Serpulina pilosicoli sp. nov., the Agent of Porcine Intestinal Spirochetosis

DARREN J. TROTT,^{1*} THADDEUS B. STANTON,² NEIL S. JENSEN,² GERALD E. DUHAMEL,³
JOHN L. JOHNSON,⁴ AND DAVID J. HAMPSON¹

School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6150, Australia¹; Physiopathology Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010²; Department of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0905³; and Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0305⁴

Phenotypic and genetic traits of porcine intestinal spirochete strain P43/6/78^T (= ATCC 51139^T) (T = type strain), which is pathogenic and weakly beta-hemolytic, were determined in order to confirm the taxonomic position of this organism and its relationships to previously described species of intestinal spirochetes. In BHIS broth, P43/6/78^T cells had a doubling time of 1 to 2 h and grew to a maximum cell density of 2×10^9 cells per ml at 37 to 42°C. They hydrolyzed hippurate, utilized D-glucose, D-fructose, sucrose, D-trehalose, D-galactose, D-mannose, maltose, N-acetyl-D-glucosamine, D-glucosamine, pyruvate, L-fucose, D-cellobiose, and D-ribose as growth substrates, and produced acetate, butyrate, ethanol, H₂, and CO₂ as metabolic products. They consumed substrate amounts of oxygen and had a G+C content (24.6 mol%) similar to that of *Serpulina hyodysenteriae* B78^T (25.9 mol%). Phenotypic traits that could be used to distinguish strain P43/6/78^T from *S. hyodysenteriae* and *Serpulina innocens* included its ultrastructural appearance (each strain P43/6/78^T cell had 8 or 10 periplasmic flagella, with 4 or 5 flagella inserted at each end, and the cells were thinner and shorter and had more pointed ends than *S. hyodysenteriae* and *S. innocens* cells), its faster growth rate in liquid media, its hydrolysis of hippurate, its lack of β-glucosidase activity, and its metabolism of D-ribose. DNA-DNA relative reassociation experiments in which the S1 nuclease method was used revealed that P43/6/78^T was related to, but was genetically distinct from, both *S. hyodysenteriae* B78^T (level of sequence homology, 25 to 32%) and *S. innocens* B256^T (level of sequence homology, 24 to 25%). These and previous results indicate that intestinal spirochete strain P43/6/78^T represents a distinct *Serpulina* species. Therefore, we propose that strain P43/6/78 should be designated as the type strain of a new species, *Serpulina pilosicoli*.

Porcine intestinal spirochetosis or spirochetal diarrhea is a disease of swine that are between 4 and 20 weeks old but typically occurs within 7 to 14 days after weaning (7, 39). The clinical signs of this disease include mucus-containing, usually nonbloody diarrhea; poor feed conversion; and depressed growth rates (1, 7, 31, 40). The characteristic histological feature that distinguishes porcine intestinal spirochetosis is a dense mat or false brush border of spirochete cells which are closely packed parallel to one another and are attached by one end to the colonic epithelium (4, 7, 14, 40). Such cells are not typical of infections caused by *Serpulina hyodysenteriae* (the agent of swine dysentery), but have been found in humans colonized by intestinal spirochetes, where their clinical significance is unclear (8, 22).

The etiologic agent of porcine intestinal spirochetosis was first described in 1980 by Taylor et al. (40). These investigators successfully isolated an intestinal spirochete, designated strain P43/6/78^T (T = type strain), and reproduced clinical signs and lesions typical of the disease in pigs that were orally challenged with pure cultures of this bacterium. Colonies of this spirochete were found to be weakly beta-hemolytic on blood agar media. Electron microscopy revealed that strain P43/6/78^T cells had a typical spirochete ultrastructure and that each cell had 8, 10, or 12 periplasmic flagella (4 to 6 periplasmic flagella were inserted into the protoplasmic cylinder at each end of the cell, with the free ends overlapping in the middle of the cell) (12, 40). This feature distinguished P43/6/78^T from *S. hyodys-*

enteriae and the nonpathogenic spirochete *Serpulina innocens*, which typically have 16 to 24 and 20 to 28 periplasmic flagella per cell, respectively (18, 30). Despite its apparent significance as a swine pathogen, strain P43/6/78^T has not been extensively characterized in terms of its physiological and biochemical properties, and its taxonomic status has not been conclusively determined previously.

In this study, we determined ultrastructural, physiological, and genomic characteristics of strain P43/6/78^T. Our results, together with the results of previous investigators, support the hypothesis that strain P43/6/78^T should be assigned to a new *Serpulina* species. We propose the name *Serpulina pilosicoli* for this spirochete. A major objective of this study was to obtain information that will be useful for studying this bacterium and for identifying other strains of *S. pilosicoli*.

MATERIALS AND METHODS

Bacterial strains and media. *S. pilosicoli* P43/6/78^T (= ATCC 51139^T) was originally obtained as a stock culture from D. J. Taylor, University of Glasgow, Glasgow, Scotland. *S. hyodysenteriae* B78^T (= ATCC 27164^T) and *S. innocens* B256^T (= ATCC 29796^T) were obtained from the National Animal Disease Center culture collection. Cells were routinely grown at 38°C in 7 ml of stirred BHIS broth under an atmosphere that initially contained N₂ and O₂ (99:1), as described previously (36). Heart infusion broth containing heat-treated (56°C, 30 min) serum (HS broth) was used to determine which growth substrates were utilized (36). Bacterial growth was measured by monitoring the culture optical density at 620 nm (OD₆₂₀) by using a path length of 18 mm and a Bausch & Lomb Spectronic 70 spectrophotometer. Bacterial population densities were equated to culture optical densities by determining direct microscope counts with a Petroff-Hausser counting chamber.

Phase-contrast microscopy and electron microscopy. Wet mount preparations of *S. pilosicoli* P43/6/78^T and *S. hyodysenteriae* B78^T were prepared as described previously (37) and were photographed with an Olympus phase-contrast micro-

* Corresponding author. Phone: 619 360 2247. Fax: 619 310 4144. Electronic mail address: dtrott@numbat.murdoch.edu.au.

scope equipped with an Olympus model PM-10AD automatic photomicrography system. Images were recorded on Kodak TMAX 100 film.

Actively dividing cells of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T were prepared for transmission electron microscopy by centrifuging 1.0-ml portions of cultures at 2,000 × g and then resuspending the cells in 500-μl portions of 10 mM sodium phosphate buffer (pH 7). A 20-μl sample of each suspension was negatively stained with an equal volume of 2% phosphotungstic acid (pH 6 for strain B78^T and pH 7 for strain P43/6/78^T), mounted on a carbon-reinforced 200-mesh copper grid coated with 2% Parlodion, and examined at 80 kV with a Phillips model 410 transmission electron microscope. Cell lengths and diameters were determined from electron micrographs of five individual cells.

Growth characteristics. *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T cells were cultured in BHIS broth, and their optimal growth temperatures were determined by incubating culture tubes at a 45° angle at 32, 37, 42, and 47°C in a Lab-line orbital shaker at 200 rpm. The ability of each strain to grow in the presence of different O₂ concentrations (1, 2, 5, 7, 15, and 20%) was determined with freshly inoculated cultures by removing an appropriate volume of gas from the headspace with an airtight syringe and injecting an equal volume of air. The pH range for growth was determined by adjusting the pH of the medium with 1 M HCl or 1 M NaOH in 0.2-pH unit increments prior to inoculation. The final pH of each inoculated medium containing glucose was determined when the cultures reached their maximum population densities (OD₆₂₀). Population doubling times were estimated from standard growth curves. The strength of beta-hemolysis was determined by stab inoculating individual colonies into a segment of a Trypticase soy agar plate supplemented with 5% defibrinated bovine blood. The plates were then incubated at 37°C in a Coy anaerobic chamber filled with N₂-H₂-CO₂ (85:10:5).

Biochemical tests. Gelatinase activity was determined by using the modified zymography method of Kleiner and Stetler-Stevenson (20). Cells of the three spirochete strains and a gelatinase-producing strain of *Staphylococcus aureus* (strain ATCC 25923) were grown in 7 ml of BHIS broth supplemented with 4% gelatin (OD₆₂₀ 1.2 to 1.6). The bacterial cells then were harvested by centrifugation at 12, 100 × g for 10 min at 4°C and washed with 7 ml of TBS buffer (10 mM Tris, 0.85% NaCl; pH 7.4). The resulting suspensions were centrifuged at 12, 100 × g for 10 min at 4°C, and the cells of each strain were suspended in 2 ml of TBS buffer and sonicated at 50 W on ice three times (30 s each) with a Branson model 250 Sonifier. The cellular debris was pelleted by centrifugation at 12, 100 × g for 20 min at 4°C, and the supernatant was collected and stored at -70°C. A 25-μl sample of each supernatant was mixed with an equal volume of 0.2× sample buffer (0.4 M Tris [pH 6.8], 5% sodium dodecyl sulfate [SDS], 20% glycerol, 0.03% bromophenol blue), and the resulting preparation was loaded into the wells of an SDS-polyacrylamide gel (10 by 10 by 0.1 cm; 4% [wt/vol] polyacrylamide stacking gel, 12% [wt/vol] polyacrylamide resolving gel containing 0.1% gelatin). Electrophoresis and staining of the gel were performed as recommended by Kleiner and Stetler-Stevenson (20).

Hydrolysis of esculin in HS broth cultures was determined by the method of Holdeman et al. (9); a *Staphylococcus aureus* ATCC 25923 culture and uninoculated media were included as controls. Hydrolysis of hippurate in BHIS broth cultures supplemented with 1% sodium hippurate was determined as described by Kinyon and Harris (18); uninoculated medium was included as a control. H₂S production was determined by using BHIS broth containing no L-cysteine or resazurin. A dry, sterile strip of Whatman no. 1 filter paper that had been presoaked in hot 5% lead acetate was suspended 1 cm above the level of the medium. The controls used included an H₂S-producing strain of *Salmonella typhimurium* (strain 798) and *Escherichia coli* HB101, which did not produce H₂S. The tubes were incubated at 38°C and were observed twice daily; a black discoloration that extended up the filter paper strip indicated that lead sulfide was being formed from evolved H₂S. Enzyme profiles were determined by using the API ZYM system (13). The production of indole in broth cultures was determined as described previously (24).

Substrate utilization. Before individual substrates were examined, the minimum serum concentration that supported maximum growth in HS broth containing 0.4% (wt/vol) glucose was found to be 7% for both *S. pilosicoli* P43/6/78^T and *S. innocens* B256^T. This concentration was used in media to determine which growth substrates were utilized. Potential growth substrates were prepared as filter-sterilized stock solutions (14%, wt/vol) and added to the medium to a final concentration of 0.4%. Compounds that could not be filter sterilized were added to the basal medium before autoclaving. A 1% (vol/vol) inoculum of washed cells grown in BHIS broth was transferred into 7 ml of HS broth containing a potential substrate, and the resulting preparation was incubated at 38°C for 5 days or until the maximum cell growth (highest culture optical density) was observed. If growth was detected, a 0.1-ml sample was transferred into fresh media containing the same substrate. Growth yields (maximum OD₆₂₀ values) were determined from these subcultures. When growth on a particular substrate only slightly exceeded (i.e., was <0.3 OD₆₂₀ unit greater than) growth in medium without added substrate, cell densities were determined by direct counting. A substrate was considered a substrate that supported growth if the number of cells in a culture containing the substrate was more than fivefold greater than the number of cells in a culture containing no added substrate.

Analysis of fermentation products. Nongaseous end product contents were determined by using cultures of *S. pilosicoli* P43/6/78^T and *S. innocens* B256^T

growing in 7 ml of HS broth supplemented with 0.4% glucose. A Hewlett-Packard model 5890 gas chromatograph equipped with a model 7671A automatic sampler was used to identify and quantify volatile fatty acids and alcohols (36).

Gases in culture atmospheres were identified and quantified by using a Gow-Mac series 580 gas chromatograph. The culture medium was acidified to release CO₂ by injecting 0.1 ml of 0.1 M HCl into the culture through the rubber stopper. A 3-ml sample was removed from the headspace with an airtight syringe and injected into the sample loop (0.5 ml) of the gas chromatograph. H₂ was detected by using nitrogen as the carrier gas, and CO₂, O₂, and N₂ were detected by using helium as the carrier gas. The amount of N₂ in the culture atmosphere was considered fixed. Consequently, the amount of N₂ was determined and used as an internal standard to quantify other gases.

Antibiotic susceptibility. The susceptibilities of the three spirochete strains to 20 different antibiotics were determined by the agar dilution method of Kitai et al. (19). The antibiotics and concentrations that were tested are shown in Table 1. The antibiotics used included antibiotics commonly used for treatment of swine dysentery (carbadox, lincomycin, tiamulin, tylosin), as well as antibiotics used in selective media for isolating *S. hyodysenteriae* (spectinomycin, vancomycin, colistin, rifampin, spiramycin) (21). The ranges of antibiotic concentrations used were based on the mean inhibitory concentrations determined for *S. hyodysenteriae* (19). The antibiotics were prepared as 2,000-μg/ml stock solutions, filter sterilized, and added to sterile Trypticase soy agar containing 5% defibrinated bovine blood (48°C) before the plates were poured. Cultures of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T were grown in BHIS broth until they reached an OD₆₂₀ of 1.2 to 1.6 (approximately 3 × 10⁸ to 1 × 10⁹ cells per ml depending on the strain) and diluted with sterile TBS (pH 7.4) to a culture density of 10⁷ cells per ml. A 10-μl sample of each suspension was deposited onto each antibiotic plate. The mean concentration of antibiotic which inhibited growth (prevented the appearance of hemolytic zones) after 4 days of incubation in a Coy chamber at 37°C was determined by using at least three replicates of each antibiotic dilution. Bacteria were inoculated onto plates without antibiotics as controls. Carbadox was obtained from Pfizer, New York, N.Y., and tiamulin was obtained from Fermenta, Kansas City, Mo. All other antibiotics were obtained from Sigma Chemical Co., St. Louis, Mo.

DNA analysis. For DNA homology studies, DNA was extracted from cells of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T as described previously (3, 16). Levels of DNA-DNA reassociation for the three strains were determined by the S1 nuclease method (15-17).

To determine the DNA guanine-plus-cytosine (G+C) content, purified DNA was diluted in 0.3 ml of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) so that the final concentration was approximately 50 mg/ml. The G+C content was determined by the thermal denaturation method (27). DNA from *S. hyodysenteriae* B78^T (G+C content, 25 to 26 mol%) was used as the standard.

RESULTS

Phase-contrast microscopy and electron microscopy. Phase-contrast micrographs of *S. hyodysenteriae* B78^T and *S. pilosicoli* P43/6/78^T are shown in Fig. 1A and B, respectively. Compared with cells of strain B78^T, strain P43/6/78^T cells generally were shorter and thinner and their ends were less tapered. The cell dimensions were sufficiently different that it was possible to differentiate the cells of the two species by light microscopy.

Morphological characteristics determined from measurements obtained from electron micrographs of the spirochetes are shown in Table 2. The strain B78^T cells tended to taper to a blunt surface at both ends (Fig. 2A), and 8 to 12 periplasmic flagella were inserted in a V-shaped arrangement at each end of each cell, so that there were 16 to 24 periplasmic flagella per cell (Fig. 2B). The strain B256^T cells were identical to the strain B78^T cells except that they had 20 to 26 periplasmic flagella per cell. Compared with the cells of the other strains, *S. pilosicoli* P43/6/78^T cells were shorter and thinner with fewer waves, and each cell had at least one abruptly pointed end (Fig. 3A). Four or five periplasmic flagella were inserted in a line at each end of each cell (Fig. 3B), so that there were 8 or 10 periplasmic flagella per cell.

Growth characteristics. *S. pilosicoli* P43/6/78^T cells grew to the maximum population density when they were incubated at 37 and 42°C, whereas the growth of *S. hyodysenteriae* B78^T and *S. innocens* B256^T cells was limited slightly at 42°C (the maximum culture OD₆₂₀ was less than 1.0). No culture grew at 32 or 45°C. Compared with strain B78^T and B256^T cells, strain P43/6/78^T cells tolerated a slightly higher level of O₂ in the

TABLE 1. Antibiotic susceptibilities of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T

Antibiotic	Concn tested (µg/ml)	Susceptibility (µg/ml) ^a		
		<i>S. pilosicoli</i> P43/6/78 ^T	<i>S. hyodysenteriae</i> B78 ^T	<i>S. innocens</i> B256 ^T
Ampicillin	1, 10, 50, 100	10	R	1
Carbadox ^b	0.1, 1, 5, 10	0.1	0.1	0.1
Chloramphenicol	1, 5, 10, 50	5	5	5
Colistin ^c	50, 100, 200, 500	R	R	R
Erythromycin	5, 20, 50, 100	100	100	100
Kanamycin	10, 20, 50, 100	20	50	10
Lincomycin ^b	1, 10, 50, 100	1	1	1
Metronidazole	0.1, 1, 10, 20	0.1	0.1	0.1
Nalidixic acid	50, 100, 200, 500	200	500	200
Neomycin	10, 50, 100, 200	100	200	200
Novobiocin	10, 20, 50, 100	20	100	50
Polymixin B	50, 100, 200, 500	R	R	R
Rifampin ^c	10, 20, 50, 100, 200	20	R	20
Spectinomycin ^c	100, 200, 500	R	R	R
Spiramycin ^c	10, 20, 50, 100, 200	50	R	R
Streptomycin	10, 20, 50, 100	50	50	10
Tetracycline	1, 5, 10, 20	1	1	1
Tiamulin ^b	0.1, 1, 5, 10	0.1	0.1	0.1
Tylosin ^b	5, 20, 50, 100	5	R	20
Vancomycin ^c	100, 200, 500	R	R	R

^a Values are the lowest concentrations of antibiotics which inhibited hemolysis as determined by the agar dilution method of Kitai et al. (19). R, no inhibition of growth (zone of hemolysis) at the highest concentration tested.

^b Antibiotic commonly used in the treatment of swine dysentery.

^c Antibiotic commonly used in selective media for the growth and identification of *S. hyodysenteriae*.

culture headspace (7 instead of 5%), grew over a broader pH range (pH 5.6 to 8.0 instead of pH 6.0 to 8.0 for B78^T and pH 6.5 to 8.0 for B256^T), and produced a lower final pH after growth in BHIS broth (pH 5.6 instead of pH 5.9). Strain P43/

6/78^T also had a faster doubling time (1 to 2 h) than strains B78^T and B256^T (3 to 5 h) in BHIS broth.

Biochemical tests. Two bands of gelatin digestion (at 80 and 106 kDa) appeared after electrophoresis of cell lysates of the gelatinase-producing organism *Staphylococcus aureus* ATCC 25923. As determined by zymography, none of the spirochete lysates contained proteins that exhibited detectable gelatinase activity. All three spirochete species hydrolyzed esculin. Although both *S. hyodysenteriae* B78^T and *S. innocens* B256^T were negative in the test for hippurate hydrolysis, a strong positive reaction was obtained with *S. pilosicoli* P43/6/78^T. As determined by the H₂S litmus test, *Salmonella typhimurium* 798 produced large amounts of H₂S, each of the spirochete strains produced a moderate amount, and *E. coli* HB101 and the uninoculated media were both negative for this characteristic. The API ZYM profile of strain P43/6/78^T revealed that this organism was α-glucosidase negative, α-galactosidase positive, and β-glucosidase negative.

Substrate utilization. All three spirochete species used soluble sugars, pyruvate, D-glucosamine, and N-acetyl-D-glucosamine as energy sources (Table 3). Both strain P43/6/78^T cells and strain B256^T cells were able to metabolize the same mono- and disaccharides as strain B78^T cells. In addition, P43/6/78 and B256^T cells were able to use D-cellobiose and L-fucose for growth. Of the three spirochetes, only P43/6/78^T was able to use the pentose sugar D-ribose as a growth substrate. P43/6/78^T grew to higher cell densities than either B78^T or B256^T in HS broth containing no added substrate (OD₆₂₀, 1.0 [equivalent to 10⁸ to 3 × 10⁸ cells per ml]). The spirochetes apparently used unidentified substrates in the serum, as no growth occurred in HS broth alone.

End products. All three species produced large amounts of acetate and small amounts of butyrate (Table 4). In addition, P43/6/78^T and B256^T produced small amounts of ethanol. All three organisms also produced approximately twice as much H₂ as CO₂. Oxygen added to the headspace atmosphere of cultures at concentrations of 1 to 7% (vol/vol) was consumed during growth (<0.05% O₂ was present at the maximum cell

FIG. 1. Phase-contrast light micrographs showing cells of *S. hyodysenteriae* B78^T (A) and *S. pilosicoli* P43/6/78^T (B). Bar = 7 µm.

TABLE 2. Characteristics useful for distinguishing *S. pilosicoli*, *S. hyodysenteriae*, and *S. innocens*

Characteristic	<i>S. pilosicoli</i> P43/6/78 ^T	<i>S. hyodysenteriae</i> B78 ^T	<i>S. innocens</i> B256 ^T
Avg cell length	6.27 ± 0.98	9.78 ± 1.87	9.40 ± 1.85
Cell diameter (µm) ^a	0.27 ± 0.03	0.35 ± 0.02	0.36 ± 0.03
No. of periplasmic flagella per cell ^b	8–12	16–24	20–26
Growth rate (h) ^c	1–2	3–5	3–5
% DNA reassociation with <i>S. pilosicoli</i> DNA	100	24	32
MEE group ^d	VI	I	III
16S rRNA-specific sequence	5'-AGUUUUUUCCGCUUCA-3' ^e	5'-CGGAACGCCCUCCGAGUACUUGUAAGUC-3' ^f	5'-CGGAACGCCCUCCGAGUACUUGAGAGUC-3' ^g
Enteropathogenicity for swine	Diarrhea	Severe mucohemorrhagic diarrhea	Non-pathogenic
Cell association with intestinal mucosa	Attached by one end to epithelium	Overlying mucosa, populates mucus	Unknown
Beta-hemolysis	Weak	Strong	Weak
Indole production	–	+	–
Hippurate hydrolysis	+	–	–
α-Glucosidase activity ^h	–	+	–
α-Galactosidase activity ^h	+	–	+
β-Glucosidase activity ^h	–	+	+
Utilization of the following growth substrates:			
D-Ribose	+	–	–
L-Fucose	+	–	+
D-Cellobiose	+	–	+
Growth in HS broth with no added substrate (maximum OD ₆₂₀)	+++ (1.0)	+(0.35)	++ (0.60)

^a Mean ± standard deviation of five measurements.^b Each *S. pilosicoli* P43/6/78^T cell had 4 to 6 periplasmic flagella inserted at each end of the cell; each *S. hyodysenteriae* B78^T cell had 8 to 12 periplasmic flagella inserted at each end of the cell; and each *S. innocens* B256^T cell had 10 to 13 periplasmic flagella inserted at each end of the cell.^c Population doubling times of stirred cultures in BHIS broth in a 99% N₂–1% O₂ atmosphere at 38°C.^d See references 23, 25, and 38.^e This signature sequence has been used to design a PCR test which is specific for *S. pilosicoli* P43/6/78^T and other closely related intestinal spirochetes that have four to six periplasmic flagella at each end of each cell (29).^f Data from reference 35.^g An API ZYM analysis was performed by the method described by Hunter and Wood (13).

FIG. 2. (A) Electron micrograph of negatively stained whole cell of *S. hyodysenteriae* B78^T. The ends of the cell are blunt. Bar = 1.0 μm . (B) Electron micrograph of one end of a cell, showing 12 flagella and insertion discs in a V-shaped arrangement. Bar = 0.2 μm .

FIG. 3. (A) Electron micrograph of a negatively stained whole cell of *S. pilosicoli* P43/6/78^T. The ends of the cell are pointed. Bar = 0.5 μm. (B) Electron micrograph of one end of a cell, showing five flagella and insertion discs in a row. Bar = 0.2 μm.

density). In separate experiments, P43/6/78^T cells yielded the same end products in similar proportions when D-ribose was used in place of D-glucose as the carbohydrate source. When no substrate was included, P43/6/78^T cells produced acetate, ethanol, H₂, and CO₂ and did not produce butyrate.

Antibiotic susceptibility. The antibiotic susceptibilities of the three spirochete strains are shown in Table 1. Similar susceptibility patterns were obtained for all three strains with each antibiotic, although *S. innocens* B256^T and *S. pilosicoli*

P43/6/78^T cells were susceptible to lower concentrations of ampicillin, kanamycin, nalidixic acid, novobiocin, rifampin, spiramycin, and tylosin than strain B78^T cells were.

DNA analysis. The relative levels of DNA-DNA reassociation are shown in Table 5. Moderate levels of sequence homology were observed between the DNAs of *S. hyodysenteriae* B78^T and *S. innocens* B256^T (46 and 38%). *S. pilosicoli* P43/6/78^T DNA exhibited 24 to 25% homology with strain B78^T DNA and 26 to 32% homology with strain B256^T DNA. As

TABLE 3. Comparison of growth substrates for *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T

Compound ^a	Growth response ^b		
	<i>S. pilosicoli</i> P43/6/78 ^T	<i>S. hyodysenteriae</i> B78 ^{Tc}	<i>S. innocens</i> B256 ^T
None	(1.0)	(0.35)	(0.60)
D-Glucose	+(≥2.0)	+(1.3)	+(1.5)
D-Fructose	+(≥2.0)	+(1.3)	+(1.4)
Sucrose	+(≥2.0)	+(1.3)	+(1.2)
D-Trehalose	+(≥2.0)	+(1.3)	+(1.2)
D-Galactose	+(1.5)	+(0.6)	+(0.85)
D-Mannose	+(1.6)	+(0.7)	+(1.0)
Maltose	+(1.5)	+(0.6)	+(1.2)
N-Acetyl-D-glucos- amine	+(≥2.0)	+(1.2)	+(1.4)
D-Glucosamine	+(1.8)	+(1.1)	+(1.2)
Pyruvate	+(1.5)	+(0.9)	+(0.9)
L-Fucose	+(1.5)	—	+(0.9)
D-Cellobiose	+(≥2.0)	—	+(1.3)
D-Ribose	+(≥2.0)	—	—

^a The substrates that were not metabolized by any isolate included L-arabinose, D-fucose, D-raffinose, D-melibiose, L-rhamnose, D-xylose, galactosamine, N-acetylglucosamine, cellulose, esculin, soluble starch, pectin, mucin, glycogen, polygalacturonic acid, glutathione, glycerol, mannitol, acetate, lactate, succinate, and 50× MEM amino acids.

^b —, no detectable growth above the background level in medium without added substrate; +, substrate utilized by growing cells. The numbers in parentheses are the maximum culture OD₆₂₀ values obtained in media containing the substrates.

^c Data from reference 36.

determined by the thermal denaturation method, the G+C contents of the DNAs of *S. pilosicoli* P43/6/78^T and *S. hyodysenteriae* B78^T were 24.6 and 25.9 mol%, respectively.

DISCUSSION

The intestinal spirochetes that form weakly beta-hemolytic colonies on Trypticase soy blood agar have not been well characterized and, except for *Brachyspira aalborgi* (11) and *S. innocens* (18, 33), have not been given official taxonomic designations previously. As determined by DNA-DNA relative reassociation experiments, the only strains identified as *S. innocens* strains are strains B256^T and 4/71 (33, 35). Swine intestinal spirochete strain P43/6/78^T and other strains of weakly beta-hemolytic intestinal spirochetes have been compared by

TABLE 4. End products of growing cells of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T

Product ^a	Amt produced (μmol/ml of medium) ^b		
	<i>S. pilosicoli</i> P43/6/78 ^T	<i>S. hyodysenteriae</i> B78 ^{Tc}	<i>S. innocens</i> B256 ^T
Acetate	23.3	12.0	18.0
Butyrate	1.5	1.7	1.8
Ethanol	2.4	ND ^d	0.9
CO ₂	11.3	10.3	13.1
H ₂	22.4	24.0	22.0

^a The following compounds were assayed for but were not produced by any strain: formate, lactate, propionate, isobutyrate, valerate, succinate, butanol, and propanol.

^b The culture medium used was HS broth supplemented with 0.4% glucose, and the atmosphere was 1% O₂–99% N₂. O₂ (1%) injected into the headspace atmosphere before inoculation was completely consumed by all three strains. The amount of oxygen consumed was estimated to be 2 μmol/ml of culture broth.

^c Data from reference 36.

^d ND, not detected.

TABLE 5. Levels of relative reassociation of *S. pilosicoli* P43/6/78^T, *S. hyodysenteriae* B78^T, and *S. innocens* B256^T DNAs

Source of labeled DNA	% Reassociation with unlabeled DNA from ^a :		
	<i>S. pilosicoli</i> P43/6/78 ^T	<i>S. hyodysenteriae</i> B78 ^T	<i>S. innocens</i> B256 ^T
<i>S. pilosicoli</i> P43/6/78 ^T	100	25	26
<i>S. hyodysenteriae</i> B78 ^T	24	100	46
<i>S. innocens</i> B256 ^T	32	38	100

^a Reassociation values were determined by the S1 nuclease method (15, 17).

performing multilocus enzyme electrophoresis (MEE) (23, 25) and a random amplified polymorphic DNA analysis (3), as well as phenotypic characterization (5) and DNA-DNA reassociation (3, 26, 30) analyses. The results of these studies suggested that P43/6/78^T is related to other intestinal spirochete strains that have 8 to 12 periplasmic flagella per cell (4 to 6 flagella at each end) and have been isolated from pigs (25), humans (23), dogs (2, 3), and birds (28) with intestinal disorders similar to the intestinal spirochetosis caused by P43/6/78^T in swine. The results of a MEE analysis revealed that these spirochetes should be placed within a single electrophoretic group (23, 28). Since the electrophoretic profiles of the spirochetes in this group were distinct from those of *S. innocens*, *S. hyodysenteriae*, and *B. aalborgi*, the provisional name "*Anguillina coli*" was suggested for P43/6/78^T and the other closely related spirochetes (23, 25).

More recently, intestinal spirochetes belonging to different MEE groups were compared in a 16S rRNA sequence analysis (38). It was found that swine intestinal spirochete strain P43/6/78^T and two other intestinal spirochete strains (human strains HRM-7 and Wes-B) belonging to the "*A. coli*" MEE group exhibited 99.7 to 99.8% 16S rRNA sequence similarity with each other and 98 to 98.6% 16S rRNA sequence similarity with *S. hyodysenteriae* B78^T and *S. innocens* B256^T. These results supported the results of Fellström and coworkers, who determined partial 16S ribosomal DNA sequences for members of a heterogeneous group of porcine intestinal spirochetes and suggested that the isolates placed in the "*A. coli*" group exhibited high levels of sequence homology with *S. hyodysenteriae* and *S. innocens* (6). *S. hyodysenteriae* B78^T and *S. innocens* B256^T exhibited 99.5% 16S rRNA sequence similarity with each other and 72.7 to 76.1% 16S rRNA sequence similarity with the gastrointestinal spirochetes *Treponema bryantii* RUS-1^T and *Treponema succinifaciens* 6091^T (35). On the basis of these results, it was suggested that the "*A. coli*" group should be considered a *Serpulina* species (6, 38). It was also recommended that these spirochetes should be investigated for distinguishing phenotypic properties and that their phylogenetic relationships should be assessed by other techniques, especially DNA homology estimates (38).

On the basis of the results of previous investigations and the findings of this study, we concluded that intestinal spirochete strain P43/6/78^T should be assigned to the genus *Serpulina* and represents a distinct species, for which we propose the name *Serpulina pilosicoli*. Strain P43/6/78 (= ATCC 51139) is the type strain of *S. pilosicoli*.

S. pilosicoli P43/6/78^T has a number of genomic and phenotypic properties in common with *S. hyodysenteriae* and *S. innocens*. The 16S rRNA sequences of these spirochetes are similar (levels of similarity, 98.2 to 99.5%) (38), and the G+C contents of their DNAs are also similar (24 to 26 mol%). All three species use soluble sugars, amino sugars, and pyruvate for growth (Table 3); consume O₂ in substrate amounts; and pro-

duce acetate, butyrate, H_2 , and CO_2 during glucose metabolism (Table 4). Interestingly, the ratio of H_2 to CO_2 is 2:1, suggesting that all three spirochetes have NADH:ferredoxin oxidoreductase activity (32). These organisms are aerotolerant anaerobes that can grow in atmospheres that initially contain 1 to 5% (vol/vol) O_2 , and they possess NADH oxidase (34) and superoxide dismutase activities (23). The level of DNA-DNA relative reassociation between P43/6/78^T and either *S. hyodysenteriae* B78^T or *S. innocens* B256^T is 24 to 36%, and the levels of DNA-DNA reassociation between *S. hyodysenteriae* B78^T and *S. innocens* B256^T are 38 to 46% (Table 5). In a previous study, the swine intestinal spirochete *T. succinifaciens* 6091^T exhibited 2% DNA-DNA relative reassociation with *S. hyodysenteriae* B78^T (35). Although a standard range of DNA homology values has not been established, 24 to 46% seems to be a reasonable range of DNA homology values for including strains in the same genus. We believe that the characteristics described above support placement of strain P43/6/78^T in the genus *Serpulina*.

S. pilosicoli P43/6/78^T cells superficially resemble *B. aalborgi* 513A^T cells in that both spirochetes have fewer than six periplasmic flagella inserted at each end of each cell and produce weakly beta-hemolytic colonies (11, 23). Unfortunately, the physiological and biochemical properties of *B. aalborgi* have not been studied in sufficient detail to allow comparisons between this human intestinal spirochete and other species of intestinal spirochetes (11). *B. aalborgi* 513A^T exhibited 96.5% 16S rRNA sequence homology with *S. hyodysenteriae* B78^T and *S. innocens* B256^T (10, 38) and 96.3% 16S rRNA sequence homology with *S. pilosicoli* P43/6/78^T (38). The MEE profile of *B. aalborgi* 513A^T is distinct from the MEE profiles of other intestinal spirochetes (23). While the 16S rRNA sequence data suggest that *B. aalborgi* 513A^T is phylogenetically related to species of intestinal spirochetes belonging to the genus *Serpulina* (10, 38), additional methods of comparison (for example, DNA-DNA relative reassociation and more extensive phenotypic characterization) will have to be used before the genetic relatedness of *B. aalborgi* and the other intestinal spirochetes can be fully evaluated.

Phenotypic characteristics that distinguish *S. pilosicoli* P43/6/78^T from *S. hyodysenteriae* B78^T and *S. innocens* B256^T are shown in Table 2. Under the same conditions, *S. pilosicoli* P43/6/78^T cells were smaller, had fewer flagella, and grew more rapidly than the cells of the other species. Ribose was the only growth substrate that distinguished *S. pilosicoli* P43/6/78^T from *S. hyodysenteriae* B78^T and *S. innocens* B256^T. Only *S. pilosicoli* cells hydrolyzed hippurate and were beta-glucosidase negative, two biochemical characteristics that have been used by previous researchers to distinguish intestinal spirochetes associated with intestinal spirochetosis (3, 5). Once additional *S. pilosicoli* strains are identified by genomic and phylogenetic comparisons with P43/6/78^T, it will be possible to assess whether these phenotypic properties are unique to *S. pilosicoli* and thus are useful for identifying this new species.

At this time, the most clear-cut method for differentiating *S. hyodysenteriae*, *S. innocens*, and *S. pilosicoli* is the DNA-DNA relative reassociation method (3, 35). Analyses and comparisons of 16S rRNA sequences are both useful and essential for classifying spirochetes on the "*Serpulina-Brachyspira*" branch of the 16S rRNA dendrogram. The 16S rRNA sequence of *S. pilosicoli* P43/6/78^T contains a short "signature" sequence of nucleotides which has been used to design a PCR that is specific for other human and porcine spirochetes associated with intestinal spirochetosis (29). However, 16S rRNA sequence analysis alone cannot be used to differentiate species within the genus *Serpulina*, as all spirochete strains that have been char-

acterized thus far exhibit significant levels of 16S rRNA sequence identity (38). On the basis of MEE analysis results, *S. pilosicoli* P43/6/78^T was assigned (along with other porcine and human strains) to an electrophoretic group (group VI) which was distinct from the electrophoretic groups containing *S. hyodysenteriae* strains (group I) and *S. innocens* strains (group III) (23, 38). However, at this time, it has not been established whether all of the spirochetes belonging to electrophoretic group VI belong to a single species (i.e., are *S. pilosicoli* strains) (23).

S. pilosicoli P43/6/78^T is a pathogenic spirochete that causes porcine intestinal spirochetosis, a diarrheal disease of swine characterized by the attachment of large numbers of spirochetes by one end to the colonic epithelium. Recent studies have revealed that other intestinal spirochete strains recovered from intestinal spirochetosis-like conditions in pigs (5, 6, 25, 30), humans (23, 26), birds (28), and dogs (2, 3) are related to *S. pilosicoli* P43/6/78^T. It has recently been shown that some of these strains (human intestinal spirochete strain WesB and porcine intestinal spirochete strain 1648) attach by one end to the colonic epithelium and cause watery diarrhea and stunting in 1-day-old specific-pathogen-free chicks (41). Additional analyses of these spirochetes to determine their phylogenetic, genomic, and phenotypic similarities to *S. pilosicoli* P43/6/78^T should reveal whether *S. pilosicoli* is an enteropathogenic spirochete with a broad host range. It will be particularly important to determine if all human intestinal spirochetes that are distinct from the nonpathogenic organism *B. aalborgi* belong to *S. pilosicoli*, as such a finding would support previous suggestions that these organisms may act as diarrheal agents (23, 41). Such studies are currently in progress.

Description of *Serpulina pilosicoli* sp. nov. *Serpulina pilosicoli* (pi.lo.si'co.li.) L. adj. *pilosum*, hairy, napped, shaggy; G neu⁺. n. colon, colon; N. L. gen. n. *pilosicoli*, of a hairy colon (referring to the fact that infection and attachment by this intestinal spirochete [intestinal spirochetosis] result in a microscopic appearance that resembles a hairy covering on the surface of the colon). Typical morphology and motility of members of the genus *Serpulina*. Cells are 4 to 12 μ m long and 0.2 to 0.3 μ m in diameter. There are 8, 10, or occasionally 12 periplasmic flagella per cell; 4 to 6 periplasmic flagella are inserted at each end of each cell, and free ends of these flagella overlap in the middle of the cell. At least one end of each cell tapers abruptly to a distinct point. Weakly beta-hemolytic on Trypticase soy blood agar. Isolated from swine with diarrhea (40).

Enteropathogenic when pure cultures are inoculated orally into swine (40). Each cell attaches by one end to the colonic epithelium. Causes watery, mucoid diarrhea (40). Similar strains may cause diarrhea in humans (26), dogs (3), and poultry (28).

Grows at 37 to 42°C in Trypticase soy broth or brain heart infusion broth supplemented with serum (minimum concentration, 7%) in the presence of an O_2 - N_2 (1:99) atmosphere and reaches a maximum culture density of 2×10^9 cells per ml. Tolerates low O_2 tensions (<7%) in sealed culture tubes and consumes substrate amounts of O_2 . NADH oxidase positive (37). The population doubling time is 1 to 2 h in BHIS broth at 38°C.

The growth substrates utilized include D-glucose, D-fructose, sucrose, D-trehalose, N-acetyl-D-glucosamine, D-glucosamine, D-mannose, maltose, pyruvate, L-fucose, D-cellobiose, and D-ribose. The substrates that do not support growth include L-arabinose, D-fucose, D-raffinose, D-melibiose, L-rhamnose, D-xylose, galactosamine, N-acetylgalactosamine, cellulose, esculin, soluble starch, pectin, mucin, glycogen, glucuronic acid, polygalacturonic acid, glutathione, glycerol, mannitol, acetate, lactate,

succinate, and amino acids. Limited growth occurs in HS broth supplemented with the minimum concentration of serum required and no additional substrate.

Hydrolyzes hippurate. Negative for indole. α -Glucosidase negative, α -galactosidase positive, and β -glucosidase negative. Growing cells produce acetate (23.3 μ mol/ml of medium), small amounts of butyrate and ethanol (1.5 and 2.4 μ mol/ml of medium, respectively), CO₂ (11.3 μ mol/ml of medium), and H₂ (22.4 μ mol/ml of medium) in HS broth supplemented with 0.4% glucose in the presence of an O₂-N₂ (1:99) atmosphere.

Susceptible to ampicillin, carbadox, chloramphenicol, lincomycin, metronidazole, tetracycline, tiamulin, and tylosin. Moderately susceptible to erythromycin, kanamycin, neomycin, novobiocin, rifampin, spiramycin, and streptomycin. Resistant to colistin, nalidixic acid, polymixin B, spectinomycin, and vancomycin.

Exhibits 24 to 25% DNA homology with *S. hyodysenteriae* B78^T and 26 to 32% DNA homology with *S. innocens* B256^T as determined by the S1 nuclease method. The G+C content of strain P43/6/78^T DNA is 25 $\pm$ 1 mol% (as determined by the thermal denaturation method). Exhibits 98.5% 16S rRNA sequence homology with *S. hyodysenteriae* B78^T and 99.2% 16S rRNA sequence homology with *S. innocens* B256^T. Has a unique 16S rRNA signature sequence (5'-AGUUUUUUCGC UUCA-3') (29).

The type strain is strain P43/6/78, which has been deposited in the American Type Culture Collection as strain ATCC 51139.

ACKNOWLEDGMENTS

This work was funded by the Australian Pig Research and Development Corporation, the Australian Research Council, and Murdoch University. D.J.T. was the recipient of an Australian Pig Research and Development Corporation postgraduate scholarship.

We thank Sophy Oxberry, Sam Humphrey, Herb Cook, Judi Stasko, Michelle R. Mathiesen, and Julianna Toth for providing technical assistance. We gratefully acknowledge the assistance of Judith Maitland, Arts Department, University of Western Australia, Perth, Western Australia, Australia, and Thomas McAdoo, Department of Foreign Languages and Literatures, Virginia Polytechnic Institute and State University, Blacksburg, who helped us select the specific epithet of *S. pilosicoli* and provided advice regarding nomenclature.

REFERENCES

- Andrews, J. J., and L. J. Hoffman. 1982. A porcine colitis caused by a weakly β -hemolytic treponeme (*Treponema innocens*?), p. 395–402. In Proceedings of the 25th American Association of Veterinary Laboratory Diagnosticians, Nashville, Tenn.
- Duhamel, G. E., B. D. Hunsaker, M. R. Mathieson, and R. A. Moxley. Intestinal spirochaetosis and giardiasis in a beagle pup with diarrhoea. Vet. Pathol., in press.
- Duhamel, G. E., N. Muniappa, M. R. Mathieson, J. L. Johnson, J. Toth, R. O. Elder, and A. R. Doster. 1995. Certain canine weakly β -hemolytic intestinal spirochetes are phenotypically and genotypically related to spirochetes associated with human and porcine intestinal spirochetosis. J. Clin. Microbiol. 33:2212–2215.
- Duhamel, G. E., M. Ramanathan, I. Gardner, M. A. Anderson, R. L. Walker, and D. J. Hampson. 1993. Intestinal spirochetosis of swine associated with weakly β -hemolytic spirochetes distinct from *Serpulina innocens*, p. 53. In Proceedings of the 36th American Association of Veterinary Laboratory Diagnosticians, Las Vegas, Nev.
- Fellström, C., and A. Gunnarsson. 1995. Phenotypic characterisation of intestinal spirochaetes isolated from pigs. Res. Vet. Sci. 59:1–4.
- Fellström, C., B. Pettersson, M. Uhlén, A. Gunnarsson, and K. Johansson. 1995. Phylogeny of *Serpulina* based on sequence analyses of the 16S rRNA gene and comparison with a scheme involving biochemical classification. Res. Vet. Sci. 59:5–9.
- Girard, C., T. Lemarchand, and R. Higgins. 1995. Porcine colonic spirochetosis: a retrospective study of eleven cases. Can. Vet. J. 36:291–294.
- Harland, W. A., and F. D. Lee. 1967. Intestinal spirochaetosis. Br. Med. J. 3: 718–719.
- Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.). 1977. Anaerobic laboratory manual. Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg.
- Hooley, J. V., S. P. Barrett, C. S. Read, and P. Barber. 1994. Phylogeny of the human intestinal spirochetes inferred from 16S rDNA sequence comparisons. FEMS Microbiol. Lett. 117:345–350.
- Hovind-Hougen, K., A. Birch-Andersen, R. Henrik-Nielsen, M. Orholm, J. O. Pedersen, P. S. Teglbjærg, and E. H. Thaysen. 1982. Intestinal spirochetosis: morphological characterization and cultivation of the spirochete *Brachyspira aalborgi* gen. nov., sp. nov. J. Clin. Microbiol. 16:1127–1136.
- Hovind-Hougen, K., P. Høgh, and A. Birch-Andersen. 1990. Morphological heterogeneity among spirochetes isolated from cases of swine dysentery. Zentralbl. Bakteri. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 274:1–15.
- Hunter, D., and T. Wood. 1979. An evaluation of the API ZYM system as a means of classifying spirochaetes associated with swine dysentery. Vet. Rec. 104:383–384.
- Jacques, C., C. Girard, R. Higgins, and G. Goyette. 1989. Extensive colonization of the porcine colonic epithelium by a spirochete similar to *Treponema innocens*. J. Clin. Microbiol. 27:1139–1141.
- Johnson, J. L. 1985. DNA reassociation and RNA hybridization of bacterial nucleic acids. Methods Microbiol. 18:33–74.
- Johnson, J. L. 1994. Similarity analysis of DNAs, p. 655–682. In P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
- Johnson, J. L., C. F. Phelps, C. S. Cummins, J. London, and F. Gasser. 1980. Taxonomy of the *Lactobacillus acidophilus* group. Int. J. Syst. Bacteriol. 30: 53–68.
- Kinyon, J. M., and D. L. Harris. 1979. *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae*. Harris et al. Int. J. Syst. Bacteriol. 29:102–109.
- Kitai, K., M. Kashiwazaki, Y. Adachi, T. Kume, and A. Arakawa. 1979. In vitro activity of 39 antimicrobial agents against *Treponema hyodysenteriae*. Antimicrob. Agents Chemother. 15:392–395.
- Kleiner, D. E., and W. G. Stetler-Stevenson. 1994. Quantitative zymography: detection of picogram quantities of gelatinases. Anal. Biochem. 218:325–329.
- Kunkle, R. A., and J. M. Kinyon. 1988. Improved selective medium for the isolation of *Treponema hyodysenteriae*. J. Clin. Microbiol. 26:2357–2360.
- Lee, F. D., A. Kraszewski, J. Gordon, J. G. R. Howie, D. McSeveney, and W. A. Harland. 1971. Intestinal spirochaetosis. Gut 12:126–133.
- Lee, J. I., and D. J. Hampson. 1994. Genetic characterisation of intestinal spirochaetes, and their association with disease. J. Med. Microbiol. 40:365–371.
- Lee, J. I., D. J. Hampson, B. G. Combs, and A. J. Lymbery. 1993. Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae*, and comparison of methods for their subspecific differentiation. Vet. Microbiol. 34:35–46.
- Lee, J. I., D. J. Hampson, A. J. Lymbery, and S. J. Harders. 1993. The porcine intestinal spirochaetes: identification of new genetic groups. Vet. Microbiol. 34:273–285.
- Lee, J. I., A. J. McLaren, A. J. Lymbery, and D. J. Hampson. 1993. Human intestinal spirochaetes are distinct from *Serpulina hyodysenteriae*. J. Clin. Microbiol. 31:16–21.
- Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol. 12:195–206.
- McLaren, A. J., D. J. Trott, D. E. Swayne, J. W. Stoutenburg, and D. J. Hampson. 1994. Characterization of avian intestinal spirochetes, p. 66. In Proceedings of the North Central Avian Disease Congress, Des Moines, Iowa.
- Park, N. Y., C. Y. Chung, A. J. McLaren, R. F. Atyeo, and D. J. Hampson. 1995. Polymerase chain reaction for identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis. FEMS Microbiol. Lett. 125:225–230.
- Ramanathan, M., G. E. Duhamel, M. R. Mathieson, and S. Messier. 1993. Identification and partial characterisation of a group of weakly β -hemolytic intestinal spirochetes of swine distinct from *Serpulina innocens* isolate B256. Vet. Microbiol. 37:53–64.
- Spearmann, J. G., G. Nayar, and M. Sheridan. 1988. Colitis associated with *Treponema innocens* in pigs. Can. Vet. J. 29:747.
- Stanton, T. B. 1989. Glucose metabolism and NADH recycling by *Treponema hyodysenteriae*, the agent of swine dysentery. Appl. Environ. Microbiol. 55:2365–2371.
- Stanton, T. B. 1992. Proposal to change the genus designation *Serpulina* to *Serpulina* gen. nov. containing the species *Serpulina hyodysenteriae* comb. nov. and *Serpulina innocens* comb. nov. Int. J. Syst. Bacteriol. 42:189–192.
- Stanton, T. B., B. L. Hanzelka, and N. S. Jensen. 1995. Survey of intestinal spirochetes for NADH oxidase activity. Microb. Ecol. Health Dis. 8:93–100.
- 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.
- Stanton, T. B., and D. F. Lebo. 1988. *Treponema hyodysenteriae* growth under

- various culture conditions. *Vet. Microbiol.* **18**:177–190.
37. **Stanton, T. B., and D. C. Savage.** 1983. *Roseburia cecicola* gen. nov., sp. nov., a motile, obligately anaerobic bacterium from a mouse cecum. *Int. J. Syst. Bacteriol.* **33**:618–627.
38. **Stanton, T. B., D. J. Trott, J. I. Lee, A. J. McLaren, D. J. Hampson, B. J. Paster, and N. S. Jensen.** Differentiation of intestinal spirochaetes by multilocus enzyme electrophoresis and 16S rRNA comparisons. Submitted for publication.
39. **Taylor, D. J.** 1992. Spirochaetal diarrhoea, p. 584–587. *In* A. D. Leman, B. E. Straw, W. L. Mengeling, S. D'Allaire, and D. J. Taylor (ed.), *Diseases of swine*. Iowa State University Press, Ames.
40. **Taylor, D. J., J. R. Simmons, and H. M. Laird.** 1980. Production of diarrhoea and dysentery in pigs by feeding pure cultures of a spirochaete differing from *Treponema hyodysenteriae*. *Vet. Rec.* **106**:326–332.
41. **Trott, D. J., A. J. McLaren, and D. J. Hampson.** 1995. Pathogenicity of human and porcine intestinal spirochetes in day-old specific-pathogen-free chicks: an animal model of intestinal spirochetosis. *Infect. Immun.* **63**:3705–3710.