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Campylobacter showae sp. nov., Isolated from the Human Oral Cavity

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Nine *Campylobacter*-like strains were isolated from human gingival crevices and characterized. These strains were gram-negative, straight rods that were motile by means of multiple unipolar flagella. They were asaccharolytic and preferred an anaerobic atmosphere rather than a microaerophilic atmosphere for growth, and their growth was stimulated by formate and fumarate. These strains were biochemically similar to *Campylobacter curvus* and *Campylobacter rectus*, but were clearly distinguishable from these organisms by the number of flagella (two to five flagella at one end of the cell), by being catalase positive, by their whole-cell protein profiles, by their Western blot (immunoblot) patterns, and on the basis of DNA-DNA homology data. They could also be differentiated from the other species of the genus *Campylobacter*. The nine *Campylobacter*-like strains were compared with two strains (FDC 286 and VPI 10279) representing a previously described but unnamed *Wolinella* sp. The nine isolates and strains FDC 286 and VPI 10279 were found to be members of a single species. The 16S rRNA sequences of two strains of the newly identified species were compared with the rRNA sequences of 21 reference *Campylobacter*, *Wolinella*, and *Helicobacter* species in order to generate a phylogenetic tree. We propose the name *Campylobacter showae* for the newly identified strains; strain SU A4 (= ATCC 51146) is the type strain of this new species.

Previously, we isolated several *Wolinella*-like strains from human subgingival dental plaque (16) and subsequently proposed *Wolinella curva* subsp. *intermedius* as a new subspecies for two of the isolates (SU A4^T [T = type strain] and SU A5) (4). These organisms could be differentiated at least at the subspecies level by morphological, biochemical, antigenic, and DNA-DNA homology methods from the other human oral *Wolinella* species, *W. curva* and *Wolinella recta*, which recently have been transferred to the genus *Campylobacter* as *Campylobacter curvus* and *Campylobacter rectus*, respectively, by Vandamme et al. (24, 25). In order to more fully describe the status of a new species in the genus *Campylobacter*, seven additional strains were isolated; thus, a total of nine strains, including SU A4^T and SU A5, were characterized. These nine isolates were compared with *Wolinella* sp. strains FDC 286 (22) and VPI 10279 (14) and strains of other *Campylobacter* species (19, 20, 24, 25). A 16S rRNA sequence analysis was used to determine the phylogenetic position of strains SU A4^T and VPI 10279. The nine isolates and strains FDC 286 and VPI 10279 are members of a new species, for which we propose the name *Campylobacter showae*.

MATERIALS AND METHODS

Bacterial strains and characterization. The sources and accession numbers of the isolates and reference strains examined in this study are shown in Table 1. The nine *Campylobacter*-like strains were isolated from the dental plaque of gingival crevices of nine apparently healthy adults on plates containing modified CBRCA (26), which is a selective medium for *Campylobacter*, *Fusobacterium*, *Bacteroides*, *Selenomonas*, and *Veillonella* strains and contains reinforced clostridial agar (Oxoid Ltd., Hampshire, England), 5% horse blood, 0.03% China blue, 0.2% sodium formate, 0.3% sodium fumarate, and 0.5 μg of menadion per ml. The strains were maintained on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% horse blood, 0.2% sodium formate, and 0.3% sodium fumarate (basal medium) and were incubated at 37°C anaerobically in an 80% N_2–10% CO_2–10% H_2 atmosphere. Growth under microaerophilic conditions, which were provided by a hydrogen and carbon dioxide generator envelope (GasPak Anaerobic System; BBL Microbiology Systems, Cockeysville, Md.), was tested in a GasPak jar without a catalyst on blood agar plates supplemented with 0.2% sodium formate and 0.3% sodium fumarate. Cell morphology was determined by transmission electron microscopy by using either platinum-carbon shadowing, negative staining, or ultrathin sections of the bacterial cells (7, 16). Catalase activity was tested by dropping a 3% hydrogen peroxide solution directly on bacteria grown on a plate containing brucella agar (Difco). Oxidase activity was determined with cytochrome oxidase test strips (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Indoxyl acetate hydrolysis was determined by the impregnated disc method (5). Arylsulfatase activity was determined by hydrolysis of indoxyl sulfate in an indicator medium (27). Urease activity was determined by using Christensen (2) urease test agar (Remel, Lenexa, Kans.). Alkaline phosphatase and gamma-glutamyl transpeptidase activities were determined by hydrolysis of 0.1% solutions of *p*-nitrophenylphosphate or gamma-glutamyl-nitroanalide (Rapid NH System; Innovative Diagnostic Systems, Inc., Atlanta, Ga.). Reduction of nitrate and nitrite and production of hydrogen sulfide were determined by conventional methods (9). Lysine and ornithine decarboxylase activities were determined by using decarboxylase broth (Remel). Resistance to nalidixic acid and cephalothin was analyzed by using 50- and 30-μg discs (Showa Yakuhin Kako Co., Ltd., Tokyo, Japan), respectively. Growth in the presence of 0.01% Janus green, 0.005% basic fuchsin, 0.0005% crystal violet, 0.01% safranine, 0.025% methyl orange, 0.05% so-

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TABLE 1. Strains, sources, and accession numbers of sequences

Strain or taxon	Source[a]	Culture collection[b]	GenBank accession no.[c]
Campylobacter-like strains			
SU A4[Td]	Gingival crevice	ATCC 51146^T (= CCUG 30254^T)	L06974[e]
SU A5[d]	Gingival crevice		
SU A43[d]	Gingival crevice		
SU A1221[d]	Gingival crevice		
SU B312[d]	Gingival crevice		
SU B329[d]	Gingival crevice		
SU Ig4[d]	Gingival crevice		
SU Ig9[d]	Gingival crevice		
SU Ig24[d]	Gingival crevice		
Wolinella sp. strain FDC 286[d]	Gingival crevice		
Wolinella sp. strain VPI 10279[d]	Root canal	CCUG 11641	L06975[e]
Campylobacter curvus		ATCC 35224^T	L04313
Campylobacter curvus		SU C10	L06976[e]
Campylobacter concisus		FDC 484^T (= ATCC 33237^T)	L04322
Campylobacter concisus		FDC 288	L06977[e]
Campylobacter concisus		FDC 569	
Campylobacter rectus		ATCC 33238^T	L04317
Campylobacter rectus		CCUG 19168	L06973[e]
Campylobacter sputorum biovar *sputorum*		VPI S17	
Campylobacter sputorum biovar *bubulus*		ATCC 33491	L04319
Campylobacter fetus subsp. *fetus*		ATCC 27374^T	L04314
Campylobacter coli		CCUG 11283^T	L04312
Campylobacter hyointestinalis		ATCC 35217^T	M65010
Campylobacter jejuni subsp. *jejuni*		CCUG 11284^T	L04315
Campylobacter lari		CCUG 23947^T	L04316
Campylobacter mucosalis		CCUG 6822^T	L06978[e]
Campylobacter species[f]		PGC 40-6AT	L04318
Bacteroides gracilis		ATCC 33236^T	L04320
Bacteroides ureolyticus		ATCC 33387^T	L04321
Wolinella succinogenes		ATCC 29543^T	M88159
Helicobacter pylori		ATCC 43504^T	M88157
Helicobacter felis		ATCC 49179^T	M37642
Helicobacter mustelae		ATCC 43772^T	M35048
Helicobacter muridarum		ATCC 49282^T	M80205

[a] Source of isolate in a human oral cavity. The nine *Campylobacter*-like strains were isolated from the healthy adults at Showa University.

[b] Culture collections in which strains have been deposited or from which strains were obtained. ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; FDC, Forsyth Dental Center, Boston, Mass.; PGC, Procter & Gamble Co., Cincinnati, Ohio; SU, Showa University, Tokyo, Japan; VPI, Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg.

[c] The 16S rRNA sequences of some strains are available for electronic retrieval from GenBank under the accession numbers indicated. Through cross-distribution, these sequences should also be available from European (EMBL) and Japanese (DDBJ) data bases.

[d] Strain included in *C. showae*.

[e] Sequence determined in this study.

[f] One of several genetically similar isolates from pigs which was used for 16S rRNA sequence analysis.

dium fluoride (NaF), 0.1% sodium deoxycholate, 1% oxgall, 1% glycine, and 2 and 3.5% NaCl was tested by using methods described by Tanner et al. (19, 20). The guanine-plus-cytosine (G+C) contents of DNAs were determined by reversed-phase high-performance liquid chromatography (HPLC) (4, 17).

SDS-PAGE. Bacterial cells were collected from basal medium agar plates and suspended in distilled water. After washing, the cells were resuspended and then lysed with 4% sodium dodecyl sulfate (SDS). The lysates were diluted with distilled water and mixed with equal volumes of sample buffer containing 0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.01% bromophenol blue (18). Samples of whole-cell proteins were loaded onto gels. Prestained SDS-polyacrylamide gel electrophoresis (PAGE) standards (type HMW; Bio-Rad Laboratories, Richmond, Calif.) were used as molecular weight markers. Discontinuous SDS-PAGE in a 10% separating gel and a 4% stacking gel (16 by 16 cm; thickness, 0.75 mm) was carried out with dual gels at 30 mA for 4 to 5 h (6). After electrophoresis, one of the gels was silver stained (21), and the other was soaked in a transfer buffer containing 0.1 M Tris-HCl (pH 7.5), 0.2 M glycine, and 1% methanol for at least 30 min for subsequent electrotransfer.

Western blotting of SDS-PAGE gels. The soluble proteins from whole bacterial cells were transferred electrophoretically from each SDS-PAGE gel to a nitrocellulose membrane filter by using a Horiz-Blot apparatus (Atto Co., Tokyo, Japan) at 180 mA for 90 min. The antisera used as the first antibodies for Western blotting (immunoblotting) were prepared by immunizing rabbits with intact live cells of strain SU A5, *C. rectus* ATCC 33238^T, or *C. curvus* ATCC 35224^T as described by Badger and Tanner (1). Goat anti-rabbit immunoglobulin G–horseradish peroxidase conjugate (Bio-Rad Laboratories) was used as the second antibody. The antigens on the filter bound to the antibodies were detected by using an Immuno-blot horseradish peroxidase assay system (Bio-Rad Laboratories) according to the manufacturer's instructions.

DNA isolation and purification. The DNAs were prepared as previously described (4). Briefly, bacterial cells were harvested and washed by centrifugation, suspended in sa-

line-EDTA (0.15 M NaCl, 0.1 M EDTA; pH 8.0), and lysed by incubating them with SDS at a final concentration of 1.0% at 60°C for 10 min. Then DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), precipitated with cold ethanol, and dissolved in Tris-EDTA buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA). The partially purified DNA was then digested with RNase A (0.5 mg/ml) at 37°C for 30 min and with proteinase K (100 μg/ml) at 37°C for 2 h, and the enzyme was inactivated by shaking the preparation with an equal volume of chloroform-isoamyl alcohol (24:1). The DNA was precipitated with 2 volumes of ethanol and dissolved in Tris-EDTA buffer. The concentration and purity of DNA were determined by measuring A_{234}, A_{260}, and A_{280}.

DNA-DNA hybridization. The DNAs of strain SU A4^T, *C. rectus* ATCC 33238^T, and *C. curvus* ATCC 35224^T were labeled with [*methyl*-1′,2′-^{3}H]dTTP (tetrasodium salt; Du Pont, Inc., NEN Products, Boston, Mass.) by using a nick translation system (Du Pont, Inc.). The specific activities of the labeled DNAs were between 1.2×10^7 and 1.4×10^7 cpm/μg of DNA. DNA-DNA hybridization experiments were carried out by using a membrane filter method (10, 23). Sheared and denatured DNAs were fixed on a nitrocellulose membrane filter (9 by 12 cm) by aspirating preparations through wells of a microfiltration apparatus (Bio-Rad Laboratories). After air dying, the filter was baked at 80°C for 2 h. The fixed DNAs were prehybridized at 42°C for 2 to 4 h in prehybridization buffer containing 50% formamide, 2 × SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, and 200 μg of denatured salmon sperm DNA per ml and subsequently hybridized at 42°C overnight in a hybridization mixture (50% formamide, 2× SSC, 1× Denhardt's solution, 100 μg of denatured salmon sperm DNA per ml) containing 100 μl of a ^{3}H-labeled DNA solution. After hybridization, the filter was washed twice with 2× SSC, and a section of six wells corresponding to each strain was cut out. The filter section was dissolved in methylcellosolve, Aquasol-2 (Du Pont, Inc.) was added, and the radioactivity was measured with a liquid scintillation counter. Hybridization of salmon sperm DNA was used as a negative control with each DNA probe. The DNA-DNA hybridization experiments were repeated at least three times.

Isolation and purification of rRNA. rRNA was isolated from bacterial cells and partially purified by a modification of the procedure of Pace et al. (11), as previously described (12).

16S rRNA sequencing and data analysis. rRNA was sequenced by the modified Sanger dideoxy chain termination technique in which primers complementary to conserved regions were elongated with avian myeloblastosis virus reverse transcriptase (8). A program set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation, and phylogenetic tree construction for 16S rRNA data was written in Microsoft Quick BASIC for use on IBM PC-AT and compatible computers; rRNA sequences were entered and aligned as previously described (12). Similarity matrices were constructed by comparing only those base positions for which there were data for 90% of the sequences. Phylogenetic trees were constructed by the neighbor-joining method (13, 15).

GenBank accession numbers. The GenBank accession numbers for all strains examined in this study are shown in Table 1.

RESULTS AND DISCUSSION

Cell morphology and biochemical characteristics. A total of nine *Campylobacter*-like strains were isolated from human gingival crevices and examined. As determined by electron microscopy, the cells were straight rods approximately 0.5 to 0.8 μm wide and 2 to 5 μm long with round ends. Each cell had two to five unsheathed unipolar flagella that were 15 to 20 nm in diameter (Fig. 1A through D), whereas each *C. curvus* and *C. rectus* cell had only a single polar flagellum (data not shown). Although this feature is unique among the campylobacters, DNA-DNA homology and 16S rRNA sequencing data clearly place these organisms in the genus *Campylobacter*. Therefore, the definition of the genus *Campylobacter* should be emended to include species with single flagella and species with multiple flagella. In ultrathin sections (Fig. 1E and F), the structure of the cell wall appeared to be typical of gram-negative rods, in which the cell wall is composed of an outer membrane, a periplasmic space, and an inner membrane. The cells were not covered with either a crystalline surface layer (3) or the hexagonal arrangement of macromolecular subunits (7) observed in *C. rectus*.

The following characteristics were shared by all nine *Campylobacter*-like strains (except where noted): colonies on blood agar plates supplemented with 0.2% sodium formate and 0.3% sodium fumarate were 1 to 2 mm in diameter, translucent, and convex; rapid darting motility occurred; the organisms did not form spores; the DNA G+C content was 44 to 46 mol%; growth occurred in anaerobic and microaerophilic atmospheres, but the organisms preferred anaerobic conditions; growth occurred at 42°C but not at 25°C; growth was stimulated by the addition of formate and fumarate to the medium; growth occurred in the presence of sodium fluoride (0.5 g/liter), Janus green (0.1 g/liter), basic fuchsin (50 mg/liter), crystal violet (5 mg/liter) (eight of nine strains), safranine (0.1 g/liter), methyl orange (0.25 g/liter), glycine (10 g/liter) (six of nine strains), and oxgall (10 g/liter) (six of nine strains); fumarate was reduced to succinate; the organisms were oxidase positive and urease negative; nitrate and nitrite were reduced; hydrogen sulfide was produced; the organisms were not able to oxidize or ferment carbohydrates; and the organisms were resistant to nalidixic acid. Strains SU A4^T and SU A5 were negative for alkaline phosphatase activity, hippurate hydrolysis, lysine and ornithine decarboxylase activities, and gamma-glutamyl transferase activity and positive for arylsulfatase activity and hydrolysis of indoxyl acetate. The major features distinguishing the *Campylobacter*-like strains from other species of genus *Campylobacter* are shown in Table 2.

SDS-PAGE. To distinguish phenotypically similar species, one-dimensional SDS-PAGE was carried out. The bacterial whole-cell proteins extracted from the nine *Campylobacter*-like strains, two strains of *C. rectus*, six strains of *C. curvus*, and *Wolinella succinogenes* were determined. The electrophoretic patterns, comprising 40 to 50 bands, were highly reproducible, and the intraspecies protein profiles for the *Campylobacter*-like strains, *C. rectus*, and *C. curvus* were similar (data not shown). The protein profiles of the nine *Campylobacter*-like strains were similar to each other (Fig. 2, lanes 1 through 9) but were different from the profiles of the three reference strains, *C. rectus* ATCC 33238^T, *C. curvus* ATCC 35224^T, and *W. succinogenes* ATCC 29543^T (Fig. 2, lanes 10 through 12), because of two characteristic bands in the profiles of the nine isolates (Fig. 2, lanes 1 through 9, two large arrows). *C. rectus* was characterized by

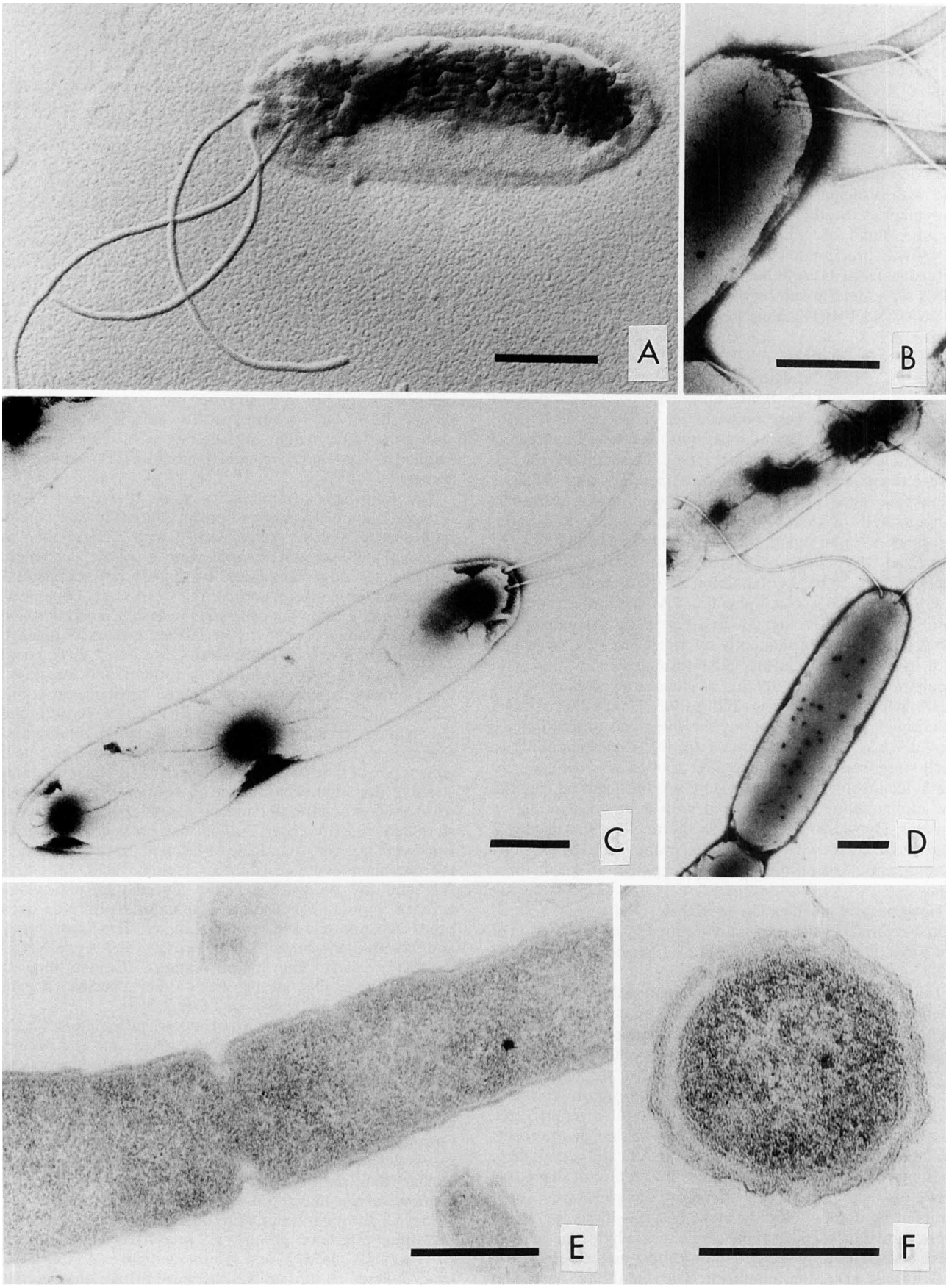

FIG. 1. Transmission electron micrographs of strain SU A5. (A) Platinum-carbon shadowing. (B through D) Negatively stained preparations. (E) Ultrathin section. (F) High-magnification micrograph of ultrathin section. Bars = 0.5 μm.

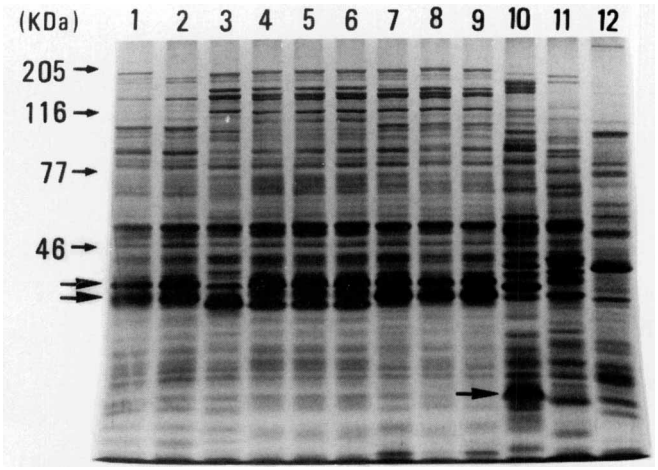

FIG. 2. Electrophoretic protein patterns of *Campylobacter*-like strains and the reference strains as determined by silver staining. Lanes 1 through 9, *Campylobacter*-like strains SU A4^T (lane 1), SU A5 (lane 2), SU A43 (lane 3), SU A1221 (lane 4), SU B312 (lane 5), SU B329 (lane 6), SU Ig4 (lane 7), SU Ig9 (lane 8), and SU Ig24 (lane 9); lane 10, *C. rectus* ATCC 33238^T; lane 11, *C. curvus* ATCC 35224^T; lane 12, *W. succinogenes* ATCC 29543^T. The large arrow in lane 10 indicates the unique bands of *C. rectus*. The two large arrows on the left (lanes 1 through 9) indicate the characteristic bands of the *Campylobacter*-like strains.

TABLE 2. Characteristics which differentiate *C. showae* from other *Campylobacter* species[a]

Taxon	No. of flagella	Catalase activity	Urease activity	Nitrite reduction	H_2 requirement[b]	H_2S production	Hydrolysis of:		Growth at:		Growth in the presence of:			Susceptibility to:		G+C content (mol%)
							Hippurate	Indoxyl acetate	25°C	42°C	3.5% NaCl	1% Glycine	0.05% NaF	Nalidixic acid	Cephalothin	
C. showae (nine strains)	2–5	+	−	+	+	+	−	+	−	+	−	6/9[c]	+	R	S	44–46
C. rectus	1	−	−	+	+	+	−	+	−	W	−	+	−	S	S	45–46
C. curvus	1	−	−	+	+	+	−	+	−	+	−	+	−	R[d]	S	45–46
C. concisus	1	−	−	+	+	+	−	−	−	+	−	+	+	R	R	37–41
C. mucosalis	1	−	−	+	+	+	−	−	−	+	−	+	+	R	S	36–38
C. sputorum biovar sputorum	1	−	−	+	−	+	−	−	−	+	−	+	+	S	S	30–31
C. sputorum biovar bubulus	1	−	−	+	−	+	−	−	−	+	+	+	+	S	S	29–30
C. sputorum biovar fecalis	1	+	−	+	−	+	−	−	−	+	−	+	+	R	S	30–32
C. fetus subsp. *fetus*	1	+	−	−	−	−	−	−	+	−	−	+	ND	R	S	33–35
C. fetus subsp. *venerealis*	1	+	−	−	−	−	−	−	+	−	−	−	ND	R	S	33–34
C. hyointestinalis	1	+	−	−	V	+	−	+	+	+	−	+	ND	R	S	33–36
C. jejuni subsp. *jejuni*	1	+	−	−	−	−	+	+	−	+	−	+	+	S	R	30–33
C. jejuni subsp. *doylei*	1	V	−	−	−	−	V	+	−	−	−	+	ND	S	S	30–31
C. coli	1	+	−	−	−	−	−	+	−	+	−	+	+	S	R	30–33
C. lari	1	+	V	−	−	−	−	+	−	+	−	+	ND	R	R	30–32

[a] Data were obtained from references 19, 20, 22, 24, and 25 and our study. +, positive reaction; −, negative reaction; W, weak reaction; V, variable reaction; S, susceptible; R, resistant; ND, not determined.
[b] H_2 required for microaerophilic growth.
[c] The values were positive for the nine isolates tested.
[d] Susceptible according to Vandamme and De Ley (24).

a unique band (Fig. 2, lane 10, large arrow). To clearly differentiate the protein profiles, Western blotting was used.

Western blotting of SDS-PAGE gels. Whole-cell proteins from the nine *Campylobacter*-like strains and three reference strains separated by SDS-PAGE were transferred onto nitrocellulose membrane filters for Western blotting. The filters were incubated with antisera against strain SU A5 (Fig. 3A), *C. rectus* ATCC 33238^T (Fig. 3B), and *C. curvus* ATCC 35224^T (Fig. 3C). The Western blot patterns of the nine *Campylobacter*-like strains were relatively homogeneous. The principal protein bands between 116 and 205 kDa appeared to be specific to the *Campylobacter*-like strains (Fig. 3A, lanes 1 through 9), whereas the bands in the middle indicated by the two large arrows were reactive with anti-*C. rectus* and anti-*C. curvus* sera, as well as anti-SU A5 serum (Fig. 3A through C). These cross-reactive bands, corresponding to the two major bands of *Campylobacter*-like strains determined by SDS-PAGE, appeared to be common antigens also present in *C. rectus* and *C. curvus*. The bands between 116 and 205 kDa for *C. rectus* (Fig. 3B and C, lane 10, upper arrow) were cross-reactive with anti-*C. rectus* and anti-*C. curvus* sera, but not with anti-SU A5 serum. Low-molecular-weight bands of *C. rectus* (Fig. 3B, lane 10, lower arrow) and *C. curvus* (Fig. 3C, lane 11, lower arrow) were specific to anti-*C. rectus* and anti-*C. curvus* sera, respectively. On the whole, the Western blot patterns of *Campylobacter*-like strains were characteristic and were distinct from the patterns of the phenotypically closely related organisms *C. rectus* and *C. curvus*.

DNA homology. The results of DNA-DNA hybridization experiments are shown in Table 3. The DNA homology data show that the nine *Campylobacter*-like strains and *Wolinella* sp. strains FDC 286 and VPI 10279 were closely related, with levels of DNA homology of more than 74%, and formed a single group. On the other hand, the levels of DNA homology for this group ranged from 20 to 41% in comparisons with ^{3}H-labeled DNA from *C. rectus* ATCC 33238^T and were

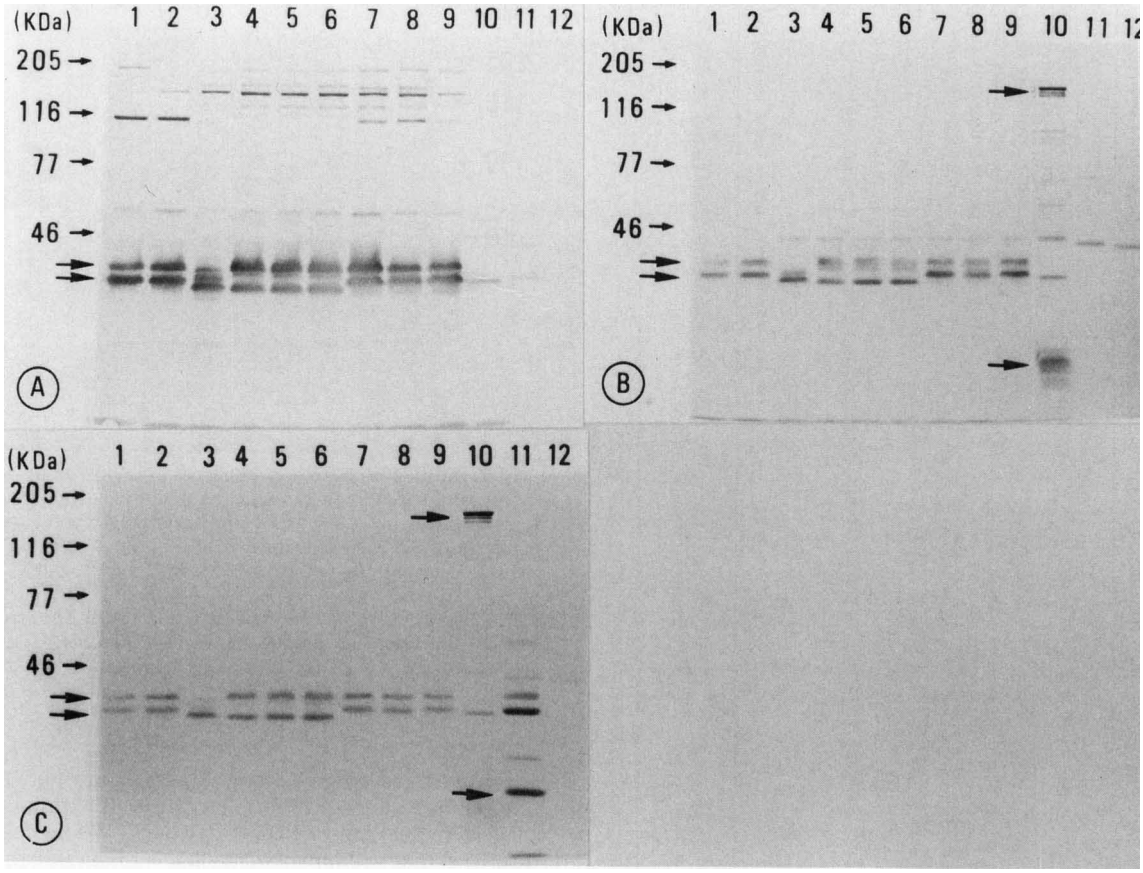

FIG. 3. Western blot patterns of the SDS-PAGE gel which is shown in Fig. 2. (A) Proteins incubated with anti-SU A5 serum. The two large arrows on the left indicate the common bands found in the nine *Campylobacter*-like strains. (B) Proteins incubated with anti-*C. rectus* serum. The two large arrows on the left indicate common bands found in the nine *Campylobacter*-like strains. In lane 10, the upper arrow indicates bands common to anti-*C. rectus* and anti-*C. curvus* sera, and the lower arrow indicates the bands specific to anti-*C. rectus* serum. (C) Proteins incubated with anti-*C. curvus* serum. The two large arrows on the left indicate common bands found in the nine *Campylobacter*-like strains. The large arrow in lane 10 indicates bands common to anti-*C. rectus* and anti-*C. curvus* sera, and the large arrow in lane 11 indicates the bands specific to anti-*C. curvus* serum.

less than 11% in comparisons with ^{3}H-labeled DNA from *C. curvus* ATCC 35224^T. The levels of DNA homology between strain SU A4^T and *Campylobacter concisus* FDC 569 or *W. succinogenes* ATCC 29543^T were much lower. Because of the high DNA-DNA homology values within this group (more than 74%) and the low homology values with strains of other *Campylobacter* species (less than 41%), the nine *Campylobacter*-like strains and *Wolinella* sp. strains FDC 286 and VPI 10279 should be regarded as belonging to a single new species. Our results are consistent with the previous findings of Tanner et al., who reported that strains FDC 286 and VPI 10279 represented an additional unnamed species in the genus *Wolinella*, which could be differentiated from *C. curvus*, *C. rectus*, and *W. succinogenes* by phenotypic characteristics and DNA-DNA homology data (22). The 16S rRNA sequence data compared with the 16S rRNA sequence data for other *Campylobacter* species strongly support placing this new species in the genus *Campylobacter*.

16S rRNA sequences. Essentially complete sequences (1,480 bases) were obtained for strains SU A4^T and VPI 10279 and are available by electronic retrieval from GenBank under the accession numbers listed in Table 1. The 16S rRNA sequences of the following four additional *Campylo-*

TABLE 3. Levels of DNA-DNA homology among the nine *Campylobacter*-like strains, other *Campylobacter* strains, and related bacteria

Strain	% Homology with ^{3}H-labeled DNA from:		
	Strain SU A4^T	*C. rectus* ATCC 33238^T	*C. curvus* ATCC 35224^T
Campylobacter-like strains			
SU A4^T	100	20	5
SU A5	100	29	3
SU A43	82	34	7
SU A1221	74	32	7
SU B312	91	41	11
SU B329	90	36	10
SU Ig4	100	39	10
SU Ig9	82	33	8
SU Ig24	82	33	7
Wolinella sp. strain FDC 286	74	38	6
Wolinella sp. strain VPI 10279	80	36	5
C. rectus ATCC 33238^T	43	100	6
C. rectus CCUG 19168	49	73	3
C. curvus ATCC 35224^T	4	13	100
C. concisus FDC 569	<1	<1	<1
W. succinogenes ATCC 29543^T	<1	<1	<1

TABLE 4. Similarity matrix based on 16S rRNA sequence comparisons

Taxon	% of similarity or difference[a]																						
	C. showae SU A4^T	*C. showae* VPI 10279	*C. rectus* ATCC 33238	*C. rectus* CCUG 19168	*C. curvus* ATCC 35224	*C. curvus* SU C10	*B. gracilis*	*C. sputorum* biovar bubulus	*C. concisus* FDC484	*C. concisus* FDC288	*C. mucosalis*	*C. fetus* subsp. *fetus*	*C. hyolintestinalis*	*Campylobacter* sp. strain PGC 50-6AT	*C. coli*	*C. jejuni*	*C. lari*	*B. ureolyticus*	*W. succinogenes*	*H. pylori*	*H. felis*	*H. mustelea*	*H. muridarum*
C. showae SU A4^T		99.9	98.7	98.7	96.1	97.2	95.2	94.9	95.2	95.1	93.9	93.3	92.3	92.3	92.8	92.0	91.8	91.5	86.4	84.5	83.7	85.1	85.4
C. showae VPI 10279	0.1		98.6	98.7	96.1	97.2	95.1	94.8	95.2	95.1	93.9	93.2	92.2	92.2	92.7	91.9	91.8	91.5	86.6	84.7	83.9	85.3	85.5
C. rectus ATCC 33238	1.3	1.4		99.5	96.3	97.3	95.6	95.0	95.7	95.6	94.1	93.7	92.8	92.9	93.3	92.5	92.3	91.8	86.4	84.2	83.3	84.9	85.0
C. rectus CCUG 19168	1.3	1.4	0.5		96.4	97.2	95.1	94.9	96.0	95.9	94.4	93.7	92.8	92.8	93.3	92.5	92.6	91.7	86.7	84.1	83.5	85.2	85.3
C. curvus ATCC 35224	4.0	4.1	3.7	3.7		98.6	94.3	95.0	96.9	96.9	94.6	94.1	93.2	93.9	93.3	92.5	92.0	90.6	86.7	85.0	84.2	85.8	85.3
C. curvus SU C10	2.9	2.9	2.7	2.9	1.4		94.5	95.1	96.5	96.4	95.6	94.2	93.2	93.4	93.6	92.8	92.4	90.6	86.6	85.0	83.9	85.8	85.4
B. gracilis	4.9	5.0	4.6	5.0	5.9	5.7		94.0	94.8	94.7	93.6	93.7	92.8	91.5	91.9	91.3	91.1	91.5	85.5	83.9	83.1	84.5	84.3
C. sputorum biovar *bubulus*	5.3	5.3	5.2	5.3	5.1	5.1	6.3		95.1	94.8	93.2	93.2	92.3	92.8	93.0	92.4	91.9	91.9	86.2	85.4	85.0	87.2	86.7
C. concisus FDC 484	4.9	4.9	4.4	4.1	3.1	3.6	5.4	5.1		99.8	96.1	95.4	94.4	94.1	94.2	93.5	93.7	91.7	86.6	85.3	84.9	86.2	86.6
C. concisus FDC 288	5.1	5.1	4.6	4.2	3.1	3.7	5.5	5.4	0.2		96.0	95.3	94.4	94.1	94.2	93.5	93.7	91.7	86.2	85.1	84.7	85.9	86.3
C. mucosalis	6.3	6.3	6.2	5.8	5.6	4.6	6.7	7.1	4.0	4.1		96.2	94.7	93.6	94.2	93.7	93.6	92.1	85.9	85.0	85.2	85.6	85.6
C. fetus subsp. *fetus*	7.1	7.1	6.6	6.6	6.1	6.0	6.5	7.1	4.8	4.9	3.9		98.2	95.6	95.6	94.5	94.3	92.7	86.3	85.4	85.1	86.2	85.9
C. hyointestinalis	8.1	8.2	7.6	7.6	7.1	7.1	7.5	8.1	5.8	5.8	5.5	1.8		95.6	95.5	94.5	94.3	92.6	85.5	84.7	84.2	85.6	85.3
Campylobacter sp. strain PGC 40-6AT	8.2	8.3	7.5	7.6	6.3	6.9	9.0	7.5	6.1	6.1	6.6	4.5	4.5		96.3	95.2	94.8	92.2	85.7	84.4	84.1	85.5	85.3
C. coli	7.6	7.7	7.1	7.0	7.0	6.7	8.6	7.3	6.0	6.1	6.0	4.6	4.6	3.8		98.6	97.5	93.6	86.7	85.5	84.8	86.8	86.2
C. jejuni	8.5	8.6	7.9	7.9	7.9	7.6	9.2	8.0	6.8	6.8	6.6	5.7	5.7	4.9	1.5		98.5	93.2	86.8	85.8	85.2	87.1	86.2
C. lari	8.7	8.7	8.2	7.8	8.4	8.0	9.4	8.5	6.6	6.6	6.7	6.0	5.9	5.4	2.6	1.5		92.8	87.1	85.8	85.7	87.4	86.5
B. ureolyticus	9.0	9.1	8.7	8.8	10.0	10.0	9.0	8.5	8.8	8.8	8.4	7.6	7.8	8.3	6.7	7.1	7.6		85.9	84.2	83.8	85.9	85.0
W. succinogenes	15.0	14.8	15.0	14.7	14.6	14.8	16.1	15.3	14.7	15.2	15.6	15.1	16.1	15.9	14.7	14.5	14.2	15.6		91.0	90.6	94.0	93.6
H. pylori	17.4	17.1	17.8	17.8	16.7	16.8	18.2	16.2	16.4	16.6	16.7	16.2	17.2	17.5	16.1	15.8	15.7	17.8	9.6		95.4	94.0	93.1
H. felis	18.3	18.1	18.9	18.6	17.8	18.1	19.2	16.7	16.8	17.1	16.5	16.6	17.7	17.8	17.0	16.4	15.9	18.3	10.1	4.8		93.6	92.8
H. mustelae	16.6	16.4	16.8	16.5	15.8	15.7	17.3	14.1	15.3	15.6	16.0	15.3	16.0	16.1	14.6	14.2	13.7	15.6	6.2	6.3	6.7		95.7
H. muridarum	16.3	16.1	16.7	16.3	16.4	16.3	17.6	14.7	14.8	15.1	16.0	15.6	16.4	16.4	15.2	15.2	14.9	16.8	6.6	7.2	7.6	4.4	

[a] The values on the upper right are uncorrected percentages of similarity. The numbers on the lower left are percentages of differences corrected for multiple base changes by the method of Jukes and Cantor (12).

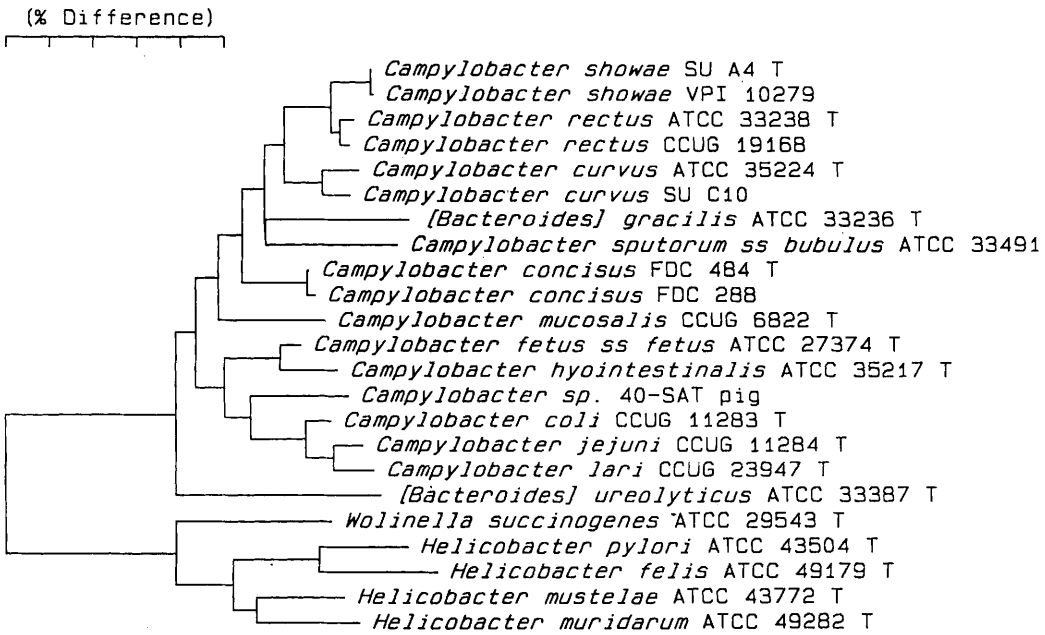

FIG. 4. Phylogenetic tree for 23 strains of *Campylobacter*, *Helicobacter*, and *Wolinella* species based on 16S rRNA sequence similarity data. The scale bar represents a 5% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting any two species.

bacter strains have been deposited in the GenBank data base: *C. curvus* SU C10, *C. concisus* FDC 288, *C. rectus* CCUG 19168, and *Campylobacter mucosalis* CCUG 6822^T. The sequences of strains SU A4^T and VPI 10279 differed from one another by a single base and were 98.7% similar to the sequence of *C. rectus*.

Phylogenetic analysis based on 16S rRNA sequence comparisons. The 16S rRNA sequences of strains SU A4^T and VPI 10279 were aligned with the 16S rRNA sequences of 21 reference strains of *Campylobacter*, *Helicobacter*, and *Wolinella* species. A similarity matrix (Table 4) was determined by comparing the base positions for which 90% of the sequences had data and could be unambiguously aligned. The data matrix in Table 4 is based on comparisons at 1,400 base positions. A phylogenetic tree determined from this matrix by using the neighbor-joining method is shown in Fig. 4. As determined by 16S rRNA sequence similarity, strains SU A4^T and VPI 10279 were most closely related to *C. rectus*; however, they differed from *C. rectus* at 1.35% of the positions, which was about the same level of difference observed between *Campylobacter jejuni* and *Campylobacter coli* or between *C. jejuni* and *Campylobacter lari*. These data showed that the *Campylobacter*-like strains and *Wolinella* sp. strains FDC 286 and VPI 10279 should be placed in the genus *Campylobacter*, as there is sufficient genetic distance to warrant a new species, in addition to the DNA-DNA homology data. Strain CCUG 19168, which was identified as *C. rectus*-like, was closely related to the type strain of *C. rectus*. Strain SU C10, which was identified as *C. curvus* on the basis of DNA-DNA homology and phenotypic characteristics (4), differed at 1.4% of the positions from the type strain of *C. curvus*; this suggests that the species *C. curvus* may be genetically heterogeneous. As indicated previously, *Bacteroides gracilis* and *Bacteroides ureolyticus* are genetically misnamed (12). A more extensive discussion of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella* phylogeny based on a 16S rRNA sequence analysis of more than 45 strains will be presented elsewhere.

Description of *Campylobacter showae* sp. nov. *Campylobacter showae* (show'ae. L. n. *showae*, referring to Showa University, where several strains of the species were first isolated). Gram-negative, straight rods. Cells are 0.5 to 0.8 μm wide and 2 to 5 μm long with round ends and two to five unipolar flagella. The flagella are unsheathed. Grows in a microaerophilic atmosphere in the presence of fumarate with formate or H_2, but prefers to grow under anaerobic conditions. Succinate is produced from fumarate. Oxidase positive. Most strains decompose hydrogen peroxide. Asaccharolytic. Reduces nitrate and nitrite. Arylsulfatase positive. Does not produce lysine or ornithine decarboxylase, alkaline phosphatase, urease, or gamma-glutamyl transferase or hydrolyze hippurate. H_2S is produced. Can be differentiated from other *Campylobacter* species as shown in Table 2. Because of the limited number of reliable biochemical traits which can be used to differentiate closely related *Campylobacter* species, serologic, DNA probe, or protein profile tests may be required to positively identify isolates of this species. Strains were isolated from human dental plaque and from infected root canals. Pathogenicity is unknown. The G+C content of the DNA is 44 to 46 mol% as determined by HPLC. The type stain is strain SU A4 (= ATCC 51146). The description of the type strain does not differ from the description of the species.

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