescreened with *S. innocens* B256.

One clone (pBT9001 with a 10.2-kb *S. hyodysenteriae* DNA insert) which reacted strongly with *S. hyodysenteriae* 5380 but weakly with *S. innocens* B256 was selected for further analysis. The clone was restricted with the restriction

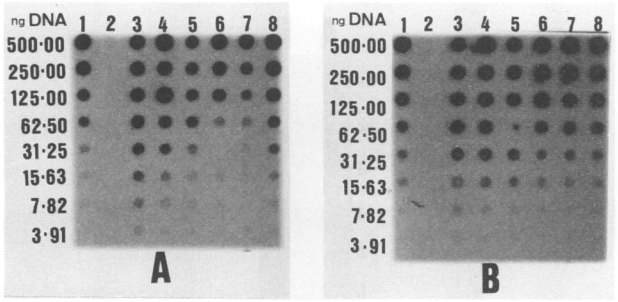

FIG. 1. Hybridization of the 0.75-kb (A) and 1.1-kb (B) DNA probes to purified DNA. Lanes 1 and 2, DNA prepared from *S. hyodysenteriae* 5380 and *S. innocens* B256, respectively; lanes 3 to 8, DNA prepared from *S. hyodysenteriae* B78, B204, B169, A1, B8044, and B6933, respectively.

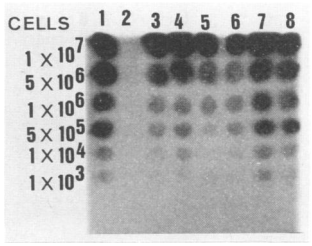

FIG. 2. Hybridization of the 1.1-kb DNA probe to DNA from various strains of *S. hyodysenteriae* lysed in situ. Lanes 1 and 2, DNA prepared from *S. hyodysenteriae* 5380 and *S. innocens* B256, respectively; lanes 3 to 8, DNA prepared from *S. hyodysenteriae* B78, B204, B169, A1, B8044, and B6933, respectively.

enzymes *Hin*dIII and *Eco*RI, and it produced fragments ranging in size from 0.2 to 7.0 kb. Each DNA fragment was evaluated for hybridization to *S. hyodysenteriae* 5380 and *S. innocens* B256 (data not shown). Two fragments, a 0.75- and a 1.1-kb fragment, hybridized strongly to *S. hyodysenteriae* 5380 but not to *S. innocens* B256. These *S. hyodysenteriae* fragments did not hybridize to pBR322 DNA (data not shown). These two DNA fragments were further subcloned into pGEM3Z, and they yielded two recombinant plasmids with inserts of 0.75 and 1.1 kb (pBT9002 and pBT9003). These two clones were present 4.1 kb apart on the recombinant plasmid pBT9001, and they did not hybridize to each other (data not shown). These two clones were selected for further analysis.

Hybridization of the probes with *Serpulina* sp. chromosomal DNA. The insert fragments in pBT9002 and pBT9003 were purified, labeled, and used to probe DNA isolated from the *S. hyodysenteriae* serotype reference strains and *S. innocens* B256 (Table 1). Purified DNAs from the *S. hyodysenteriae* reference strains and *S. innocens* B256 were spotted onto a nylon membrane in twofold dilutions ranging from 500 to 3.90 ng and were then hybridized with the 0.75-kb probe (Fig. 1A) and the 1.1-kb probe (Fig. 1B). All hybridizations were performed under high-stringency conditions at 65°C. Both DNA probes hybridized to the *S. hyodysenteriae* reference strains but not to *S. innocens* B256 (Fig. 1A and B, lanes 2) and could detect as little as 7.82 ng of purified *S. hyodysenteriae* DNA. The 1.1-kb DNA probe hybridized to all *S. hyodysenteriae* reference strains with equal intensity (Fig. 1B), whereas the 0.75-kb probe hybridized less strongly to serotypes 4 and 5 (Fig. 1A, lanes 6 and 7, respectively).

Colony blot hybridization with the *S. hyodysenteriae*-specific probes. The specificity of the DNA probes was further examined by using whole cells from *Serpulina* spp. Strains of *S. hyodysenteriae* and *S. innocens* were applied as whole cells in twofold dilutions ranging from 10^7 to 10^3 onto a nylon membrane and lysed in situ with sodium hydroxide. Cells were hybridized with the 1.1-kb DNA probe under high-stringency conditions (65°C) (Fig. 2). The probe hybridized specifically to the *S. hyodysenteriae* strains but not to *S. innocens* B256 (Fig. 2, lane 2). The detection limit was 10^3 cells for the 1.1-kb probe. The 0.75-kb probe was tested and found to hybridize specifically to the *S. hyodysenteriae* strains, but the detection limit was slightly lower, at 10^4 cells (data not shown).

The 1.1-kb DNA probe was further evaluated for specificity by testing it against a variety of strains and serotypes of *S. hyodysenteriae*, *S. innocens*, and a variety of enteric species from swine and/or humans (Table 3). Normal swine flora such as a *Bacteroides* sp., a *Eubacterium* sp., and a *Fusobacterium* sp. did not react with the *S. hyodysenteriae* DNA probes (Fig. 3, lanes D9 to F9). Whole cells (5×10^5) were applied to a nylon membrane, lysed with sodium hydroxide, and hybridized with the 1.1-kb probe at 65°C (Fig. 3). The 1.1-kb DNA probe hybridized to all *S. hyodysenteriae* strains but not to *S. innocens* strains or to the enteric pathogens (Fig. 3). A similar result was obtained with the 0.75-kb DNA probe (data not shown).

TABLE 3. *Serpulina* spp. and enteric pathogens analyzed by colony dot blot hybridization as shown in Fig. 3

Row	Organism in dot blot lane:								
	1	2	3	4	5	6	7	8	9
A		B78	B204	B169	A1	70A	*Klebsiella pneumoniae*	*Campylobacter jejuni*	*E. coli*[a]
B	5380	L7273	8841[b]	8441[b]	Q5392	598[b]	*Salmonella enteritidis*	RMIT 32A	*E. coli*[c]
C	B256[b]	1415	2612	Yugo	Will A	594[b]	*Streptococcus pyogenes*	*Yersinia enterocolitica*	*E. coli*[d]
D	*E. coli* DH1	WA2528	933	PM88	Will B	VDI	*Klebsiella oxytoca*		*Bacteroides* sp.
E	B8044	1165	34286C	4/71[b]	8930	8929	*Salmonella typhimurium*		*Fusobacterium* sp.
F	B6933	27164	924	G strain	Vella	9706	*Streptococcus faecalis*		*Eubacterium* sp.
G	B234	320-91A	600	S80	Black 2	*E. coli*[e]	*Shigella sonnei*		5380
H		350	4943	320-91B	3460	*Shigella flexneri*	*Campylobacter coli*		

[a] Enterotoxigenic *E. coli* O41:K85ac.
[b] *S. innocens* strain.
[c] Enterotoxigenic *E. coli* O149:K91:K88.
[d] Enterotoxigenic *E. coli* O141:K85ab.
[e] Enteropathogenic *E. coli* 103/1-1.

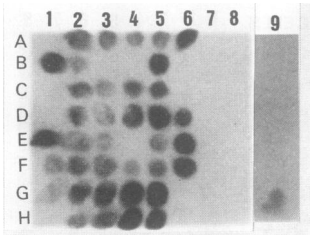

FIG. 3. Colony dot blot hybridization of the 1.1-kb probe with 5 × 10^5 cells of the *Serpulina* sp. strains and enteric pathogens in Table 3. Rows and lanes are as indicated in Table 3.

Detection of *S. hyodysenteriae* DNA in fecal samples. Fecal samples were examined to determine the efficacies of both of the DNA probes for the detection of *S. hyodysenteriae* in fecal material. Fecal matter collected from a swine with no previous history of swine dysentery was experimentally seeded with 1 × 10^4 to 5 × 10^6 whole *S. hyodysenteriae* 5380 cells and examined with both probes. Unseeded fecal material was used as a control. The fecal samples were prepared and spotted onto a nylon membrane and lysed with sodium hydroxide. Control fecal samples, *S. hyodysenteriae*-seeded feces, and whole *S. hyodysenteriae* cells were prepared in parallel, and all samples were hybridized with the 1.1-kb DNA probe under high-stringency conditions (65°C). The results showed that a minimum of 10^4 *S. hyodysenteriae* cells per 0.1 g of feces could be detected (Fig. 4, lane 1). A similar result was obtained with the 0.75-kb probe (data not shown). No hybridization was detected in the control feces with either the 1.1-kb DNA probe (Fig. 4, lane 2) or the 0.75-kb DNA probe (data not shown).

Fecal samples were collected from the following sources: (i) two pigs experimentally challenged with another virulent *S. hyodysenteriae* strain (PM88) and showing clinical signs of swine dysentery, and (ii) two swine naturally infected with swine dysentery. *S. hyodysenteriae* was recovered from all fecal samples and identified by beta-hemolysis, biochemical tests, and serotyping.

Fecal samples (0.1 g) from these pigs (stored at −70°C for 1 month from experimentally challenged swine and 18 months from naturally infected swine) were prepared, spotted onto a nylon membrane, and lysed with sodium hydroxide. The DNA on the filter was hybridized with both the 1.1-kb (Fig. 5, lane A) and 0.75-kb (Fig. 5, lane B) DNA

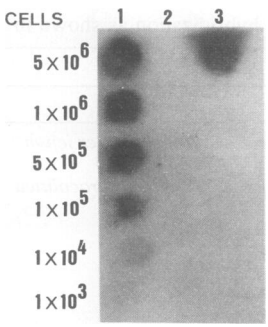

FIG. 4. Colony dot blot hybridization of the 1.1-kb DNA probe with seeded and control swine feces and *S. hyodysenteriae* 5380 cells. Lane 1, 0.1 g of swine feces seeded with *S. hyodysenteriae* 5380 cells (numbers on the left); lane 2, normal swine feces; lane 3, 10^5 purified *S. hyodysenteriae* 5380 cells.

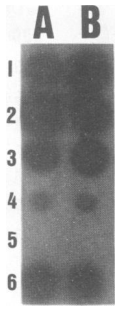

FIG. 5. Colony dot blot hybridization of the 1.1-kb (A) and 0.75-kb (B) DNA probes with swine feces from a swine dysentery outbreak. Rows 1 to 4, feces from infected swine PM88, PM97, W37, and Y94; row 5, normal swine feces; row 6, 10^7 *S. hyodysenteriae* 5380 cells.

probes under high-stringency conditions. *S. hyodysenteriae* was detected in both naturally infected swine (Fig. 5, lane A, rows 1 and 2) and experimentally challenged swine (Fig. 5, lane A, rows 3 and 4), but no *S. hyodysenteriae* was detected with feces from control (noninfected) swine (Fig. 5, row 5). *S. hyodysenteriae* was recovered from the freshly collected fecal samples from both of the experimentally challenged swine and samples from the two swine naturally infected with swine dysentery. However, *S. hyodysenteriae* was not recovered from fecal samples after storage at −70°C for 18 months.

DISCUSSION

At present, the detection and identification of *S. hyodysenteriae* in fecal specimens from pigs with suspected swine dysentery require isolation of the organism on artificial medium and confirmation by serotyping or protein profile analysis followed by Western blotting (25, 26, 32). These procedures are time-consuming, labor-intensive, and costly. DNA probes have been described for many infectious diseases, and one advantage of DNA probes is that they decrease the time required for detection of microorganisms (30). Hybridization of whole chromosomal *S. hyodysenteriae* DNA cannot be used for the differentiation of *S. hyodysenteriae* from *S. innocens* because of a high level of DNA-DNA homology at 40% (27), and so a more specific method of identification is required. In this study, we report the development of two DNA probes specific for *S. hyodysenteriae* which do not react with the nonpathogenic intestinal spirochete *S. innocens* or with other enteric pathogens.

The DNA probes have been shown to be highly specific for *S. hyodysenteriae* and have been used to detect a large number of *S. hyodysenteriae* isolates, including all the serotype reference strains and strains from each of the serotypes from various geographical locations, including the United States, Mexico, Poland, Yugoslavia, and Australia (21 isolates) (Table 1). Both DNA probes hybridized specifically to the large number of *S. hyodysenteriae* strains, representing all the serotypes tested.

The probes did not react with *S. innocens* B256 (the reference strain) or the other *S. innocens* strains examined (Fig. 3). Pure *S. hyodysenteriae* cells can be detected at a level of more than 10^4 organisms, and specific strains can be detected at levels near 10^3 organisms. Importantly, neither probe reacted to the wide range of enteric pathogens examined. This is an important observation, as pig enteric disease

may be attributed not only to *S. hyodysenteriae* but also to *Campylobacter* spp., enteropathogenic *E. coli* and enterotoxigenic *E. coli* strains of three serotypes, and *Salmonella* spp.

The 1.1- and 0.75-kb probes were sensitive enough to hybridize with a minimum of 7.82 ng of purified *S. hyodysenteriae* DNA and to detect *S. hyodysenteriae* serotype reference strains at a level of 7.82 ng of purified DNA (Fig. 1).

The level of *S. hyodysenteriae* in feces of swine with clinical signs of swine dysentery is reported to be on the order of 2×10^6 to 2×10^{10} CFU/g of fecal material (18). The detection level attained with the DNA probes against *S. hyodysenteriae* in seeded feces was 10^4 CFU (Fig. 4). This detection limit is 100-fold below the level seen in pigs with clinical signs of swine dysentery and represents the level likely to occur in carrier animals without overt clinical signs, and it emphasizes the potential of these probes for use in detection of *S. hyodysenteriae* during early stages of infection.

Use of these probes on fecal specimens from swine experimentally challenged with *S. hyodysenteriae* PM88 and also from fecal specimens (stored at −70°C for 18 months) from naturally infected swine with clinical signs of swine dysentery showed that the probes could successfully detect the presence of *S. hyodysenteriae* in fecal material (Fig. 5) from clinical specimens from both swine with the experimental infection and those infected during a natural outbreak. These data provide evidence that *S. hyodysenteriae* can be detected retrospectively in frozen swine fecal specimens with the *S. hyodysenteriae* DNA probe.

The levels of *S. hyodysenteriae* detected with the DNA probes in feces from two experimentally challenged swine were different, indicating different amounts of *S. hyodysenteriae* in the feces (Fig. 5, rows 3 and 4). Clinical postmortem observations support this observation, and a greater level of mucohemorrhagic colitis was observed in swine W37 (Fig. 5, row 3) than swine Y94 (Fig. 5, row 4) (24).

Jensen et al. (11) have reported the development of oligodeoxyribonucleotide probes directed to *S. hyodysenteriae* rRNA. A possible disadvantage of the short oligonucleotide probes lies in the 2-nucleotide difference in the 28-base 16S rRNA sequences of *S. hyodysenteriae* and *S. innocens*. The 17-base sequence probe developed has a 1-base difference between *S. hyodysenteriae* and *S. innocens* and was 5 to 10 times less sensitive than the 28-base sequence probe. The change of only one of these two nucleotides will affect the sensitivity of the oligodeoxynucleotide probes and may render the probes ineffective. The probes developed in this study are relatively large (0.75 and 1.1 kb), and minor nucleotide changes would not affect the sensitivity or specificity as determined by hybridization. Also, only a small number of reporter molecules may be attached to the oligodeoxynucleotides, whereas a greater number of labeled molecules may be incorporated into the 0.75- and 1.1-kb DNA probes, increasing the specific activities of the probes. The 1.1-kb probe was able to detect 1×10^3 purified *S. hyodysenteriae* cells, in comparison with 2.5×10^3 cells detected by the 28-base probe. The relative difference in the sensitivities of the two-probe systems (DNA-RNA versus DNA-DNA) makes the 1.1- and 0.75-kb DNA probes suitable for the detection of *S. hyodysenteriae*.

The preparation of fecal specimens for hybridization analysis with the two probes is neither complex nor time-consuming. The entire processing time from specimen delivery to application on the nylon membrane filter was less than 10 min.

The studies reported in this paper demonstrate that a specific DNA probe can be used to rapidly detect *S. hyodysenteriae*, both after isolation and in fecal material. The total time required for detection of *S. hyodysenteriae* from fecal samples was only 24 h. This method has a significant advantage over conventional isolation and characterization methods, which may take up to 2 weeks to complete. Importantly, the use of a probe specific to *S. hyodysenteriae* allows for the sensitivity of current methods without the necessity for recovery of live culture from freshly collected specimens or specimens which have been frozen (during lengthy periods) for biochemical tests such as API ZYM (9) or α-arabinosidase (3), which require actively growing culture. Techniques such as DNA hybridization and the polymerase chain reaction may also be used in conjunction with nonisotopic detection methods to provide increased sensitivity and rapid detection of *S. hyodysenteriae* directly from clinical specimens.

Further investigations are currently under way to increase sensitivity in detecting *S. hyodysenteriae* by employing the polymerase chain reaction from sequence data of the 1.1-kb DNA probe.

REFERENCES

1. **Alderton, M. (Royal Melbourne Institute of Technology).** 1992. Personal communication.
2. **Baum, D. H., and L. A. Joens.** 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. **25**:792–796.
3. **Bélanger, M., and M. Jacques.** 1991. Evaluation of the An-Ident system and an indole spot test for the rapid differentiation of porcine treponemes. J. Clin. Microbiol. **29**:1727–1729.
4. **Bolivar, F., R. C. Rodrigues, P. J. Greene, M. C. Betlach, H. C. Haynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterisation of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
5. **Collins, D. M., and D. E. Ross.** 1984. Restriction endonuclease analysis of *Campylobacter* strains with particular reference to *Campylobacter fetus* ss *fetus*. J. Med. Microbiol. **18**:117–124.
6. **Combs, B., D. J. Hampson, J. R. L. Mhoma, and J. R. Buddle.** 1989. Typing of *Treponema hyodysenteriae* by restriction enzyme analysis. J. Clin. Microbiol. **19**:351–359.
7. **Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon.** 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.
8. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
9. **Hudson, M. J., T. J. L. Alexander, and R. J. Lysons.** 1976. Diagnosis of swine dysentery: spirochetes which may be confused with *Treponema hyodysenteriae*. Vet. Rec. **99**:498–500.
10. **Hunter, D., and T. Wood.** 1979. An evaluation of the APIZYM system as a means of classifying spirochaetes associated with swine dysentery. Vet. Rec. **28**:383–384.
11. **Jensen, N. S., T. A. Casey, and T. B. Stanton.** 1990. Detection and identification of *Treponema hyodysenteriae* by using oligodeoxynucleotide probes complementary to 16S rRNA. J. Clin. Microbiol. **28**:2717–2721.
12. **Joens, L. A., and R. D. Glock.** 1979. Experimental infection in mice with *Treponema hyodysenteriae*. Infect. Immun. **25**:757–760.
13. **Joens, L. A., D. L. Harris, and D. H. Baum.** 1979. Immunity to swine dysentery in recovered pigs. Am. J. Vet. Res. **40**:1352–1354.
14. **Joens, L. A., D. L. Harris, J. M. Kinyon, and M. L. Kaeberle.** 1978. Microtitration agglutination for detection of *Treponema hyodysenteriae* antibody. J. Clin. Microbiol. **8**:293–298.

15. **Joens, L. A., J. G. Songer, D. L. Harris, and R. D. Glock.** 1978. Experimental infection with *Treponema hyodysenteriae* in guinea pigs. Infect. Immun. **22:**132–135.
16. **Kinyon, J. M., and D. L. Harris.** 1979. *Treponema innocens*, a new species of intestinal bacteria, and an emended description of the type strain of *Treponema hyodysenteriae* Harris et al. Int. J. Syst. Bacteriol. **29:**102–109.
17. **Kinyon, J. M., D. L. Harris, and R. D. Glock.** 1977. Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. Infect. Immun. **15:**638–646.
18. **Kunkle, R. A., and J. M. Kinyon.** 1988. Improved selective medium for the isolation of *Treponema hyodysenteriae*. J. Clin. Microbiol. **26:**2357–2360.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Mapother, M. E., and L. A. Joens.** 1985. New serotypes of *Treponema hyodysenteriae*. J. Clin. Microbiol. **22:**161–164.
21. **Nibbelink, S. K., and M. J. Wannemuehler.** 1991. Susceptibility of inbred mouse strains to infection with *Serpula* (*Treponema*) *hyodysenteriae*. Infect. Immun. **59:**3111–3118.
22. **Rigby, P., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.
23. **Schwan, T. G., W. J. Simpson, M. E. Schrumpf, and R. H. Karstens.** 1989. Identification of *Borrelia burgdorferi* and *B. hermsii* using DNA hybridization probes. J. Clin. Microbiol. **27:**1734–1738.
24. **Smith, S. C. (Royal Melbourne Institute of Technology).** 1992. Personal communication.
25. **Smith, S. C., L. M. Barret, T. Muir, W. L. Christopher, and P. J. Coloe.** 1991. Application and evaluation of enzyme-linked immunosorbent assay and immunoblotting for detection of antibodies to *Treponema hyodysenteriae*. Epidemiol. Infect. **107:**285–296.
26. **Smith, S. C., F. Roddick, S. Ling, N. L. Gerraty, and P. J. Coloe.** 1990. Biochemical and immunochemical characterisation of strains of *Treponema hyodysenteriae*. Vet. Microbiol. **24:**29–41.
27. **Stanton, T. B., N. S. Jensen, T. A. Casey, L. A. Tordoff, F. E. Dewhirst, and B. J. Paster.** 1991. Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. Int. J. Syst. Bacteriol. **41:**50–58.
28. **Sueyoshi, M., Y. Adachi, and S. Shoya.** 1987. Enteropathogenicity of *Treponema hyodysenteriae* in young chicks. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **266:**469–477.
29. **Taylor, D. J., and T. J. L. Alexander.** 1971. The production of dysentery in swine feeding cultures containing a spirochete. Br. Vet. J. **127:**58–61.
30. **Tenover, F. C.** 1988. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev. **1:**82–101.
31. **Walfield, A. M., P. A. Hanff, and N. A. Lovett.** 1982. Expression of *Treponema pallidum* antigens in *Escherichia coli*. Science **216:**522–523.
32. **Wright, J. C., G. R. Wilt, R. B. Reed, and T. A. Powe.** 1989. Use of an enzyme-linked immunosorbent assay for detection of *Treponema hyodysenteriae* infection in swine. J. Clin. Microbiol. **27:**411–416.
33. **Zuerner, R. L., and C. A. Bolin.** 1988. Repetitive sequence element cloned from *Leptospira interrogans* serovar hardjo type hardjo-bovis provides a sensitive diagnostic probe for bovine leptospirosis. J. Clin. Microbiol. **26:**2495–2500.