Anaerobiospirillum thomasii sp. nov., an Anaerobic Spiral Bacterium Isolated from the Feces of Cats and Dogs and from Diarrheal Feces of Humans, and Emendation of the Genus *Anaerobiospirillum*

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Thirty-seven similar strains isolated from feces of cats and dogs and from human diarrheal feces had characteristics of the genus *Anaerobiospirillum*. These organisms were distinguished from the only previously described *Anaerobiospirillum* species, *Anaerobiospirillum succiniciproducens*, by producing acid from adonitol but not from fructose, raffinose, or sucrose and by the lack of α -glucosidase. The G+C contents of the DNAs of the new strains were 39 to 42 mol%. The results of morphological, physiological, DNA G+C content, and DNA homology studies support the proposal that the description of the genus *Anaerobiospirillum* should be emended so that a new species can be included in the genus. The new species *Anaerobiospirillum thomasii* is proposed, with strain A273/88 (= NCTC 12467) as the type strain.

The genus *Anaerobiospirillum*, containing the single species *Anaerobiospirillum succiniciproducens*, was described by Davis et al. in 1976 (1) as a genus of anaerobic spiral bacteria with bipolar tufts of flagella. Later, *A. succiniciproducens* was implicated as a cause of human bacteremia (5). In 1983 *Anaerobiospirillum*-like organisms were isolated from two patients with diarrhea (2). This led to further studies which demonstrated that both *A. succiniciproducens* and *Anaerobiospirillum*-like organisms are part of the normal fecal flora of cats and dogs and that *Anaerobiospirillum*-like organisms may cause diarrhea in humans (3).

In this study I compared the phenotypic characteristics, DNA base compositions, and levels of DNA-DNA homology of *Anaerobiospirillum*-like organisms and *A. succiniciproducens*. I propose that the description of the genus *Anaerobiospirillum* be emended so that a new species, *Anaerobiospirillum thomasii*, can be included in the genus.

MATERIALS AND METHODS

Strains examined and media. The strains used in this study are listed in Table 1. The human strains were isolated during routine diagnostic examination of blood and fecal samples. Details concerning the clinical strains and the methods of isolation used for animal and human strains have been described previously (3).

All strains were grown on fastidious anaerobe agar (Lab M, Topley House, Bury, England) with added horse blood (50 ml/liter) and were incubated at 37°C in a Don Whitley Mark III anaerobic cabinet.

Cell morphology and tests for biochemical properties. The procedures used to determine cell morphology, carbohydrate fermentation, substrate hydrolysis (as determined by the API ZYM technique), and volatile and nonvolatile acid end products of glucose fermentation (as determined by gas chromatography) have been described previously (3). For electron microscopy 1% formalin was added to a 24-h broth culture. The suspension was negatively stained with 1% phosphotungstic acid (pH 6.5), and grids were examined with a Philips model 420 transmission electron microscope.

DNA base composition. DNA was extracted and prepared from 48-h plate cultures (11). The guanine-plus-cytosine (G+C) contents of the DNAs of four strains of *A. succiniciproducens* and 13 strains of the *Anaerobiospirillum*-like organism were determined by the thermal denaturation method (4). *Escherichia coli* type strain NCTC 9001 was used as a reference as it has a known G+C content of 51.1 mol% (8).

DNA-DNA hybridization. RNA-free DNA was extracted by using a combination of the methods of Owen and Borman (7) and Pitcher et al. (11). Cells were

removed from plates and suspended in 240 μ l of RNase/SET (50 mg of RNase A per ml in SET buffer [150 mM NaCl, 15 mM EDTA, 60 mM Tris-HCl; final pH, 8.3]), 10 μ l of 25% sodium dodecyl sulfate was added, and the mixture was incubated in a 37°C water bath for 30 min. After incubation, 250 μ l of GES reagent (60 g of guanidium thiocyanate, 3.7 g of EDTA, and 1.7 ml of 30% sarcosyl in 100 ml) was mixed with the suspension, which was gently swirled until lysis was complete. Then 250 μ l of cold ammonium acetate (7.5 M aqueous solution) and 500 μ l of cold chloroform (96 ml of chloroform, 4 ml of 2-pentanol) were added to the suspension, and the whole mixture was shaken vigorously, transferred to microcentrifuge tubes, and centrifuged at high speed for 10 min. The supernatant (approximately 740 μ l) was transferred to a fresh microcentrifuge tube to which 410 μ l of cold isopropanol was added. The solution was mixed gently by inversion to precipitate the DNA as a pellet, and this pellet was washed three times in 70% ethanol and dried under a vacuum for 20 min.

Hybridization was carried out by using the techniques described by Pitcher et al. (10) with an optimum reassociation temperature of 48°C for 16 h and a stringent washing temperature of 54°C. DNA of the *Providencia alcalifaciens* type strain NCTC 10286 was used as a heterologous control (G+C content, 43 mol%) (6).

RESULTS

Cell morphology. Spiral cells of both *A. succiniciproducens* and *A. thomasii* were usually 0.6 to 0.8 μ m in diameter and 3 to 15 μ m long, although cells up to 32 μ m long were sometimes seen. The type strain of *A. succiniciproducens* was found to be pleomorphic with few typical spiral cells. Electron microscopy revealed the presence of bipolar tufts of flagella (Fig. 1).

Phenotypic characterization. All strains grew at 37 and 42°C but not at 22°C. All strains were negative for catalase activity, oxidase activity, nitrate reduction, and hydrolysis of esculin, hippurate, and urea. Acid was produced from galactose, glucose, and maltose but not from cellobiose, mannitol, salicin, sorbitol, and trehalose. As determined by the API ZYM kit, all of the strains produced leucine arylamidase and *N*-acetyl- β -glucosaminidase, but the following enzymes were not detected: alkaline phosphatase, esterase (C-4), esterase-lipase (C-8), lipase (C-14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphohydrolase, α -D-galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase, and α -fucosidase. The major products of glucose metabolism were succinic acid and acetic acid. Differences between the two species of the genus *Anaerobiospirillum* are shown in Table 2.

DNA base composition. Within *A. succiniciproducens* the G+C contents were found to range from 42 to 44 mol%, and

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TABLE 1. *Anaerobiospirillum* strains used in this study

Strain ^a	Source ^b
<i>A. succiniciproducens</i> strains	
NCTC 11536 ^T	Dog
A196/82	Dog feces ^c
A63/86	Human feces
A148/86	Blood culture, United States ^d
A149/86	Blood culture, United States ^d
A71/88	Blood culture, Hong Kong ^e
A116/88	Cat rectal swab
A337/88	Dog feces
<i>A. thomasii</i> strains	
A30/82	Human feces
A142/82	Human feces
A352/83	Human feces
A356/83	Cat feces
A57/84	Human feces
A353/84	Human feces
A22/85	Human feces
A37/85	Human feces
A70/85	Human feces
A87/85	Human feces
A180/85	Human feces
A181/85	Human feces
A227/86	Human feces
A34/87	Human feces
A52/87	Dog feces
A114/87	Dog feces
A99/88	Cat rectal swab
A103/88	Cat rectal swab
A104/88	Dog rectal swab
A109/88	Human feces
A110/88	Human feces
A115/88	Cat rectal swab
A117/88	Cat rectal swab
A118/88	Cat rectal swab
A124/88	Cat rectal swab
A125/88	Cat rectal swab
A126/88	Cat rectal swab
A137/88	Cat rectal swab
A138/88	Cat rectal swab
A150/88	Cat rectal swab
A151/88	Dog rectal swab
A152/88	Dog rectal swab
A153/88	Dog rectal swab
A159/88	Dog rectal swab
A160/88	Cat rectal swab
A273/88 ^T (=NCTC 12467)	Human feces
A50/89	Dog feces

^a NCTC, National Collection of Type Cultures, London, United Kingdom; A, National Collection of Type Cultures Identification Service, London, United Kingdom.

^b All of the human isolates were obtained from different patients in the United Kingdom, and the cat and dog isolates were obtained from different animals examined at the same veterinary hospital, unless indicated otherwise (3).

^c M. J. Hudson, Centre for Applied Microbiology and Research, Porton Down, United Kingdom.

^d See reference 9.

^e See reference 12.

A. thomasii had a G+C range of 39 to 41 mol% (Table 3). The G+C contents of the *A. thomasii* strains not included in Table 3 were as follows: A181/85 and A137/88, 39 mol%; A109/88, A110/88, and A152/88, 40 mol%; and A70/85, A159/88, and A160/88, 41 mol%.

DNA-DNA hybridization. The results of the DNA-DNA hybridization experiments are presented in Table 3. DNAs from *A. succiniciproducens* strains were 79 to 88% related to labeled DNA from type strain NCTC 11536, but the *Anaerobiospirillum*-like organisms were 5% or less related to the same type strain (most values were the same as the <5% background value obtained with *P. alcalifaciens* NCTC 10286^T [T = type strain]). Hybridization with labeled probes prepared with DNAs from A151/88 and A273/88^T (*Anaerobiospirillum*-like organisms) demonstrated that DNAs from *Anaerobiospirillum*-like strains were 65% or more related to both DNA probes. The levels of hybridization between DNAs from *A. succiniciproducens* strains and A151/88 DNA varied from 3 to 12%, and the levels of hybridization between DNAs from *A. succiniciproducens* strains and A273/88^T DNA ranged from 2 to 10%.

The difference between the level of hybridization of probe DNA from A273/88^T with A151/88 DNA and the level of hybridization of probe DNA from A151/88 with A273/88^T DNA was 5%. The levels of hybridization for reciprocal pairs of labeled and unlabeled NCTC 11536^T DNAs and A151/88 and A273/88^T DNAs varied between 2 and 7%.

DISCUSSION

The original description of *A. succiniciproducens*, published in 1976, was based on three isolates from dogs (1). Since then, several cases of *A. succiniciproducens* bacteremia have been described (5, 9, 12). However, when the type strain of *A. succiniciproducens* was compared with two strains isolated from humans with diarrhea, differences were noticed in the biochemical test results, and the type species was not able to grow on Skirrow's *Campylobacter* isolation medium due to sensitivity to trimethoprim (2, 9). As additional strains isolated from human diarrheal feces were tested, it became clear that there was an undescribed *Anaerobiospirillum* species. The differences in phenotypic test results are shown in Table 2. Although relatively few cases of *Anaerobiospirillum* infection have been described, there are possible differences in pathogenicity between the two species, as *A. succiniciproducens* has been isolated from blood cultures but *A. thomasii* has not. Lack of isolation of *A. succiniciproducens* from human diarrheal feces is probably due to the inability of this organism to grow on selective media normally used for isolation of *Campylobacter* strains and anaerobic bacteria in clinical laboratories.

I propose, because of the similarities in cell morphology, flagellar arrangement (bipolar tufts), growth characteristics, and some phenotypic characteristics and the differences in DNA-DNA hybridization values and DNA G+C contents of *A. succiniciproducens* and the *Anaerobiospirillum*-like organisms, that the definition of the genus *Anaerobiospirillum* be emended to include a new species, *A. thomasii*.

TABLE 2. Differentiation between *A. succiniciproducens* and *A. thomasii*

Species	No. of strains	Acid production from:						API substrates		
		Adonitol	Fructose	Inulin	Lactose	Raffinose	Sucrose	β-D-Galactoside	α-D-Glucoside	α-Maltoside
<i>A. succiniciproducens</i>	8	— ^a	+	+	7	+	+	+	+	4
<i>A. thomasii</i>	37	+	—	—	—	—	—	—	—	—

^a —, all strains are negative; +, all strains are positive. The numbers are the numbers of strains that are positive.

FIG. 1. Electron micrograph of negatively stained cells of *A. thomasi* NCTC 12467^T, showing bipolar tufts of flagella. Bar = 1 μ m.

It should be noted that the original description of *A. succiniciproducens* (1) stated that this organism is galactose negative. However, all of the strains tested in this study, including the type strain, were galactose positive.

Emended description of the genus *Anaerobiospirillum* Davis, Cleven, Brown, and Balish 1976. Gram-negative helical rods with rounded ends, usually 0.6 to 0.8 μ m in diameter and 3 to 15 μ m long, although some cells are up to 32 μ m long. Motile by means of bipolar tufts of flagella. Cells usually occur singly. Endospores are not formed. Strictly anaerobic. Catalase and oxidase negative. Does not hydrolyze esculin, hippurate, or urea and does not reduce nitrate. Carbohydrate metabolism is fermentative. The major products from glucose metabolism are succinic and acetic acids; traces of lactic and formic acids may also be formed. The optimal growth temperature is 37 to 42°C. Found in feces of dogs and cats and as a pathogen in humans, causing septicemia and/or diarrhea. The DNA G+C content range is 39 to 44 mol%. The type species is *A. succiniciproducens* (type strain, NCTC 11536 [= ATCC 29305]).

Description of *Anaerobiospirillum thomasi* sp. nov. *Anaerobiospirillum thomasi* (thom'as.i.i. N. L. gen. n. *thomasi*, of Thomas, named after M. E. M. Thomas [Public Health Laboratory Service, 1947 to 1984], whose interest in and enthusiasm for uncommon medical bacterial infections led to the initiation of this study). Cells are gram-negative helical rods (0.5 to 0.7 by 5 to 32 μ m). Flagella are arranged in bipolar tufts. Growth occurs at temperatures between 33 and 43°C; the optimal temperature is 37°C. Flat colonies with even, sometimes spreading edges are 1 to 2 mm in diameter after 24 to 48 h on horse blood agar. Hemolysis does not occur. Strictly anaerobic. Carbohy-

drate metabolism is fermentative. Acid is produced from adonitol, galactose, glucose, and maltose. Acid is not produced from cellobiose, fructose, inulin, lactose, mannitol, raffinose, salicin, sorbitol, sucrose, and trehalose. The following tests are nega-

TABLE 3. DNA base compositions and levels of homology between [³H]dCTP-labeled DNAs and unlabeled DNAs

Source of unlabeled DNA ^a	G+C content (mol%)	% Homology with labeled DNA from:		
		NCTC 11536 ^T	A151/88	A273/88 ^T
<i>A. succiniciproducens</i> strains				
NCTC 11536 ^T	44	100	7	6
A196/82	44	88	5	9
A63/86	ND ^b	81	7	10
A148/86	44	81	5	6
A71/88	42	79	3	2
A116/88	ND	80	12	6
<i>A. thomasi</i> strains				
A273/88 ^T (= NCTC 12467 ^T)	41	4	97	100
A52/87	ND	4	103	93
A117/88	39	3	97	97
A227/86	41	2	102	101
A114/87	40	0	82	70
A138/88	41	5	65	77
A151/88	ND	2	100	92

^a The level of hybridization between *P. alcalifaciens* NCTC 10286^T and each probe was less than 5%.

^b ND, not determined.

tive: catalase, oxidase, nitrate reduction, and hydrolysis of esculin, hippurate, and urea. The enzymes detected with an API ZYM kit are leucine arylamidase and *N*-acetyl- β -glucosaminidase. The enzymes not detected with an API ZYM kit include acid and alkaline phosphatases, esterase (C-4), esterase-lipase (C-8), lipase (C-14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphohydrolase, α -D-galactosidase, β -D-galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase, and α -fucosidase. The major products of glucose metabolism are succinic acid and acetic acid. The G+C content of the DNA is 39 to 42 mol%. Isolated from cat and dog feces and as a pathogen in human diarrheal feces. The type strain is strain NCTC 12467.

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