Chemotaxonomic Analyses of *Bacteroides gracilis* and *Bacteroides ureolyticus* and Reclassification of *B. gracilis* as *Campylobacter gracilis* comb. nov.

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The cellular fatty acids, respiratory quinones, and proteins of the generically misnamed taxa *Bacteroides gracilis* and *Bacteroides ureolyticus* were analyzed and compared with the corresponding chemotaxonomic features of their closest relatives, the campylobacters. Our results and previously published data for genotypic and phenotypic characteristics were used in a polyphasic approach to reconsider the classification of these organisms. We transfer *B. gracilis* to the genus *Campylobacter* as *Campylobacter gracilis* comb. nov. *B. ureolyticus* can be considered a campylobacter on genotypic grounds; in contrast, the proteolytic metabolism and fatty acid components of this taxon exclude it from the genus *Campylobacter*. We prefer to consider this taxon a species *incertae sedis* pending the isolation and characterization of additional *B. ureolyticus*-like bacteria.

Clinical isolates of gram-negative bacilli that produce pitting or corroding growth on agar surfaces were first described by Henriksen (15) and Eiken (8). The name *Bacteroides corrodens* was proposed by Eiken (8) for these organisms, which were considered anaerobes. In fact, the *Bacteroides corrodens* group comprised several taxa (18). One subgroup contained facultatively anaerobic organisms having guanine-plus-cytosine (G+C) contents of 56 to 58 mol%, and this subgroup was subsequently named *Eikenella corrodens* (16). Strains belonging to this subgroup did not produce urease. Strains belonging to a second subgroup produced urease, had G+C contents of 28 to 30 mol%, and were considered true anaerobes. This subgroup was referred to as "*Bacteroides corrodens*, anaerobic" and later was named *Bacteroides ureolyticus* (17), although its inclusion in the genus *Bacteroides* was dubious (40). *B. ureolyticus* strains have been isolated from patients with superficial ulcers, soft-tissue infections, nongonococcal nonchlamydial urethritis, and periodontal disease (6, 7, 10, 11). The pathogenicity of *B. ureolyticus* is difficult to assess because for the most part *B. ureolyticus* strains are recovered from mixed infections. Nevertheless, the presence of this organism in high numbers in mixed infections and the strong proteolytic activity which results in tissue destruction suggest that *B. ureolyticus* is pathogenic (6, 39).

The name *Bacteroides gracilis* was proposed by Tanner et al. for another group of agar-corroding bacteria (37). *B. gracilis* strains have G+C contents of 44 to 46 mol%, and this species was originally also considered an anaerobic bacterium (37). *B. gracilis* strains have been isolated from gingival crevices and from visceral, head, and neck infections in humans (19). The association of *B. gracilis* with serious deep-tissue infections and its high level of antibiotic resistance suggest that this organism may be an important pathogen whose importance is underestimated (19).

For many years the genus *Bacteroides* has been known to be

phenotypically as well as genotypically extremely heterogeneous, and major taxonomic revisions of this genus have been proposed (32–34). Species previously known as *Bacteroides* species are now included in the genera *Anaerorhabdus*, *Capnocytophaga*, *Fibrobacter*, *Megamonas*, *Mitsuokella*, *Porphyromonas*, *Prevotella*, *Rikenella*, *Ruminobacter*, *Sealdella*, and *Tissierella* (34). The taxonomic positions of several other species are still not known. rRNA homology studies have revealed that two of these other species, *B. ureolyticus* and *B. gracilis*, are closely related to members of the emended genus *Campylobacter* (29, 43). It has also been shown that, like campylobacters, *B. ureolyticus* and *B. gracilis* are microaerophils, not anaerobes (13). Both of these species were included in the family *Campylobacteraceae* together with the genera *Campylobacter* and *Arcobacter* by Vandamme and De Ley (41). Although genotypic data have clearly shown that *B. ureolyticus* and *B. gracilis* are generically misnamed, until now they have not been reclassified.

Below, respiratory quinone, protein, and fatty acid composition data for these organisms and for the biochemically similar organisms *Wolinella succinogenes*, *Campylobacter rectus*, and *Campylobacter curvus* are presented.

MATERIALS AND METHODS

Bacterial strains. The strains which we used and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Cellular fatty acid and isoprenoid quinone analyses. Cells for fatty acid and isoprenoid quinone analyses were grown on agar plates containing mycoplasma base broth supplemented with formate, fumarate, and hemin (30). The plates were incubated at 37°C for 4 days in an atmosphere containing approximately 80% N₂, 10% CO₂, and 10% H₂, and cells were removed with gentle scraping. Cellular fatty acids were liberated by saponification, processed, and analyzed as their methyl esters by using high-resolution capillary gas-liquid chromatography as described previously (25, 27). Isoprenoid quinones were extracted from 100-mg portions of lyophilized cells and analyzed by reverse-phase high-performance liquid chromatography (RPHPLC) (24). The identities of individual fatty acids, aldehydes, dimethylacetals, and quinones were confirmed by mass spectrometry (22, 25).

PAGE of whole-cell proteins. Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins, a densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed as described previously (47, 48). All reference strains were grown for 3 days at 37°C on

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TABLE 1. Strains used and their sources

Strain ^a	Source (year and/or place of isolation), if known
<i>Bacteroides gracilis</i> ATCC 33236 ^T	Human gingival sulcus (1977, United States)
<i>B. gracilis</i> FDC 20A1	Human gingival sulcus (1992, United States)
<i>B. gracilis</i> FDC 406 (= LMG 14731)	Human gingival sulcus (1974, United States)
<i>B. gracilis</i> LMG 7616	Human gingival crevice (United States)
<i>B. ureolyticus</i> ATCC 33387 ^T (= LMG 6451 ^T)	Amniotic fluid (Canada)
<i>B. ureolyticus</i> ATCC 43604	Human urethritis (United Kingdom)
<i>B. ureolyticus</i> ATCC 33481	
<i>B. ureolyticus</i> LMG 7789	Human chancroid (1980, Sweden)
<i>B. ureolyticus</i> LMG 8448	Human chancroid (1980, Sweden)
<i>B. ureolyticus</i> LMG 8449	Human chancroid (1980, Sweden)
<i>B. ureolyticus</i> LMG 8450	Human chancroid (1980, Sweden)
<i>Campylobacter concisus</i> FDC 484 ^T (= LMG 7788 ^T)	Human gingival sulcus (1974, United States)
<i>C. concisus</i> LMG 7789	Human periodontal pocket (1974, United States)
<i>C. curvus</i> ATCC 35224 ^T (= CDC D2608 ^T = LMG 7609 ^T)	Human jaw abscess (1984, United States)
<i>C. curvus</i> CDC D2712	Human oral cavity (1984)
<i>C. curvus</i> CDC D4319	Human oral cavity (1984)
<i>C. curvus</i> CDC D4321	Oral cavity (1987, United States)
<i>C. curvus</i> CDC D4322	Groin abscess (1987, United States)
<i>C. curvus</i> LMG 7610	Human dental root canal (Sweden)
<i>C. mucosalis</i> CCUG 6822 ^T (= LMG 6448 ^T)	Porcine small intestine (1972, United Kingdom)
<i>C. mucosalis</i> LMG 8265	Porcine intestine (1972, United Kingdom)
<i>C. rectus</i> ATCC 33238 ^T (= CDC D2083 ^T = LMG 7613 ^T)	Human periodontal pocket (1974, United States)
<i>C. rectus</i> CDC D4326	Human oral cavity (1984, United States)
<i>C. rectus</i> CDC D4328	Human oral cavity (1986, United States)
<i>C. rectus</i> CDC D4329	Tooth abscess (1986, United States)
<i>C. rectus</i> CDC D4330	Oral cavity (1986, United States)
<i>C. rectus</i> CDC D4331	Jaw abscess (1987, United States)
<i>C. rectus</i> CDC D4332	Blood (1987, United States)
<i>C. rectus</i> LMG 7614	Human periodontitis (United States)
<i>C. showae</i> ATCC 51146 ^T (= LMG 12635 ^T)	Gingival crevice (Japan)
<i>C. showae</i> LMG 8543	Human dental root canal (United States)
<i>C. showae</i> LMG 12676	Gingival crevice (Japan)
<i>Wolinella succinogenes</i> ATCC 29543 ^T (= LMG 7608 ^T)	Bovine rumen (1960, United States)

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; FDC, Forsyth Dental Center, Boston, Mass.; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium.

Mueller-Hinton agar (catalog no. CM 337; Oxoid, Ltd., Basingstoke, United Kingdom) supplemented with 5% (vol/vol) horse blood and were incubated at 36 to 37°C in a microaerobic atmosphere containing approximately 5% O₂, 3.5% CO₂, 7.5% H₂, and 84% N₂.

16S rRNA data analysis. The 16S rRNA sequences of the strains analyzed in this study have been described previously (5, 9, 23, 29, 35). A program set for data entry, editing, sequence alignment, secondary-structure comparison, similarity matrix generation, and dendrogram construction for 16S rRNA data was written in Microsoft QuickBASIC for use on IBM PC and compatible computers (29). RNA sequences were entered and aligned as previously described (29). Our sequence database contains approximately 300 sequences determined in our laboratory and another 200 sequences obtained from GenBank or the Ribosomal Database Project (28). The reference strains used in the 16S rRNA analysis are shown in Table 2. Similarity matrices were constructed from the aligned sequences by using only those sequence positions at which 90% of the strains had data. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (20). Phylogenetic trees were constructed by using the neighbor-joining method of Saitou and Nei (31). Bootstrapping of neighbor-joining trees was performed by using the program MEGA (21) with 500 resamplings and pairwise elimination of incomplete data.

Nucleotide sequence accession numbers. The GenBank nucleotide sequence and culture collection accession numbers for the strains used in the 16S rRNA sequence comparisons are shown in Table 2.

RESULTS AND DISCUSSION

Isoprenoid quinone analysis. Figure 1 shows an RPHPLC chromatogram of the isoprenoid quinone fraction from *B. ureolyticus* ATCC 33387^T (T = type strain). The retention times of the first two peaks, designated MK-5 and MK-6, were exactly the same as the retention times of standard MK-5 and MK-6, respectively. The retention time of the peak designated *MK-6 was exactly the same as the retention time of methyl-substituted menaquinone 6 (2,[5 or 8]-dimethyl-3-farnesyl-

farnesyl-1,4-naphthoquinone) from *Campylobacter fetus*, the so-called thermoplasmaquinone (2, 25). Fractions corresponding to each of these three peaks were collected from RPHPLC preparations and analyzed by mass spectrometry. We confirmed that peaks MK-5 and MK-6 were menaquinones since the mass spectrum of each peak had a base peak ion at *m/e* 225 and an intense ion at *m/e* 187, representing the naphthoquinone nucleus (2, 25). The mass spectrum of the *MK-6 peak is shown in Fig. 2. This spectrum had a base peak ion at *m/e* 239 and a smaller ion at *m/e* 201, which is consistent with the presence of an additional methyl group on the naphthoquinone ring (2). This fragmentation pattern, together with a prominent molecular ion at *m/e* 594, confirmed that *MK-6 was 2,[5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone. Prominent molecular ions (M⁺) were observed at *m/e* 512 for MK-5 and at *m/e* 580 for MK-6. The M⁺ ions were verified by chemical ionization spectra, which had intense (M+1)⁺ ions at the expected mass values of *m/e* 513, *m/e* 581, and *m/e* 595 for MK-5, MK-6, and *MK-6, respectively.

All of the *B. ureolyticus*, *B. gracilis*, *C. curvus*, *C. rectus*, and *W. succinogenes* strains tested contained only menaquinones. The identities of the menaquinones and the relative amounts detected in each species are shown in Table 3. *MK-6 was present as a significant compound in all species and was the major menaquinone in all species except *W. succinogenes*, which contained MK-6 as its major component. All of the strains were regrown and retested for quinones, and the results were essentially identical. Moreover, the menaquinone patterns obtained for additional strains of each species (except *W.*

TABLE 2. Strains used in 16S rRNA sequence studies and their accession numbers

Taxon	Strain ^a	GenBank accession no. ^b
Epsilon subclass of the Proteobacteria		
<i>Arcobacter butzleri</i>	CCUG 10373	L14626
<i>Arcobacter cryaerophilus</i>	CCUG 17801 ^T	L14624
<i>Arcobacter nitrofigilis</i>	CCUG 15893 ^T	L14627
<i>Arcobacter skirrowii</i>	CCUG 10374 ^T	L14625
<i>Bacteroides gracilis</i>	ATCC 33236 ^T	L04320
<i>Bacteroides ureolyticus</i>	ATCC 33387 ^T	L04321
<i>Campylobacter coli</i>	CCUG 11238 ^T	L04312
<i>Campylobacter concisus</i>	ATCC 33237 ^T	L04322
<i>Campylobacter curvus</i>	ATCC 35224 ^T	L04313
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	ATCC 27374 ^T	L04314
<i>Campylobacter helveticus</i>	NCTC 12570 ^T	U03022
<i>Campylobacter hyointestinalis</i>	ATCC 35217 ^T	M65010
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	CCUG 11284 ^T	L04315
<i>Campylobacter lari</i>	CCUG 23947 ^T	L04316
<i>Campylobacter mucosalis</i>	CCUG 6822 ^T	L06978
<i>Campylobacter mucosalis</i> -like	CCUG 20705	L14629
<i>Campylobacter rectus</i>	ATCC 33238 ^T	L04317
<i>Campylobacter showae</i>	ATCC 51146 ^T	L06974
<i>Campylobacter sputorum</i> biovar <i>bubulus</i>	ATCC 33491	L04319
<i>Campylobacter</i> sp.	PGC 40-6AT	L04318
<i>Campylobacter upsaliensis</i>	CCUG 14913 ^T	L14628
<i>Helicobacter canis</i>	NCTC 12739 ^T	L13464
<i>Helicobacter felis</i>	ATCC 49179 ^T	M37642
<i>Helicobacter hepaticus</i>	ATCC 51448 ^T	U07574
<i>Helicobacter mustelae</i>	ATCC 43772 ^T	M35048
<i>Helicobacter pametensis</i>	ATCC 51478 ^T	M88147
<i>Helicobacter pylori</i>	ATCC 43504 ^T	M88157
<i>Wolinella succinogenes</i>	ATCC 29543 ^T	M88159
Delta subclass of the Proteobacteria		
<i>Desulfovibrio desulfuricans</i>	ATCC 27774	M34113
<i>Myxococcus xanthus</i>	MD207	M34114
Gamma subclass of the Proteobacteria		
<i>Escherichia coli</i>		J01695
<i>Pseudomonas aeruginosa</i>	ATCC 25330	M34133
Beta subclass of the Proteobacteria		
<i>Neisseria gonorrhoeae</i>	NCTC 8375 ^T	X07714
<i>Eikenella corrodens</i>	ATCC 23834 ^T	M22512
Alpha subclass of the Proteobacteria		
<i>Agrobacterium tumefaciens</i>	DSM 30105	M11223
<i>Rickettsia rickettsii</i>	ATCC VR-891	M21293
Bacteroides group		
<i>Prevotella loeschii</i>	ATCC 15930 ^T	L16481
<i>Prevotella melaninogenica</i>	ATCC 25845 ^T	L16469
<i>Bacteroides fragilis</i>	ATCC 25285 ^T	M11656
<i>Bacteroides vulgatus</i>	ATCC 8482 ^T	M58762
<i>Porphyromonas asaccharolytica</i>	ATCC 25260 ^T	L16490
<i>Porphyromonas gingivalis</i>	ATCC 33277 ^T	L16492
Spirochetes		
<i>Borrelia burgdorferi</i>	ATCC 35210 ^T	M59293
<i>Spirochaeta aurantia</i>	ATCC 25082 ^T	M57740
<i>Treponema pallidum</i>	Nichols	M34266

^a NCTC, National Collection of Type Cultures, London, United Kingdom; PGC, The Procter and Gamble Co. Cincinnati, Ohio. For other abbreviations see Table 1, footnote a.

^b The 16S rRNA sequences of strains are available for electronic retrieval from the GenBank database under the accession numbers indicated. Because of cross-distribution of databases, these sequences should also be available from EMBL and DDBJ.

succinogenes, which contains only a single strain) were similar to the patterns obtained for the type strains shown in Table 2; there were only small quantitative differences among the strains of each species (data not shown). Thus, if the mena-

FIG. 1. RPHPLC chromatogram of menaquinones from *B. ureolyticus* ATCC 33387^T analyzed on a μ Bondpack C18 reverse-phase column (300 by 3.9 mm). The detector was set at 248 nm. *MK-6 is thermoplasmaquinone (2, 25).

quinone pattern of *W. succinogenes* is confirmed with additional strains, it will be possible to distinguish this organism from the other species listed in Table 2. It should be noted that the overall menaquinone patterns of all of the species listed in Table 2 are essentially identical to the patterns of one or more species belonging to the genus *Campylobacter* (25, 26). To our knowledge, *MK-6 is a unique menaquinone which has been found only in members of rRNA superfamily VI (43).

Cellular fatty acid analysis. The fatty acid compositions of all of the strains which we studied are shown in Table 4. The fatty acid compositions of *C. rectus* and *B. ureolyticus* differed from the fatty acid compositions of *C. curvus*, *W. succinogenes*, and *B. gracilis* because *C. rectus* and *B. ureolyticus* lacked aldehydes and dimethylacetyls. *C. curvus*, *W. succinogenes*, and *B. gracilis* contained a 16-carbon dimethylacetyl (16:0 DMA) which was not present in *C. rectus* and *B. ureolyticus*. The overall fatty acid compositions of *C. curvus* and *W. succinogenes* were similar to each other and differed from the fatty acid composition of *C. rectus* by the presence of small amounts

FIG. 2. Electron impact mass spectrum of *MK-6 (2,5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone) isolated from *B. ureolyticus* ATCC 33387^T.

of 16:0 DMA and 3-OH-16:0 (Table 4). The fatty acid composition of *B. gracilis* was clearly different, because this species contained significant amounts of a 16-carbon aldehyde (16:0 ALD) and an 18-carbon dimethylacetal (18:1 DMA). The presence of large amounts of 18:1 ω 7c and 3-OH-16:0 and the low levels of 16:1 ω 7c distinguish *B. ureolyticus* from all other species (Table 4).

Our data confirm previous reports that *B. gracilis* has a fatty acid composition similar to that of campylobacters, whereas the fatty acid composition of *B. ureolyticus* is clearly distinct (1, 22, 25).

Protein analysis. The cellular proteins of reference strains of *B. ureolyticus*, *B. gracilis*, *C. curvus*, *C. rectus*, and *W. succinogenes* were extracted and separated in polyacrylamide gels. The protein patterns are shown in Fig. 3, together with the protein patterns obtained for representative strains of other hydrogen-requiring *Campylobacter* species. Clearly, protein analysis is a useful method for differentiating these biochemically similar species. However, numerical comparisons of the protein elec-

trophoretic traces did not allow differentiation of species because of the extreme variability in the principal protein band region (Fig. 3) in some of the species (data not shown). It has been demonstrated previously that *B. ureolyticus* and several

TABLE 4. Cellular fatty acid compositions of the strains studied

Fatty acid ^a	% of total fatty acids in:				
	<i>C. rectus</i> (n = 8) ^{b,c}	<i>C. curvus</i> (n = 6) ^d	<i>B. gracilis</i> (n = 3) ^e	<i>B. ureolyticus</i> (n = 3) ^f	<i>W. succinogenes</i> (n = 2)
12:0	11 ^g	7	10	5	6
14:0	8	16	10	5	6
16:0 ALD	— ^h	—	12	—	—
3-OH-14:0	4	6	6	5	5
16:1 ω 7c	8	9	8	1	18
16:0	31	25	12	10	23
16:1 DMA	—	—	2	—	—
16:0 DMA	—	4	10	—	7
18:2	8	3	—	—	6
3-OH-16:0	—	1	—	5	1
18:1 ω 9c	5	2	2	1	2
18:1 ω 7c	18	22	14	61	22
18:0	4	2	2	5	2
18:1 DMA	—	—	9	—	—

^a The number before the colon is the number of carbon atoms, and the number after the colon is the number of double bonds. ALD, aldehyde; DMA, dimethylacetal; 3-OH, a hydroxyl group occurs at carbon 3; ω , double bond position from the hydrocarbon end of the chain; c, cis isomer.

^b n, number of strains tested.

^c Strains ATCC 33238^T, CDC D2083^T, CDC D4326, CDC D4328, CDC D4329, CDC D4330, CDC D4331, and CDC D4332.

^d Strains ATCC 35224^T, CDC D2608^T, CDC D2712, CDC D4319, CDC D4321, and CDC D4322.

^e Strains ATCC 33236^T, FDC 20A1, and FDC 406.

^f Strains ATCC 33387^T, ATCC 43604, and ATCC 33481.

^g Values are arithmetic means.

^h —, not detected or trace (less than 0.7%).

TABLE 3. Menaquinone contents of the strains studied

Strain	Amt of ^a :		
	MK-5	MK-6	*MK-6 ^b
<i>C. rectus</i> ATCC 33238 ^T	1	1	4
<i>C. curvus</i> ATCC 35224 ^T	T	1	4
<i>B. gracilis</i> ATCC 33236 ^T	T	2	4
<i>B. ureolyticus</i> ATCC 33387 ^T	1	2	4
<i>W. succinogenes</i> ATCC 29543 ^T	1	4	2

^a The area of the major peak on each RPHPLC chromatogram was assigned a value of 4, and the areas of the other peaks were related to this value (i.e., a value of 2 indicates that the area of a peak was 50% of the area of a peak with a value of 4; T indicates that the area of a peak was less than 10% of the area of a peak with a value of 4).

^b *MK-6 is thermoplasmaquinone.

FIG. 3. Protein profiles of all strains examined and reference strains of hydrogen-requiring *Campylobacter* species. All strain numbers are LMG (Culture Collection of the Laboratorium voor Mikrobiologie, University of Ghent, Ghent, Belgium) numbers.

Campylobacter species are characterized by the presence of a variable dense-band region which is often useful for typing purposes (4, 39, 40, 42, 44–46). Unknown strains belonging to taxa that produce such a variable dense-band region can be identified by omitting the variable-band region from the numerical analysis (4, 44) or by creating a database in which entries correspond to the various electrophoretic types within each of the species (42).

Phylogenetic position of *B. gracilis* and *B. ureolyticus*. A phylogenetic tree that includes representative strains of the different subgroups within the *Proteobacteria*, the genus *Bacteroides* and its relatives, and the spirochetes is shown in Fig. 4. This tree was generated from a similarity matrix based on a comparison of 16S rRNA sequences at 1,421 base positions (data not shown). *B. gracilis* and *B. ureolyticus* fall in the epsilon subdivision of the *Proteobacteria* along with *Campylobacter* species, not in the genus *Bacteroides*, *Prevotella*, or *Porphyromonas*. In a more detailed analysis, *B. gracilis* and *B. ureolyticus* were compared with 26 reference *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella* species. A similarity matrix based on sequence comparisons at 1,421 positions is shown in Table 5, and a phylogenetic tree based on this matrix is shown in Fig. 5. *B. gracilis* falls in the middle of a cluster containing five *Campylobacter* species, including *C. rectus* and *Campylobacter sputorum*. *B. ureolyticus* falls just outside the *Campylobacter* cluster. Bootstrapping of the neighbor-joining tree produced the values shown next to the nodes on the tree in Fig. 5 (values less than 50% are not shown). On the basis of its branching position, *B. gracilis* is clearly a member of the genus *Campylobacter*. A bootstrap value of 86% for the clade composed of the upper six species in the tree supports this conclusion. The phylogenetic position of *B. ureolyticus* is less certain. When the outgroup is changed, the position of *B. ureolyticus* occasionally changes to a position inside the *Campylobacter* cluster. A bootstrapping analysis also revealed that the *Campylobacter* clade separates from *B. ureolyticus* only 61% of the time. Thus, it is not clear from the 16S rRNA sequence analysis results whether *B. ureolyticus* branches outside the *Campylobacter* cluster (and should be placed in a new genus) or inside the *Campylobacter* cluster (and should be included in

FIG. 4. Phylogenetic tree for members of the class *Proteobacteria*, *Bacteroides* species, and spirochetes. The positions of [*B.*] *gracilis* and [*B.*] *ureolyticus* are indicated by arrowheads (the genus names are in brackets to indicate that these organisms are generically misnamed). The five divisions of the *Proteobacteria* are indicated by Greek letters. Scale bar = 10% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting two species.

the genus *Campylobacter*). *B. ureolyticus* falls in rRNA cluster I of rRNA superfamily VI (41, 43) and is clearly a member of the family *Campylobacteraceae*, which includes the genera *Campylobacter* and *Arcobacter*.

Classification of *B. gracilis* and *B. ureolyticus*. *B. gracilis* resembles campylobacters in almost all phenotypic characteristics; it is a microaerophilic, asaccharolytic organism which has biochemical characteristics that are the same as those of campylobacters (13, 37) and a DNA base ratio which is similar to the base ratios of campylobacters (37, 43). Biochemical differentiation of *B. gracilis* from some hydrogen-requiring campylobacters is difficult (36–38). In fact, only two characteristics distinguish *B. gracilis* from campylobacters, the absence of flagella and the absence of oxidase activity. Usually, campylobacters have a single flagellum at one or both ends of the cell (43); occasionally, nonmotile strains are found, whereas *Campylobacter showae* cells have bundles of two to five flagella (9). Clearly, the absence of a flagellar sheath (12) is taxonomically relevant in the genus *Campylobacter*, whereas the number of flagella is not. Similar findings have been reported for the genus *Helicobacter*. Different *Helicobacter* species may have different numbers of flagella (single flagella or tufts of flagella that are distributed polarly or laterally) (12, 43). However, the flagella of all of these species are sheathed (12, 43). Thus again, the number or position of the flagella is less significant than the flagellar structure. The second biochemical characteristic that differentiates *B. gracilis* from campylobacters is the absence of measurable oxidase activity. The pattern of cytochromes found in *B. gracilis* resembles the pattern reported for *Campylobacter* species in that *B. gracilis* possesses cytochromes *b* and *c* and CO-binding cytochrome *c* and does not possess detectable type *a* and *d* cytochromes (14). Oxidase activity as

TABLE 5. Similarity matrix based on 16S rRNA sequence comparisons^a

Species	C.re	C.sh	B.gr	C.cu	C.co	C.sp	C.mu	C.ml	C.fe	C.hy	C.sp	C.he	C.up	C.ci	C.la	C.je	B.ur	A.cr	A.su	A.wi	A.abu	A.sk	H.py	H.fe	H.he	H.ca	H.pa	H.mu
<i>Campylobacter rectus</i>	97.9	95.3	95.7	95.3	93.4	94.1	93.9	93.7	92.9	92.6	91.1	91.4	93.1	92.1	92.4	91.4	85.7	85.1	86.1	84.8	86.0	83.9	82.8	85.1	85.5	85.7	84.6	
<i>Campylobacter showae</i>	2.1	94.3	95.6	94.3	93.5	93.3	92.9	92.6	91.7	91.4	90.2	90.4	92.0	91.0	91.3	90.9	86.1	85.7	86.1	84.7	86.1	84.2	83.3	85.2	85.7	85.8	84.7	
<i>Bacteroides gracilis</i>	4.9	5.9	93.5	94.3	92.6	93.5	93.4	93.5	92.5	91.2	90.2	90.4	91.3	90.6	90.8	91.1	85.9	85.5	86.4	84.7	85.0	83.6	82.6	84.4	84.7	85.5	84.2	
<i>Campylobacter curvus</i>	4.4	4.5	6.8	96.2	94.3	94.0	94.3	93.7	92.9	93.3	91.1	91.4	92.8	91.6	92.1	90.3	85.6	85.7	86.2	85.7	86.3	84.7	83.6	85.4	86.0	86.6	85.7	
<i>Campylobacter concisus</i>	4.8	6.0	6.0	3.9	93.6	95.6	95.7	95.0	94.1	93.6	91.8	91.9	93.9	93.4	93.3	91.6	87.1	87.2	87.6	86.7	86.1	84.9	84.4	86.7	87.1	87.6	86.1	
<i>Campylobacter sputorum</i>	6.9	6.8	7.8	6.0	6.7	92.3	91.9	92.1	91.4	91.9	90.6	90.9	91.6	90.8	91.0	91.4	86.0	86.1	85.8	84.5	85.0	84.5	83.8	85.8	86.7	86.2	86.2	
<i>Campylobacter mucosalis</i>	6.2	7.0	6.8	6.3	4.6	8.1	98.0	96.2	94.9	93.6	92.1	92.9	94.1	93.4	93.6	91.9	86.8	86.5	86.3	85.7	85.6	84.6	84.6	85.5	86.0	86.7	85.4	
<i>Campylobacter mucosalis-like</i>	6.3	7.4	6.9	5.9	4.4	8.6	2.0	96.4	95.2	94.6	92.5	93.0	94.5	93.6	94.0	92.5	86.2	85.9	85.8	85.3	85.8	84.7	84.4	85.7	86.2	86.6	85.6	
<i>Campylobacter felis</i>	6.6	7.7	6.8	6.6	5.1	8.4	3.9	3.7	98.1	95.4	92.7	92.9	95.3	94.0	94.2	92.6	86.0	85.8	86.0	85.2	85.9	85.0	84.5	86.0	86.5	86.6	85.9	
<i>Campylobacter hyointestinalis</i>	7.5	8.8	7.9	7.5	6.2	9.1	5.3	5.0	1.9	95.8	93.1	92.7	95.5	94.3	94.5	92.7	86.2	85.9	85.9	85.4	85.2	84.4	83.5	85.6	85.8	86.0	85.6	
<i>Campylobacter</i> sp. Pig	7.7	9.2	9.3	7.0	6.7	8.6	6.7	5.6	4.7	4.4	93.6	92.9	96.1	94.6	95.1	92.0	85.4	85.1	85.0	84.7	85.2	84.2	83.6	84.9	85.8	86.1	85.5	
<i>Campylobacter helveticus</i>	9.5	10.5	10.5	9.5	8.7	10.0	8.4	7.9	7.7	7.3	6.7	98.1	95.7	96.2	96.6	91.6	85.9	85.6	85.9	85.5	85.8	85.4	86.5	87.4	87.3	86.6		
<i>Campylobacter upsaliensis</i>	9.2	10.2	10.3	9.1	8.5	9.7	7.5	7.3	7.5	7.7	7.5	1.9	94.8	95.3	95.8	91.2	85.7	85.4	86.0	85.2	85.9	86.0	85.9	86.1	87.1	87.5	86.8	
<i>Campylobacter coli</i>	7.3	8.5	9.3	7.5	6.3	8.9	6.1	5.7	4.9	4.6	4.0	4.4	5.4	97.7	98.6	93.3	86.5	86.3	86.0	85.8	86.3	85.4	84.4	86.4	87.2	87.4	86.7	
<i>Campylobacter lari</i>	8.3	9.6	10.0	8.9	6.9	9.9	6.9	6.7	6.2	6.0	5.6	3.9	4.9	2.3	98.7	92.5	86.8	86.5	86.2	86.2	86.7	85.7	85.2	86.5	87.3	88.0	87.4	
<i>Campylobacter jejuni</i>	8.0	9.2	9.8	8.3	7.0	9.6	6.6	6.3	6.0	5.8	5.1	3.5	4.4	1.4	1.3	92.9	87.1	86.9	86.5	86.3	86.4	85.6	84.9	86.1	87.0	87.6	87.0	
<i>Bacteroides ureolyticus</i>	9.1	9.7	9.4	10.4	8.9	9.2	8.5	7.9	7.8	7.7	8.5	8.9	9.3	7.0	7.9	7.5	86.5	86.3	86.0	85.3	85.5	84.0	83.3	85.1	86.0	86.0	85.7	
<i>Arcobacter cryaerophilus</i>	15.8	15.4	15.6	16.0	14.1	15.5	14.6	15.3	15.5	15.2	16.2	15.6	15.9	14.9	14.5	14.2	14.9	98.7	97.3	94.0	85.6	84.5	83.5	84.7	85.5	86.2	86.2	
<i>Arcobacter skirrowii</i>	16.6	15.8	16.2	15.9	14.1	15.3	14.9	15.7	15.8	15.6	16.6	16.0	16.2	15.1	14.9	14.4	15.2	1.3	97.1	94.1	85.2	84.0	83.3	84.3	85.1	85.7	85.7	
<i>Arcobacter butzleri</i>	15.4	15.4	15.0	15.3	13.6	15.8	15.1	15.7	15.5	15.7	16.7	15.6	15.5	15.4	15.2	14.8	15.5	2.8	3.0	94.4	86.1	85.3	84.2	85.6	85.9	86.6	86.6	
<i>Arcobacter nitrofigilis</i>	16.9	17.1	17.2	15.9	14.6	17.3	15.9	16.4	16.5	16.3	17.1	16.1	16.4	15.7	15.2	15.1	16.3	6.3	6.2	5.8	85.1	83.4	83.2	84.2	84.8	85.5	85.1	
<i>Wolinella succinogenes</i>	15.5	15.3	16.7	15.1	15.4	16.8	16.0	15.8	15.6	16.5	16.5	15.8	15.7	15.1	14.6	15.0	16.2	16.0	16.5	15.3	16.6	90.8	90.1	92.6	93.3	94.0	93.8	
<i>Helicobacter pylori</i>	18.1	17.7	18.5	17.1	16.8	17.4	17.2	17.1	16.7	17.5	17.8	16.2	15.5	16.3	15.9	16.0	18.1	17.4	18.0	16.3	18.8	9.8	95.4	93.3	93.9	94.4	93.8	
<i>Helicobacter felis</i>	19.5	18.9	19.7	18.6	17.5	18.3	17.3	17.5	17.4	18.6	18.5	16.8	15.6	17.4	16.4	16.9	18.8	18.6	18.9	17.7	19.0	10.6	4.7	93.1	93.2	93.8	93.3	
<i>Helicobacter hepaticus</i>	16.7	16.4	17.5	16.2	14.6	15.8	16.1	15.8	15.5	16.0	16.9	14.9	15.4	15.0	14.8	15.3	16.6	17.1	17.6	16.0	17.8	7.8	7.1	7.3	97.2	96.2	96.3	
<i>Helicobacter canis</i>	16.1	15.9	17.1	15.5	14.1	14.6	15.5	15.2	14.9	15.7	15.7	13.8	14.2	14.0	13.9	14.3	15.5	16.1	16.7	15.6	17.0	7.0	6.4	7.1	2.9	96.4	96.5	
<i>Helicobacter pametensis</i>	15.8	15.7	16.2	14.8	13.6	15.2	14.7	14.8	14.8	15.5	15.3	13.9	13.7	13.7	13.1	13.6	15.5	15.3	15.8	14.8	16.1	6.2	5.9	6.4	3.9	3.7	97.2	
<i>Helicobacter mustelae</i>	17.2	17.1	17.7	15.9	15.4	15.3	16.3	16.0	15.7	16.0	16.1	14.7	14.5	14.6	13.8	14.2	15.8	15.3	15.8	14.7	16.7	6.5	6.5	6.0	3.8	3.6	2.9	

^a The values on the upper right are uncorrected percentages of similarity, and the values on the lower left are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor. Abbreviations: C.re, *Campylobacter rectus*; C.sh, *Campylobacter showae*; B.gr, *Bacteroides gracilis*; C.cu, *Campylobacter curvus*; C.co, *Campylobacter concisus*; C.sp, *Campylobacter sputorum*; C.mu, *Campylobacter mucosalis*; C.ml, *Campylobacter mucosalis-like*; C.fe, *Campylobacter feus*; C.hy, *Campylobacter hyointestinalis*; C.sp, *Campylobacter sp. (pig)*; C.he, *Campylobacter helveticus*; C.up, *Campylobacter upsaliensis*; C.ci, *Campylobacter coli*; C.la, *Campylobacter lari*; C.je, *Campylobacter jejuni*; B.ur, *Bacteroides ureolyticus*; A.cr, *Arcobacter cryaerophilus*; A.sk, *Arcobacter skirrowii*; A.bu, *Arcobacter butzleri*; A.ni, *Arcobacter nitrofigilis*; W.su, *Wolinella succinogenes*; H.py, *Helicobacter pylori*; H.he, *Helicobacter hepaticus*; H.ca, *Helicobacter canis*; H.pa, *Helicobacter pametensis*; H.mu, *Helicobacter mustelae*.

FIG. 5. Phylogenetic tree for the epsilon subdivision of the *Proteobacteria*. The positions of [*B.*] *gracilis* and [*B.*] *ureolyticus* are indicated by arrowheads (the genus names are in brackets to indicate that these organisms are generically misnamed). The epsilon subdivision of the *Proteobacteria* corresponds to rRNA superfamily VI. The rRNA clusters within superfamily VI are the clusters described previously (43). Scale bar = 5% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting two species. The values to the left of the nodes are the percentages of times that the strains to the right of the nodes occur together by bootstrapping. Bootstrapping values less than 50% are not shown.

determined by the Kovács test is associated with cytochrome *c* and oxygen respiration, which are both present in *B. gracilis* and campylobacters. Possible explanations for the fact that oxidase activity is not detected are (i) that the reagent (tetramethylphenylenediamine) cannot penetrate the cellular membranes, and (ii) that a low-potential cytochrome *c* that cannot oxidize this reagent is present (14). However, it is unlikely that the absence of measurable oxidase activity is caused by a fundamental metabolic difference between *B. gracilis* and *Campylobacter* species.

In summary, *B. gracilis* is a *Campylobacter* species on the basis of its genotypic and phenotypic characteristics and has the same human oral niche as several *Campylobacter* species. We conclude that *B. gracilis* is a nonmotile campylobacter, and below we propose that this taxon should be transferred to the genus *Campylobacter*. The genus description of *Campylobacter* has to be emended to include species with multiple flagella (9) and species without flagella.

Reclassification of *B. ureolyticus* is not straightforward. *B. ureolyticus* resembles campylobacters in its respiratory quinone content (Table 3) and in its DNA base ratio (43). These findings are not unexpected since a 16S rRNA sequence analysis revealed that these taxa are close relatives, while respiratory quinone compositions characterize taxonomic units

at the deep phylogenetic level rather than the fine taxonomic level (3). *B. ureolyticus* differs from campylobacters in its fatty acid composition, its proteolytic metabolism, and its ability to hydrolyze urea (only a few atypical *Campylobacter lari* strains have been reported to hydrolyze urea [43]). Furthermore, the majority of *B. ureolyticus* strains are isolated from patients with superficial ulcers, soft-tissue infections, and urethritis and are therefore unlikely to be confused with campylobacters. The group of dental *B. ureolyticus*-like isolates can be readily differentiated from oral campylobacters by their ability to hydrolyze urea and by their proteolytic activity. However, this group of dental isolates has been shown to be heterogeneous, and the taxonomic structure and position of this taxon will require further investigation (7). Obviously, including *B. ureolyticus* in the genus *Campylobacter* or in a new genus is arbitrary. Including it in the genus *Campylobacter* would considerably increase the phenotypic heterogeneity of this genus, but excluding it from the genus *Campylobacter* would result in the creation of a monotypic taxon which differed from the genus *Campylobacter* only in the ability to digest casein and gelatin and in the presence of urease activity. While *B. ureolyticus* is clearly a member of the family *Campylobacteraceae*, we do not believe that it should be renamed at this time and prefer to consider this taxon a species incertae sedis pending the isolation and a thorough taxonomic characterization of additional *B. ureolyticus*-like bacteria.

Description of *Campylobacter gracilis* comb. nov. The description of *Campylobacter gracilis* (basonym, *Bacteroides gracilis* Tanner, Badger, Lai, Listgarten, Visconti, and Socransky 1981) is the same as that given previously for *B. gracilis* (37). The type strain is strain ATCC 33236.

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