Proposal of Minimal Standards for Describing New Species of the Family *Campylobacteraceae*[†]

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The International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Campylobacter* and Related Bacteria has agreed in principle on minimum requirements for the description of new species of the family *Campylobacteraceae*. These requirements, as well as methods for determining specific characteristics, are proposed as minimal standards for the description of new species. In addition to specified phenotypic characteristics, molecular data are required. The placement of a new species should be consistent with the current view on classification usually based on methods such as nucleic acid sequencing, hybridization, or protein fingerprinting.

Members of the family *Campylobacteraceae* are curved or spiral bacteria mainly encountered as commensals or parasites in humans and other warm-blooded animals. The genus *Campylobacter* was created in 1963 (27), and as a consequence of the taxonomic revisions proposed by Vandamme et al. (37), the emended genus now contains (March 1993) 13 species and four subspecies. The genus *Arcobacter* was suggested for the former species *Campylobacter cryaerophilus* and *Campylobacter nitrofigilis* (37); recently, two additional species, *Arcobacter butzleri* (12) and *Arcobacter skirrowii* (38), have been described. The inclusion of the genera *Campylobacter* and *Arcobacter* in a separate family, *Campylobacteraceae*, was suggested by Vandamme et al. (36). *Helicobacter pylori*, *Helicobacter mustelae*, *Helicobacter cinaedi*, and *Helicobacter fennelliae*, which were originally described as campylobacters (7, 37), will not be considered here.

General features of *Campylobacteraceae*. Curved or occasionally straight rods, 0.2 to 0.9 µm wide and 0.5 to 5 µm long. The rods may appear as spiral, S-, V-, or comma-shaped forms and may also occur in short or occasionally long chains. Cells may become spherical or coccoid, especially in old cultures. Non-spore-forming. Gram negative. Motile by means of single or occasionally multiple unsheathed flagella at one or both ends. Optimum temperatures of growth range from 30 to 42°C. They are usually microaerophilic and have a respiratory type of metabolism. Some species may be aerotolerant. Oxidase producing. Carbohydrates are neither fermented nor oxidized. Respiratory quinones are menaquinones. The G+C content of the DNA ranges from 28 to 46 mol%. The type genus is *Campylobacter*; at present, the only other genus is *Arcobacter*. The following key features of the genus *Arcobacter* can be used to distinguish species from those of the genus *Campylobacter*: growth at 15°C but not at 42°C, aerobic growth at 30°C, G+C contents of 27 to 30 mol%, and methyl-substituted menaquinone-6 not present as a major isoprenoid quinone (36, 37). Detailed morphological, physiological, and biochemical test results for the various species have been reported previously (8, 22, 25, 26, 29) (see also the references given in Table 1).

General comments. The description of a new species or

subspecies of *Campylobacter* or *Arcobacter* should be based on characteristics necessary for assigning the new taxon to the genus and on characteristics serving to differentiate the new taxon from existing taxa of the genus. The description should be based on as many strains as possible, preferably not fewer than 10. For critical comparisons with other species, controls consisting of type or reference strains of other species should be tested. For all test procedures, the inoculum size, composition of the gaseous atmosphere, and composition and pH of the medium should be stated. When possible, standardized, well-described tests and methods should be followed (1, 15, 16, 23, 24, 29).

Cell morphology. The shape, size, and spiral wavelength (where appropriate) of bacteria should be reported. The tendency to undergo rapid transformation to coccoid forms on exposure to air should also be indicated. Indicate whether stained preparations or wet mounts are used for microscopic observations. The number and arrangement of flagella should preferably be determined by electron microscopy; in which case, the presence or absence of flagellar sheaths should be reported.

Staining behavior. The behavior of the cells in the Gram stain must be stated.

Motility. Active, darting motility should be observed by microscopic examination of wet mounts or hanging drop preparations of young cultures in buffered saline or broth.

Colony morphology. The shape, size, and color of colonies should be described. The presence of swarming on solid media should be noted.

Growth conditions. Factors affecting growth should be tested under conditions that are near optimal unless stated otherwise.

(i) **Culture medium.** Growth on unsupplemented nutrient agar should be tested. When using blood-supplemented nutrient agar, the type and the percentage of blood used should be reported as well as any hemolytic activity observed.

(ii) **Temperature range.** The ability to grow in specified broth or agar media from standardized inocula at various temperatures should be reported with the time of incubation. The following temperatures should be used: 15, 25, 36, or 37°C and 42 or 43°C (preferably the latter).

(iii) **Gaseous requirements.** The ability of the strains to grow under aerobic conditions and under microaerobic conditions should be reported; the oxygen content should be specified in the latter instance and if formate or hydrogen is needed.

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TABLE 1. Species, subspecies, and biovars of *Campylobacteraceae*

Name	Reference(s)	Type strain designation	
		Original	Culture collection ^a
<i>Arcobacter butzleri</i>	12, 38	D2686	ATCC 49616
<i>Arcobacter cryaerophilus</i>	37	A169/B	NCTC 11885
<i>Arcobacter nitrofigilis</i>	37	C1	ATCC 33309
<i>Arcobacter skirrowii</i>	38	449/80	CCUG 10474
<i>Campylobacter coli</i> ^{ALb}	39	1407	CIP 7080
<i>Campylobacter concisus</i>	33	FDC 484	ATCC 33237
<i>Campylobacter curvus</i> (previously <i>Wolinella curva</i>)	34, 37	VPI 9584	NCTC 11649
<i>Campylobacter fetus</i> subsp. <i>fetus</i> ^{AL}	39	Mouton 1	CIP 5396
<i>Campylobacter fetus</i> subsp. <i>venerealis</i> ^{AL}	39	X/161/5	CIP 6829
<i>Campylobacter helveticus</i>	31	D5248	NCTC 12470
<i>Campylobacter hyointestinalis</i>	6	80-457-7-4	ATCC 35217
<i>Campylobacter jejuni</i> ^{AL} subsp. <i>jejuni</i>	39	Uccle 91 FB	CIP 702
<i>Campylobacter jejuni</i> subsp. <i>doylei</i>	32	093	NCTC 11951
<i>Campylobacter lari</i>	2, 40	WRI 3034/77	NCTC 11352
<i>Campylobacter mucosalis</i>	24	FS 253/72	NCTC 11000
<i>Campylobacter rectus</i> (previously <i>Wolinella recta</i>)	33, 37	FDC 371	NCTC 11489
<i>Campylobacter showae</i>	5	SW A4	ATCC 51146
<i>Campylobacter sputorum</i> biovar <i>sputorum</i> ^{AL}	25	S-17	ATCC 35980
<i>Campylobacter sputorum</i> biovar <i>bubulus</i> ^{AL}	25	Wat	CIP 53103
<i>Campylobacter sputorum</i> biovar <i>fecalis</i>	25	C-32	NCTC 11415
<i>Campylobacter upsaliensis</i>	26	C231	NCTC 11541

^a Collections in which the strains were apparently first deposited. ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection Bactérienne de l'Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, London, England; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden.

^b AL indicates that the species is on the Approved Lists of Bacterial Names (17, 28).

Anaerobic growth should be tested, and the requirement for fumarate alone, hydrogen and fumarate, or fumarate and formate should be investigated if considered appropriate (25, 30).

Biochemical properties. The tests for the following are required: (i) oxidase activity, by using any conventional method such as that described by Kovács (14); (ii) catalase activity, with percentage of reagent solution and time of observation given; (iii) acid production from glucose by oxidation and fermentation by the method of Hugh and Leifson (10); (iv) nitrate reduction in a broth or a semisolid medium or by the method of Cook (4); (v) indoxyl acetate hydrolysis, preferably by the disc method (21); (vi) H₂S production from a medium containing sodium thiosulfate, preferably as triple sugar iron (TSI) agar or sulfide-indole-motility (SIM) medium (of these two media, it has been shown that TSI agar is the more sensitive [22]); (vii) urea hydrolysis by the rapid test for preformed enzyme on Christensen's agar or in broth (19); and (viii) sodium hippurate hydrolysis by the rapid test of Hwang and Ederer (11) or an appropriate modification (9) (it is recommended that negative or equivocal results are confirmed by chromatographic assay for benzoic acid [13]).

Other tests. Tests for the following are desirable: (i) tolerance to 1.5 and 3.5% NaCl in brucella albimi broth or agar medium, (ii) anaerobic growth in the presence of 0.1% (wt/vol) trimethylamine N-oxide hydrochloride (TMAO) (2), (iii) rapid H₂S production in iron-bisulfite-pyruvate (FBP) agar (1, 29), (iv) growth in the presence of triphenyltetrazolium chloride (0.04%) (2), and (v) tolerance to 1% glycine in brucella albimi broth medium.

Resistance to antimicrobial agents. Susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg) should be determined by diffusion tests with Mueller-Hinton agar. The absence of a clear zone of inhibition should be recorded as resistance; for susceptible strains, the zone sizes should be stated.

Molecular data. Standard methods for the determination of the guanine-plus-cytosine content of DNA (melting temperature or chemical) may be used (20). Reference DNA preferably of *Escherichia coli* NCTC 9001 (G+C, 51 mol%) should be analyzed at the same time. The strain used and its estimated G+C content (moles percent) expressed relative to the reference DNA should be reported.

Determination of major cellular fatty acids and respiratory quinone content is strongly recommended (3, 41), particularly for the presence of methyl-substituted menaquinone-6.

The suggested new species and its nearest neighbors should be investigated by a method giving a broader view of genotypic relationships. Thus, relationships to other species of *Campylobacteraceae* should be determined by DNA-DNA hybridization (20), and percentage of relatedness to the type strains of the other species should be stated. It should be noted that computer-assisted analysis of one-dimensional electrophoretic protein patterns provides taxonomic discrimination comparable to that of DNA-DNA hybridization (18) and could be considered a possible alternative. If the new species is not significantly related by DNA-DNA hybridization to any species of *Campylobacteraceae*, its relation to the two genera of the family and to other allied genera, particularly *Helicobacter* and *Wolinella*, should be established by 16S rRNA sequence similarities (35) or by rRNA-DNA hybridization and thermal stability studies (37).

Ecology. The natural habitat(s) of the proposed species should be detailed as much as possible, and its pathogenicity and host range, if known, should be reported.

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