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Campylobacter nitrofigilis sp. nov., a Nitrogen-Fixing Bacterium Associated with Roots of *Spartina alterniflora* Loisel

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Obligately microaerophilic, nitrogen-fixing bacteria were found associated with roots of *Spartina alterniflora* Loisel and in root-associated sediments from salt marshes in Nova Scotia, Canada, and Georgia. These bacteria differ from previously described species and thus represent a new species. The cells of all strains which we studied are small, rigid, curved, motile, and rod shaped and have single polar flagella. Metabolism is respiratory, and the strains utilize organic and amino acids, but not carbohydrates, as sole carbon sources. Poly-β-hydroxybutyrate is not produced. These traits and the guanine-plus-cytosine contents of the deoxyribonucleic acids of these strains (28.3 ± 0.1 mol%) indicate that they are members of the genus *Campylobacter* Sebald and Véron 1963. However, these strains can be distinguished from the previously described species of *Campylobacter* by the presence of nitrogenase, by their tolerance of and apparent requirement for NaCl, by the production of pigment from tryptophan, by a combination of other biochemical traits, and by their association with plant roots. Therefore, we propose that these strains represent a new species, *Campylobacter nitrofigilis*, and we designate strain CI (= ATCC 33309) as the type strain.

Campylobacter species are commonly occurring pathogens and commensal organisms that are associated with a diverse range of animal hosts (23, 24, 31). Of particular interest in this genus is the *Campylobacter jejuni-Campylobacter coli* group (*Campylobacter fetus* subsp. *jejuni*), which increasingly is being recognized as a cause of enteritis in humans (3, 10). In addition to the human- and animal-associated campylobacters, free-living strains of *Campylobacter* have been isolated in culture (11, 15), but the taxonomic status of these organisms has not been established yet. One of the free-living strains, strain CI^T (type strain), was found associated with the roots of *Spartina alterniflora* Loisel growing in a salt marsh near Halifax, Nova Scotia, Canada; this strain was found to be capable of fixing nitrogen (15). In this paper we describe the isolation in culture of several additional nitrogen-fixing *Campylobacter* strains from *S. alterniflora* roots and root-associated sediments from a salt marsh on Sapelo Island, Georgia. We propose that these nitrogen-fixing, *Spartina*-associated strains represent a new species, *Campylobacter nitrofigilis*.

MATERIALS AND METHODS

Isolation. Roots of *S. alterniflora* Loisel were taken from a salt marsh on Sapelo Island, Georgia, and were washed free of sediment in adjacent creek water. Root pieces (length, 2 cm) were placed in tubes of semisolid diazotroph medium (15). Other tubes were seeded with root-associated sediment samples. After 72 h at room temperature, the cultures were transferred to fresh tubes of semisolid malate-salts (MS) medium (15), and nitrogenase activity was determined by acetylene reduction assays (4). After 24 h, tubes of MS medium were capped with serum stoppers, and acetylene was injected to give a final partial pressure of C_2H_2 of 2 kPa. Acetylene reduction was measured by gas chromatography, using a Shimadzu model GC-4BM instrument equipped with a flame ionization detector and a stainless steel column (0.32 by 50 cm) packed with 80- to 100-mesh Porapak T at 50°C.

Cultures that were positive for acetylene reduction were used to seed plates of molten, cooled (45°C) MS medium containing 7 g of agar per liter (27), which were incubated at 25°C. The small, white, irregularly shaped colonies which developed were transferred to tubes of semisolid MS medium, and the resulting cultures were tested for nitrogenase activity. Nitrogenase-positive cultures were streaked onto marine agar plates which were incubated under reduced oxy-

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gen tension (partial pressure of O_2, approximately 5 kPa) at 25°C. Individual colonies that formed were tested for nitrogenase activity in semisolid MS medium. Nitrogenase-positive cultures were streaked twice in succession. The pure cultures were maintained by weekly transfer in semisolid MS medium at 25°C. Young (24- to 48-h) cultures grown in MS medium were also frozen and stored at −70°C until they were needed. The Marine agar which we used had the following composition: peptone, 2.5 g; yeast extract, 0.5 g; $FePO_4$, 0.05 g; NaCl, 15.0 g; agar, 15.0 g; and distilled water, 1,000 ml. The pH of this medium was adjusted to 7.0 before autoclaving.

Physiological tests. Physiological tests were performed as described by Hylemon et al. (7), with the following exceptions. MS medium was used instead of a succinate-based medium. Unless otherwise indicated, all of the media used contained 15.0 g of NaCl per liter and 1.75 g of agar per liter. Cultures grown in liquid medium or on solid medium (15 of agar per liter) were incubated in GasPak anaerobic chambers (BBL Microbiology Systems) under reduced oxygen tensions produced with a Campypak II gas-generating envelope (BBL). All cultures were incubated at 30°C unless indicated otherwise.

Compounds which could serve as sole carbon sources were determined as described by McClung and Patriquin (15). Acidic reactions in tests with carbohydrates were determined in O/F basal medium (Difco Laboratories). The carbohydrates tested were sterilized by filtration and were incorporated into media at concentrations of 10.0 g/liter. Production of acid or neutral end products from glucose was tested in MRVP broth (Difco). Hippurate hydrolysis was determined by the method of Hwang and Ederer (6). Urease activity was also tested on Christiensen urease agar (1). Reduction of NO_3 and N_2O and growth in the presence of 2,3,5-triphenyltetrazolium chloride were determined as described by McClung and Patriquin (15). The effect of nitrate on nitrogenase activity was determined in MS medium supplemented with $NaNO_3$ at a concentration of 1.0 g/liter. The effects of nitrate, aspartate, and fumarate on anaerobic growth were tested on plates of yeast extract-peptone medium in GasPak anaerobic chambers (BBL) containing a GasPak H_2-CO_2-generating envelope (BBL). Yeast extract-peptone medium contained 1.0 g of yeast extract, 5.0 g of peptone, 15.0 g of NaCl, 15.0 g of agar, and 1,000 ml of distilled water; the pH was adjusted to 7.0 before autoclaving. Yeast extract-peptone medium was supplemented with $NaNO_3$, aspartate, or fumarate at a final concentration of 2.0 g/liter. Swarming and production of coccal forms were determined as described by Karmali et al. (9), except that nutrient agar was used instead of blood agar. Nutrient agar contained (per liter of distilled water) 5.0 g of meat extract, 3.0 g of peptone, 15.0 g of NaCl, and 15.0 g of agar; the pH was adjusted to 7.0 before autoclaving.

The ability of KCl or sucrose to replace the requirement of NaCl for growth was determined in nutrient broth (BBL). The broth was supplemented with NaCl at a concentration of 86 mM (5.0 g/liter) or 257 mM (15.0 g/liter), with 86 or 257 mM KCl, or with 172 or 514 mM sucrose. Unsupplemented nutrient broth was used as a control. The osmolarities of the media were estimated by using an Advanced Instruments model 3W osmometer.

To determine the presence of intracellular poly-β-hydroxybutyrate, late-log-phase cultures grown in nutrient broth (BBL) were extracted with commercial bleach and then with chloroform. Poly-β-hydroxybutyrate was measured by the crotonic acid method (12). *Azospirillum lipoferum* SP59b was used as a positive control for poly-β-hydroxybutyrate production.

Susceptibilities to antibiotics were assessed by disk growth inhibition tests. Plates of nutrient agar were seeded to produce bacterial lawns, and antibiotics were applied in impregnated paper disks (Difco). The plates were examined after 72 h for the presence of zones of inhibition around the disks. Minimal inhibitory concentrations of selected antibiotics were determined in semisolid MS medium. Susceptibility to 2,4-diamino-6,7-diisopropylpteridine (vibriostatic agent 0/129; Sigma Chemical Co.) was determined in semisolid MS medium.

Cells from late-log-phase cultures grown in liquid MS medium at 30°C were negatively stained with 1.0% (wt/vol) potassium phosphotungstate (pH 7.0) and observed with a JEOL model JEM-100B transmission electron microscope.

Deoxyribonucleic acids (DNAs) were extracted and purified by the method of Lee and Davis (13). The base composition of each DNA was estimated based on thermal denaturation in 10 mM potassium phosphate–1 mM ethylenediaminetetraacetate (pH 7.0) by using a model 2400 spectrophotometer (Gilford Instrument Laboratories, Inc.) equipped with a temperature programmer. *Spiroplasma* sp. strain PPS1 and *Spiroplasma citri* Maroc R8A2 were used as standards. The guanine-plus-cytosine (G+C) contents were calculated by the method of Marmur and Doty (14), assuming that the G+C content of *Spiroplasma* sp. strain PPS1 DNA is 29 mol% (15b) and the G+C of *S. citri* DNA is 26 mol% (8, 19).

Strains of the *C. jejuni-C. coli* group were tested for nitrogenase activity in MS medium (with the NaCl concentration reduced to 5.0 g/liter) by using the procedures described above.

RESULTS

Nitrogen-fixing strains of *Campylobacter* were readily isolated from roots of both short and tall *S. alterniflora* plants and from root-associated sediments in a salt marsh on Sapelo Island, Georgia (Table 1). Like the previously described nitrogen-fixing, *Spartina*-associated *Campylobacter* strains (15), the Georgia isolates were all gram-negative, slender, rigid, curved, rod-shaped organisms whose cells were 0.2 to 0.9 μm wide by 1 to 3 μm long and had single polar flagella (Fig. 1) and a characteristic rapid, corkscrew motility. Very few long, helical cells were observed.

All of these nitrogen-fixing *Campylobacter* strains from Georgia were positive for the following traits: nitrogenase, catalase, oxidase, H_2S production from cysteine, and production of pigment from tryptophan. All strains were capable of growth in media containing 0.5 to 7.0% NaCl, and all strains were able to utilize

TABLE 1. Strains used in this study

Strain no. in this study	Name	Original strain designation	Isolated from:	Reference(s)
1[T]	*Campylobacter* sp.	CI[T]	Roots of short *S. alterniflora*, Halifax, Nova Scotia, Canada	15
2	Original isolate	G1	Sediment from zone of short *S. alterniflora*, Sapelo Island, Georgia	
3	Original isolate	G4B	Sediment from zone of short *S. alterniflora*, Sapelo Island, Georgia	
4	Original isolate	G12	Sediment from zone of short *S. alterniflora*, Sapelo Island, Georgia	
5	Original isolate	G14	Sediment from zone of short *S. alterniflora*, Sapelo Island, Georgia	
6	Original isolate	G2	Roots of short *S. alterniflora*, Sapelo Island, Georgia	
7	Original isolate	G10	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
8	Original isolate	G3	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
9	Original isolate	G5	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
10	Original isolate	G6	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
11	Original isolate	G9	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
12	Original isolate	G13	Roots of tall *S. alterniflora*, Sapelo Island, Georgia	
13	*C. jejuni-C. coli* group (*C. fetus* subsp. *jejuni* ATCC 29428)[a]	H840	Diarrheic stool of child	
14–33	*C. jejuni-C. coli* group[b]		Beef, pork, and lamb carcasses	25, 26
34–42	*C. jejuni-C. coli* group[c]		Cases of human gastroenteritis	
43	*Spiroplasma citri*	Maroc R8A2	*Citrus cinensis*	19
44	*Spiroplasma* sp. strain ATCC 27556[d]	PPS1	*Calliandra haematocephala*	16
45	*Azospirillum lipoferum*[e]	SP59b	Wheat roots, Rio de Janeiro, Brazil	29

[a] ATCC, American Type Culture Collection, Rockville, Md.
[b] Obtained from N. J. Stern, Meat Science Research Laboratory, Animal Science Institute, U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center-East, Beltsville, Md.
[c] Obtained from J. C. Coolbaugh, Naval Medical Research Institute, Bethesda, Md.
[d] Obtained from R. E. McCoy, Agricultural Research Center, University of Florida, Fort Lauderdale.
[e] Obtained from P. van Berkum, Cell Culture and Nitrogen Fixation Laboratory, Plant Physiology Institute, U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center-West, Beltsville, Md.

the following compounds as sole carbon and energy sources: L-asparagine, L-aspartate, fumarate, L-glutamate, L-glutamine, α-ketoglutarate, lactate, malate, L-proline, pyruvate, and succinate. All of these nitrogen-fixing *Campylobacter* strains were negative for the following traits: Gram reaction, phosphatase, sulfatase, indole production, nitrite reduction, methyl red test, Voges-Proskauer test, fluorescent pigment production, production of pigment from phenylalanine or tyrosine, production of poly-β-hydroxybutyrate, swarming on moist media, and rapid coccal transformation. None of the strains was capable of growth at 42°C, growth in media containing 1.0% glycine or 0.1% 2,3,5-triphenyltetrazolium chloride, or growth in media containing 0.01% NaCl. None of the strains was able to hydrolyze esculin, DNA, gelatin, hippurate, or soluble starch. No strain produced an acid reaction from carbohydrates (arabinose, fructose, glucose, inositol, lactose, mannose, mannitol, sorbitol, sucrose, and xylose), and no strain was able to utilize the following compounds as sole sources of carbon and energy: β-alanine, L-arginine, citrate, L-cysteine, malonate, L-arabinose, D-fructose, D-glucose, *i*-

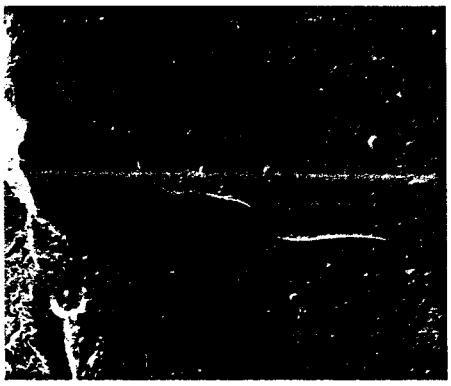

FIG. 1. Electron micrograph of freeze-etched *C. nitrofigilis* ATCC 33309^T cell. Bar = 0.5 μm. Photo courtesy of C. Bean and R. L. Steere.

inositol, lactose, D-mannose, D-mannitol, D-sorbose, sucrose, and D-xylose.

The biochemical and physiological characteristics of the nitrogen-fixing *Campylobacter* strains for which we observed differences among strains are shown in Table 2. In all strains growth was usually obligately microaerophilic, with only very weak growth detected under anaerobic conditions. However, anaerobic growth on plates containing MS medium or nutrient agar was greatly stimulated by adding either aspartate or fumarate. Nitrate did not stimulate anaerobic growth and prevented the stimulation of anaerobic growth by aspartate and fumarate in all strains except strains 4 and 5 (Table 2). Strains 4 and 5 both lacked dissimilatory nitrate reductase activity, in contrast to all other strains, which did reduce nitrate to nitrite. Nitrite was not further reduced. Nitrate (1.0 g/liter) suppressed nitrogenase activity by all strains except strains 4 and 5. On the basis of thermal denaturation, the G+C contents of the DNAs of the nitrogen-fixing *Campylobacter* strains were estimated to be 28.3 ± 0.1 mol%.

The responses of the *Campylobacter* strains to several antibiotics are shown in Table 3. The antibiotic susceptibilities of these strains were generally consistent with those reported for other members of the genus (24, 30). Certain antibiotics may be useful in species differentiation (Table 4) (31).

All strains of the *C. jejuni-C. coli* group examined grew well in MS medium, but were nitrogenase negative.

DISCUSSION

The nitrogen-fixing strains which we studied were judged to be affiliated with the *Spirillaceae* on the basis of their Gram reactions, motility, and growth as rigid, curved rods with strictly respiratory metabolism. The presence of a single polar flagellum, the lack of poly-β-hydroxybutyrate production, and the DNA base composition (28.3 ± 0.1 mol% G+C) indicate these strains should be placed in the genus *Campylobacter*. This placement in *Campylobacter* was confirmed by the physiological and biochemical characteristics of the strains, which in general are consistent with those of previously de-

TABLE 2. Biochemical characteristics of *C. nitrofigilis* for which there are strain differences

Characteristic	No. of strains positive/(n = 12)	Reaction of type strain 1	Strain(s) that gave the less common result
Urease (Hylemon et al.)[a]	10	+	2, 3
Urease (Christensen)[b]	5	+	1^T, 6, 7, 9, 12
Nitrate reduction	10	+	4, 5
Growth with 1.0% bile	9	−	1^T, 2, 3
Anaerobic growth with:			
Aspartate + nitrate	2	−	4, 5
Fumarate + nitrate	2	−	4, 5
Growth at 6°C	6	+	1^T, 3, 7, 8, 11, 12[c]
Growth at 37°C	11	−	1^T
Growth with the following compounds as sole carbon and energy sources:			
Acetate	8	+	5, 7, 11, 12
L-Alanine	10	+	4, 6
Hydroxy-L-proline	2	+	1^T, 5
L-Leucine	8	−	1^T, 2, 3, 5
L-Lysine	2	+	1^T, 5
L-Serine	2	+	1^T, 4

[a] As determined by the technique of Hylemon et al. (7).
[b] As determined by the technique of Christensen (1).
[c] Strains which grew at 6°C.

TABLE 3. In vitro antibiotic susceptibilities of *C. nitrofigilis*[a]

Antibiotic	Concn (mg/disk)	Widths of inhibition zones (mm)[b]
Cephalothin	30	16–27
Chloramphenicol	30	8–15
Erythromycin	15	9–16
Gentamicin	10	13–17
Kanamycin	30	12–17
Nalidixic acid	30	6–15
Neomycin	30	11–17
Novobiocin	30	0
Streptomycin	10	9–15
Terramycin (oxytetracycline)	30	8–20
Tetracycline	30	10–19
Tobramycin	10	11–19
Vancomycin	30	0

[a] In addition, the 12 *C. nitrofigilis* strains were tested for susceptibility to bacitracin, vibriostatic agent 0/129, penicillin G, and trimethoprim in media containing the antibiotics. The highest concentrations of antibiotics at which all strains showed growth after 72 h of incubation at 30°C and the lowest concentrations at which growth was inhibited in all strains after 72 h were as follows: bacitracin, 256 and 1,024 mg/ml, respectively; vibriostatic agent 1/129, 150 mg/ml and not determined, respectively; penicillin G, 30 and 128 mg/ml, respectively (except for strains 1^T and 6, which grew at concentrations of 8 mg/ml but were inhibited at concentrations of 16 mg/ml); and trimethoprim, 5 and 50 mg/ml, respectively.

[b] Range of the widths of the inhibition zones observed for all 12 *C. nitrofigilis* strains around disks after 72 h of incubation at 20°C.

scribed *Campylobacter* species. However, several traits of these strains are unique in the genus; these include the positive urease and nitrogenase activities, pigment production from tryptophan, and the association of the organisms with plant roots. Nevertheless, these differences are insufficient to warrant exclusion of the strains from the genus in view of the numerous similar morphological and biochemical traits (15; this study).

The DNA base compositions of the nitrogen-fixing *Campylobacter* strains, which range from 27.9 to 28.8 mol% G+C, are lower than the values obtained for other catalase-positive *Campylobacter* species, which range from 29.5 to 36 mol% (20, 23, 24, 31), and are also lower than the values obtained for the catalase-negative organisms *Campylobacter sputorum* and *Campylobacter concisus*, which range from 29 to 31 mol% and from 34 to 38 mol%, respectively (20, 28). The nitrogen-fixing *Campylobacter* strains are further distinguished from all other previously described *Campylobacter* species by the unique biochemical traits described above and also by a variety of biochemical characteristics (Table 4). Unlike *C. fetus*, the nitrogen-fixing *Campylobacter* strains grow in the presence of 3.5% NaCl, do not grow anaerobically with nitrate, and are susceptible to nalidixic acid but resistant to vancomycin. The nitrogen-fixing *Campylobacter* strains can be distinguished from the *C. jejuni-C. coli* group by their inability to grow in the presence of 1.0% glycine, to undergo rapid coccal transformation, or to swarm on moist agar, by their ability to grow at 25 but not at 42°C, and by their susceptibility to cephalothin. In contrast to *Campylobacter faecalis*, the nitrogen-fixing strains are not able to grow in the presence of 1.0% glycine or to grow anaerobically with nitrate and are able to grow at 25 but not at 42°C. Unlike *Campylobacter sputorum*, the nitrogen-fixing *Campylobacter* strains are catalase positive, cannot grow at 42°C, and cannot grow anaerobically with nitrate. The nitrogen-fixing *Campylobacter* strains differ from *C. concisus* in their positive catalase reactions, their ability to grow in 3.5% NaCl, their inability to grow anaerobically with nitrate, and their susceptibility to nalidixic acid. The nitrogen-fixing *Campylobacter* strains also differ from the strain isolated by Laanbroek et al. (11) in their ability to grow in 3.5% NaCl and in their inability to grow anaerobically with nitrate. Thus the nitrogen-fixing *Campylobacter* strains are distinct from all previously described *Campylobacter* species on the basis of DNA base composition and biochemical characteristics. Therefore, these nitrogen-fixing strains of *Campylobacter* represent a new species, for which we propose the name *Campylobacter nitrofigilis*.

The obligately microaerophilic nature of *C. nitrofigilis* has potentially important implications for the study of nitrogen-fixing bacteria. The procedures used to isolate N_2-fixing organisms commonly include steps involving aerobic culture (2) and, therefore, probably do not retain obligately microaerophilic organisms such as *Campylobacter*, as noted previously (15).

Although growth of *C. nitrofigilis* is obligately microaerophilic when oxygen is the terminal electron acceptor, both asparate and fumarate serve as terminal electron acceptors and support growth under anaerobic conditions. Nitrate does not support anaerobic growth and prevents the stimulation of anaerobic growth by aspartate and fumarate in all but the nitrate reductase-negative strains. This supports the hypothesis (15) that it is the toxic effect of the nitrite produced from nitrate reduction, rather than an inability to use nitrate as a terminal electron acceptor, that prevents stimulation of anaerobic growth by nitrate.

TABLE 4. Characteristics useful in distinguishing *C. nitrofigilis* from other *Campylobacter* species[a]

Character	*C. nitrofigilis*	*C. fetus* subsp. *fetus*[b]	*C. fetus* subsp. *venerealis*[b]	*C. jejuni-C. coli* group[b]	*C. faecalis*	*Campylobacter* sp.[c]	*C. sputorum*[b]	*C. concisus*
Nitrogenase	+[d]	−	NT	−	NT	−	NT	NT
Catalase	+	+	+	+	+	+	−	−
Urease	V	−	−	−	−	−	−	−
H_2S from cysteine	+	+	V	+	+	−	+	+
Pigment from tryptophan	+	−	−	−	−	−	−	NT
Growth with:								
1.0% Bile	V	+	−	+	V	NT	V	+
1.0% Glycine	−	+	−	+	+	−	V	NT
3.5% NaCl	+	−	−	−	V	−	V	−
0.1% Tetrazolium	−	−	−	V[e]	NT	NT	NT	NT
Growth at:								
25°C	+	+	+	−	−	+	V	NT
42°C	−	−	−	+	+	−	+	NT
Rapid coccal transformation	−	−	−	+	NT	−	NT	NT
Hippurate hydrolysis	−	−	−	+/−[f]	NT	NT	NT	NT
Swarming	−	−	−	+	NT	−	NT	NT
Susceptibility to:								
Nalidixic acid	+	−	−	+	NT	NT	NT	−
Cephalothin	+	+	+	−	NT	NT	NT	NT
Vancomycin	−	+	+	−	NT	NT	NT	−
Anaerobic growth with:								
Nitrate	−	+	+	−	+	+	+	+
Aspartate + fumarate	+	+	V	V	+	+	+	+
G+C content (mol%)	28.3	34.3	34.4	31.5/32.7	36.6	41.6	29–31	34–38

[a] Data from this study and from references 5, 9, 11, 15, 17, 18, 20, 22–24, 28, and 31.
[b] Nomenclature follows that on the Approved Lists (21).
[c] Isolated by Laanbroek et al. (11).
[d] +, Positive; −, negative; V, reactions vary among strains; NT, not tested.
[e] *C. jejuni* is weakly positive, and *C. coli* is positive (31).
[f] *C. jejuni* is positive, and *C. coli* is negative (22).

The isolation of *C. nitrofigilis* strains from high-salinity *S. alterniflora* marshes as far apart as Nova Scotia and Georgia shows that these organisms have a broad geographic range. It is not known whether the nitrogen-fixing *Campylobacter* strains are restricted to *S. alterniflora* marshes. However, nitrogen-fixing *Campylobacter* strains could not be isolated from *S. alterniflora* growing in a low-salinity marsh on Chesapeake Bay, Maryland. This, coupled with the apparent NaCl requirement of the organisms in vitro, suggests that these nitrogen-fixing *Campylobacter* strains are restricted to sites with approximately oceanic salinity. The association of *C. nitrofigilis* with the roots of a salt marsh grass contrasts with the habitat of other *Campylobacter* species, which commonly are associated with warm-blooded animals. This raises interesting questions about the evolutionary relationship between the nitrogen fixers and the other members of the genus.

Description of *Campylobacter nitrofigilis* sp. nov. *C. nitrofigilis* (ni. tro. fig' i. lis. L. n. *nitrum* native mineral salt, native alkali; L. v. *figo* to fix; L. suffix *ilis* ability; M.L.adj. *nitrofigilis* able to fix nitrogen) cells are gram-negative, curved rods 0.2 to 0.9 μm in diameter and 1 to 3 μm long. Coccoid bodies are found in old cultures but are not rapidly produced under aerobic conditions. Motile with a rapid corkscrew motion. Each cell possesses a single polar flagellum. Does not swarm. Chemoorganotrophic. Utilizes organic and amino acids as carbon sources, but not carbohydrates. Respiratory metabolism with oxygen as the terminal electron acceptor; anaerobic growth with aspartate and fumarate, but not with nitrate. Nitrate usually reduced to nitrite. Requires NaCl for growth. Grows at temperatures of 10 to 35°C but not at 42°C. Catalase, oxidase, and nitrogenase positive. Some strains are urease positive. Sulfide is produced from cysteine. A brown, water-soluble pigment is produced from tryptophan. Fluorescent pigments are not produced. The base composition of the DNA is 28.3 ± 0.1 mol% G+C (range, 27.9 to 28.8 mol% G+C). Phosphatase, sulfatase, and indole negative. Does not hydrolyze esculin, casein, DNA, gelatin, hippurate, or starch. Incapable of growth with glycine (1%, wt/vol) or 2,3,5-triphenyltetrazolium chloride

(0.1%, wt/vol). Some strains capable of growth with bile (1%, wt/vol). Poly-β-hydroxybutyrate not produced. Susceptible to cephalothin and nalidixic acid.

Source: isolated from roots of *S. alterniflora* taken from Conrad Beach, Nova Scotia, Canada, and from *S. alterniflora* roots and root-associated sediments on Sapelo Island, Georgia.

The type strain is strain CI (= ATCC 33309).

Description of the type strain. The description of the type strain is the same as that given above for the species, with the following additions. Urease positive. Reduces nitrate to nitrite. Does not grow with bile. The base composition of the DNA is 28.0 mol% G+C as determined by thermal denaturation and 32.1 mol% as determined by buoyant density.

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