Phylogenetic Relationships of The Genus *Campylobacter* and Its Relatives

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INTRODUCTION

Since some species of the genus *Campylobacter* were known to be important pathogens in humans and animals, an explosive burst of interest has continued to the present and will doubtless continue into the foreseeable future (Benjamin *et al.*, 1983; Guerrant *et al.*, 1978; Marshall *et al.*, 1984; Smibert, 1984). It is uncertain whether species of the genera *Wolinella* and *Bacteroides* cause diseases in humans and animals. However, they have many similarities with those of the genus *Campylobacter* in morphology, physiological and biochemical characteristics. Moreover, they have been recovered from extraoral infections as well as oral infections. These have made them of general medical concern (Jackson and Goodman, 1978; Tanner *et al.*, 1981; Tanner and Socransky, 1984).

It is characteristic of human mind to attempt to arrange, or classify large numbers of entities into groups on the basis of their similarities; moreover, classification allows us to discern oder in nature. Also, accurate classification and identification systems of pathogenic microorganisms are especially important for clinical microbiologists and veterinarians to correctly handle the microorganisms recovered from humans and animals.

The genus *Campylobacter* is a state of flux and may be cited as an example to illustrate that taxonomy is a dynamic science. For instance, the genera *Campylobacter* and *Wolinella* housed 15 species and 3 species, respectively. However, some species of the genus *Wolinella* have already been transferred to those of the genus *Campylobacter*, whereas some species of the genus *Campylobacter* have been elevated to the new genera *Helicobacter* and *Arcobacter*. The classification of these spriral-shaped organisms has always been somewhat difficult. Because these organisms do not catabolize carbohydrates and are inert with regard to most traditional biochemical tests used for the identification of bacteria, only a relatively small number of tests are available for the identification and classification of campylobacters and wolinellas (Roop *et al.*, 1984, 1985b; Tanner *et al.*, 1984). Consequently, classifications of the genera *Campylobacter* and *Wolinella* based on only a few physiological and biochemical tests give few means to accurately differentiate species. Although classifications based on phenotypic characteristics have been criticized as failing to reflect natural relationships, in fact they have served microbiology reasonably well. However, classification schemes based on phenotypic characteristics (e.g., comparisons of colony and cell morphology, growth characteristics, antibiotic susceptibility, biochemical tests, and guanime-plus-cytosine (G+C) content of the DNA) lack stability. This has been amply demonstrated by the many bacterial classifications that have risen and fallen over the years.

For speciation, classification of bacteria based upon DNA homology experiments has been used to provide a stable and objective basis. The DNA homology studies have shown that the current *Campylobacter* species are distinct from one another (Belland and Trust, 1982; Benjamin *et al.*, 1983; Harvey and Greenwood, 1983; He'bert *et al.*, 1983; Leaper and Owen, 1982; Owen, 1983; Owen and Leaper, 1981; Roop *et al.*, 1984, 1985; Ursing *et al.*, 1983); however, they have not ans-

wered the question of whether these species are sufficietly related to justify classification within a single genus. This has always been particularly diffult for microorganisms that have few positive biochemical traits, such as species within the genera *Campylobacter* and *Wolinella.* Among many analyses for assigning species to genera (e.g., rRNA/DNA homology, rRNA sequencing, or microcomplement fixation of glutamine synthetase), comparison of 16S ribosomal ribonucleic acid (rRNA) sequences has proven to be a powerful tool for accurate classification of microorganisms above the level of species (Fox *et al.*, 1980; Olsen *et al.*, 1986; Stackebrandt, 1985; Stackebrandt and Woese, 1984; Woese, 1985, 1987). Large data bases of sequence information currently exist for 16S rRNA molecules. This allows placement of an unknown organism or genus of uncertain affilation relative to the numerous genera that have already been examined.

GENERAL CHARACTERISTICS OF CAMPYLOBACTERS, WOLINELLAS, AND BACTEROIDES

The some strains of *Campylobacter* species are important pathogens of animals and humans; others are part of the normal flora of animals. Thus, they have been the subject of intensive investigation in veterinary and clinical microbiology.

The genus *Campylobacter*

The genus *Campylobacter* has been described by Smibert (1984) and Penner (1988) as follows: slender, nonsporeforming, gram-negative, vibroid bacteria, 0.2-0.5 μm wide and 0.5 to 5 μm long. The cells may have one or more helical turns and can be as long as 8 μm. They also appear S-shaped and gull-winged when two cells form short chains. Cells in old cultures may form spherical coccoid bodies. The cells have a polar membrane at both ends of the cell; this structure, which also occurs in several other kinds of helical or vibroid bacteria; is located directly under the cytoplasmic membrane and is linked to the cytoplasmic membrane by bar-shaped linkers. Campylobacters are motile with a characteristic corkscrew-like motion by means of a single polar flagellum at one or both ends of the cells; the flagella may be 2 to 3 times the length of the cells. Campylobacters are typically microaerophilic, although one species (*Campylobacter cryaerophila*[sic]) is aerobic. They have a strictly respiratory type of metabolism and, in the absence of other terminal electron acceptors, they typically require an O_2 concentration between 3 and 15% and a CO_2 concentration of 3 to 5%. Some species can grow under anaerobic conditions with either fumarate, formate+fumarate, or H_2+fumarate in the medium: the fumarate serves as the terminal electron acceptor and the formate or H_2 as the electron donor. Some species can use nitrate or trimethylamine oxide as a terminal electron acceptor for anaerobic respiration. Campylobacters are chemoorganotrophs; however, they neither oxidize nor ferment carbohydrates and instead obtain energy from amino acids, the salts of tricarboxylic acid cycle intermediates, the salts of other organic acids, or, in some species, H_2. They do not require blood or serum for growth. Gelatin is not hydrolyzed, and urea is hydrolyzed by only one species, *Campylobacter pylori* (now called *Helicobacter pylori*). No lipase activity occurs. Campylobacters are oxidase-positive. Some species are catalase-positive, others are catalase-negative. Nitrate is reduced to nitrite. No pigments are produced. Campylobacters are found in the reproductive organs, intestinal tract and oral cavity of humans and animals; some species are pathogenic for humans and animals. One species, *Campylobacter nitrofigilis,* is nitrogen-fixer isolated from the roots of plants growing in salt marshes. The mol% G+C of the DNA ranges from 30 to 38. The type species is *Campylobacter fetus.*

Campylobacter fetus. This species is divided into two subspecies, based upon the inability of *C. fetus* subsp. *venerealis* to grow in the presence of 1% glycine (Florent, 1959). *C. fetus* subsp. *fetus* causes orally-transmitted sporadic abortions in cattle and

sheep and blood infection in humans (Guerrant *et al.*, 1978; Mandal *et al.*, 1984; Rettig, 1979; Smibert, 1984). *C. fetus* subsp. *venerealis* causes sexually-transmitted abortion in cattle (Rettig, 1979), but is considered non-pathogenic to humans.

Campylobacter hyointestinalis. Strains have been isolated from pigs with proliferative ileitis (Gebhart *et al.*, 1983; Gebhart *et al.*, 1985), from both healthy and diarrheic cattle (Meyers *et al.*, 1984; Ursing *et al.*, 1983), and occasionally from homosexual males with proctitis (Edmonds *et al.*, 1987; Fennell *et al.*, 1987). By DNA hybridization, *C. hyointestinalis* shows a closer relationship to *C. fetus* than to any other catalase-positive *Campylobacter* species (Roop *et al.*, 1984). It is differentiated from *C. fetus* by its production of detectable H_2S in triple sugar iron (TSI) slants and by its ability to grow anaerobically with 0.1% trimethylamine-*N*-oxide hydrochloride (TMAO).

Campylobacter jejuni and *Campylobacter coli.* In the group known as the thermophilic (thermotolerant) campylobacters that prefer to grow at 42 to 43℃, *C. jejuni* and *C. coli* were recognized first. These two species are closely related by DNA hybridization, although sufficiently different as to be classified as two separate species (Roop *et al.*, 1984). Later, a third species, *C. lari,* was found to be thermophilic and closely related to *C. jejuni* and *C. coli* (see below). *C. jejuni* was initially isolated from winter scours in cattle (Smith and Orcutt, 1927). It is part of the normal intestinal flora of cattle, sheep, dogs, cats, poultry and other animals, and it is a major cause of bacterial gastroenteritis in humans (Butzler and Skirrow, 1979; Dekeyser et al., 1972; King, 1957; Luechtefeld and Wang, 1981). *C. coli* is part of the normal flora of pigs and poultry and can also cause diarrhea in humans (Butzler and Skirrow, 1979; Doyle, 1948; Luechtefeld and Wang, 1981). *C. jejuni* and *C. coli* are now being isolated as frequently as both *Salmonella* and *Shigella* species. *C. jejuni* causes two different types of diarrhea, one characterized by bloody stools like those in shigellosis, and the other by the watery stools like those caused by enterotoxin of *Vibrio cholerae. C. jejuni* can be differentiated from *C. coli* by its ability to hydrolyze hippurate (Harvey, 1980), although some hippurate-negative strains occur (Roop *et al.*, 19 84).

C. jejuni has been divided into two subspecies, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei* (Steele and Owen, 1988). The latter subspecies is differentiated by its failure to reduce nitrate, susceptibility to cephalothin, and only weak growth at 42℃. Moreover, the mol% G+C is 29% instead of 31%, and some strains are catalase-negative.

Campylobacter lari. In 1980, Skirrow and Benjamin isolated a new group of strains from the intestines of sea gulls of the genus *Larus.* These strains were thermophilic like *C. jejuni* and *C. coli* but were resistant to nalidixic acid. Strains of *C. lari* have also been found in humans, dogs, and horses, and they occasionally cause blood infections and diarrhea in humans (Benjamin *et al.*, 1983; Karmali and Fleming, 1979; Nachamkin *et al.*, 1984; Simor and Wilcox, 1987; Skirrow and Benjamin, 1980; Tauxe *et al.*, 1985). *C. lari* strains can be differentiated from *C. jejuni* and *C. coli* by their anaerobic growth in the presence of 0.1% TMAO and by their resistance to nalidixic acid.

Campylobacter mucosalis. The strains have been isolated from lesions of porcine intestinal adenomatosis (PIA) and from mouths of pigs (Lawson and Roland, 1974, 1984; Smibert *et al.*, 1984). The characteristics of *C. mucosalis* and its close relationship to *C. concisus* have been investigated in detail by Roop *et al.* (1985a, 1986b) and Tanner *et al.* (1981). *C. mucosalis* requires hydrogen of formate as an electron donor for growth; it can respire anaerobically with fumarate as the terminal electron acceptor. *C. mucosalis* can be differentiated from *C. concisus* in the susceptibility of *C. mucosalis* to cephalothin, growth at 25℃, and production of "dirty yellow" colonies (Lawson *et al.*, 1981; Roop *et al.*, 1985a).

Campylobacter concisus. This organism has been isolated from the oral cavity of human with perio-

dontal disease (Tanner *et al.*, 1981). *C. concisus* requires hydrogen or formate as an electron donor for growth; it can respire anaerobically with fumarate as the terminal electron acceptor. A chemotactic response to formate is believed to be a factor in facilitating colonization and attachment to oral surfaces (Paster and Gibbons, 1986). Resistant to cepalothin can readily differentiate *C. concisus* from *C. mucosalis* and from *C. sputorum* biovar sputorum (a commensal in the oral cavity).

Campylobacter sputorum. By DNA hybridization analysis (Roop *et al.*, 1985b), the strains previously classified as *C. sputorum* subsp. *mucosalis* were shown not to belong to *C. sputorum.* "*C. fecalis*" strains did belong to *C. sputorum,* however. *C. sputorum* is currently divided into three biovars called, sputorum, bubulus, and fecalis, which occurs as part of the normal flora of the human mouth, bovine genitalia, and sheep feces, respectively (Roop *et al.*, 1985b). Strains of all three biovars are oxidase-positive, grow in 1% glycine, and grow at 42℃ but not at 25℃. *C. sputorum* biovar sputorum and biovar bubulus are catalase-negative; the former grows in the presence of 1% oxall and the latter does not. Biovar sputorum is considered to be a commensal of the human oral cavity. *C. sputorum* biovar bubulus is commensal normally found in the preputial cavity of the male and the genital tract of the female of cattle. *C. sputorum* biovar fecalis is catalase-negative. It is isolated from bovine semen and from the vagina.

Campylobacter upsaliensis. This organism is catalase-negative or weakly catalase-positive, grows at 42℃, and is hippurate-negative. Organisms have been isolated from healthy and diarrheic dogs and cats (Gebhart *et al.*, 1984; Sandstedt *et al.*, 1983), from blood cultures of pediatric patients (Lastovica *et al.*, 1989), and from human feces (Goossens *et al.*, 1990).

Campylobacter pylori. The species is the putative causative agent of gastric and duodenal ulcers and chronic gastritis in humans (Marshall, 1986; Marshall *et al.*, 1984; Warren and Marshall, 1983). *C. pylori* grows under microaerobic atmospheres containing CO_2 and H_2 at 37℃. High humidity favors growth and the period of incubation is 3 to 4 days (McNutly, 1986). The presence of sheathed flagella (Goodwin *et al.*, 1985; Jones *et al.*, 1985), the unusual fatty acid composition (Goodwin *et al.*, 1985), and its relatedness to *Wolinella succinogenes* (Romaniuk *et al.*, 1987; Paster and Dewhirst, 1988; Thompson *et al.*, 1988) strongly indicate that the species does not belong in the genus *Campylobacter.* The name *Helicobacter pylori* was proposed for *Campylobacter pylori* by Goodwin *et al.* (1989).

Campylobacter mustelae. This species was isolated from the gastric mucosa of ferrets, *Mustela putorius furo* (Fox *et al.*, 1986; Fox *et al.*, 1988). Initially, it was thought to represent a subspeces of *C. pylori.* However, this conclusion was based on an error in DNA hybridization experiments. Subsequently, Fox *et al.* (1989) relcassified the organism in a separate species, *C. mustelae.* It was later reclassified in the genus *Helicobacter* as *Helicobacter mustelae* (Goodwin *et al.*, 1989).

Recently, organisms similar to *C. pylori* and *C. mustelae* were isolated from the stomachs of cats and dogs (Lee *et al.*, 1988) and have been classified as *Helicobacter felis* (Paster *et al.*, 1991). Strains have also been isolated from the stomach of *Macaca nemestrina* monkey (Bronsdon and Schoenknecht, 1988) and have been classified as *Helicobacter nemestrinae* (Bronsdon *et al.*, 1991). Other organisms similar to *C. pylori* have been isolated from *Papio papio* baboons (Curry *et al.*, 1987).

Campylobacter cinaedi and *Campylobacter fennelliae.* These species have been associated with proctitis, proctocolitis, enteritis and bacteremia in homosexual men (Cimolai *et al.*, 1987; Fennell *et al.*, 1984; Ng *et al.*, 1987; Pasternak *et al.*, 1984; Quinn *et al.*, 1984; Totten *et al.*, 1985). Recently, *C. cinaedi* has been isolated from blood and feces of children and adult females (Vandamme *et al.*, 1990). The two species are catalase-positive and grow microaerobically at 37℃, but not at 25℃ or 42℃. *C. cinaedi* can be differentiated from *C. fennelliae* by its ability to reduce nitrate and by its

failure to hydrolyze indoxyl acetate (Mills and Gherna, 1987). Recently, Vandamme *et al.* (1991) transferred these species t. the genus *Helicobacter* as *Helicobacter cinaedi* and *Helicobacter fennelliae.*

Campylobacter cryaerophila[sic]. The species is aerobic and catalase-positive and causes abortion in pigs, cattle, horses, and sheep. It can be isolated occasionally from human infections (Ellis *et al.*, 1977; Ellis *et al.*, 1978; Lambert *et al.*, 1987; Neill *et al.*, 1978; Neill *et al.*, 1979; Neill *et al.*, 1980; Neill *et al.*, 1985). Although *C. cryaerophila* grows at 37℃, its optimum temperature for growth is 30℃. By 16S rRNA analysis, *C. cryaerophila* species is not closely related to the genus *Campylobacter* (Thompson *et al.*, 1988). Recently, Vandamme *et al.* (1991) transferred *C. cryaerophila* to a new genus, *Arcobacter,* as *Arcobacter cryaerophilus.*

Campylobacter nitrofigilis. Ths species is aerobic, catalase-positive, and NaCl-requiring. In nitrogen-deficient media it can fix atmospheric nitrogen, but only under microaerobic conditions. The organism occurs in the roots of the salt marsh grass *Spartina alterniflora* (McClung and Patriquin, 19 80; McClung *et al.*, 1983). *C. nitrofigilis* is closely related to *C. cryaerophila* at the genus level or higher, but not closely related to the genus *Campylobacter* (Thompson *et al.*, 1988). Characteristics differentiating *C. nitrofigilis* from other *Campylobacter* species include its nitrogenase activity and its requirement for at least 1.5% NaCl (McClung *et al.*, 1983). Recently, Vandamme *et al.* (1991) transferred *C. nitrofigilis* to a new genus, *Arcobacter,* as *Arcobacter nitrofigilis* (which also contains *Arcobacter cryaerophilus).*

Campylobacter butzleri. Kiehlbauch *et al.* (1991) reported a new species that is aerotolerant, catalase-negative or weakly positive, and grows at a low temperatures. It was isolated from the stools of human and nonhuman primates with diarrhea, from necropsy of ostriches with diarrhea, and from aborted bovine and porcine fetuses. Growth of this species in the presence of 1% glycine and on MacConkey agar can be used to differentiate it from *C. cryaerophila* and *C. nitrofigilis.* It is likely that *C. butzleri* may eventually be transferred to the new genus *Arcobacter.*

The genus *Wolinella*

The genus *Wolinella* has been described by Tanner and Socransky (1984) as follows: Cells are slender, nonsporeforming, gram-negative, helical, curved, or straight unbranched cells 0.5-1.0 μm width and 2-6 μm in length. Cells show a rapid, darting motility by means of a single polar flagellum. Anaerobic, but some strains can grow in the presence of 5% O_2 but not in air (21% O_2) enriched with 10% CO_2. Hydrogen or formate is required as an electron donor for growth. Fumarate or nitrate is used as the electron acceptor. Chemoorganotrophic. Carbohydrates are neither fermented and oxidized, nor do they support growth. *Wolinella* species are oxidase-positive and catalase-negative. Strains have been isolated from the bovine rumen, from the human gingival sulcus, and from the dental root canal infections. The mol% G+C of the DNA is 42-48 (*Tm*). The genus contains three species; *W. succinogenes, W. recta,* and *W. curva.* The pathogenicities of *Wolinella* species are unknown. The type species is *Wolinella succinogenes.*

Wolinella succinogenes. The cells of this species are mainly vibroid, as suggested by the earliest name given to this organism, *Vibrio succinogenes* (Wolin *et al.*, 1961). The organism was isolated originally from bovine rumen fluid (Wolin *et al.*, 1961) but has also been isolated from humans with gingivitis, periodontic pockets, and lesions in alveolar bone (Wolin *et al.*, 1961; van Palenstein Helderman *et al.*, 1976; Smibert and Holdeman, 1976; Tanner *et al.*, 1981). Strains are oxidase-positive and catalase-negative (Tanner and Socransky, 1984). *W. succinogenes* can be differentiated from the other two *Wolinella* species by its ability to grow in the presence of crystal violet (0.005g/liter) or sodium fluoride (0.5g/liter) and by its failure to grow in the presence of 1% glycine (Tanner *et al.*, 1984).

Wolinella recta. This catalasen-negative species

is found in the gingival crevice of humans (Tanner *et al.*, 1981). Unlike the other two *Wolinella* species, *W. recta* is a straight rod and has a surface layer composed of hexagonal units. No growth occurs in the presence of 0.005% basic fuchsin, 0.032 % alizarine red S, or 0.01% sodium deoxycholate; these features differentiate *W. recta* from *Wolinella succinogenes* and *W. curva* (Tanner *et al.*, 1984).

Wolinella curva. These slightly curved rods have been isolated from lesions in the human oral cavity and from blood cultures (Tanner *et al.*, 1984). Growth in the presence of indulin scarlet (0.5g/liter) is useful for differentiating *W. curva* from other *Wolinella* species (Tanner *et al.*, 1984).

The two *Wolinella* species, *W. recta* and *W. curva,* have recently been transferred to the genus *Campylobacter* as *Campylobacter rectus* and *Campylobacter curvus* (Vandamme *et al.*, 1991).

The genus *Bacteroides*

The genus *Bacteroides* has been described as follows (Holdeman *et al.*, 1984): The cells are nonsporeforming anaerobic rods, gram-negative, and motile by peritrichous flagella or nonmotile. They are chemoorganotrophic and metabolize carbohydrates, peptone, or metabolic intermediates. Fermentation products of saccharoclastic species include combinations of succinate, acetate, lactate, formate or propionate. When *n*-butyrate is produced, isobutyrate and isovalerate also are present. The mol% G+C of the DNA ranges from 28 to 61. The type species is *Bacteroides fragilis.*

Of the many species of *Bacteroides,* only two species, *B. ureolyticus* and *B. gracilis,* have been shown to be closely related to the genus *Campylobacter* (Paster and Dewhirst, 1988). Historically, the classification and nomenclature for *B. ureolyticus* and *B. gracilis* have been confusing. Among the anaerobic, agar-pitting, gram-negative bacilli isolated from humans with periodontal disease, the nonmotile strains were placed in the genus *Bacteroides* and the motile strains into a group of "anaerobic vibrios", including the organisms now called *Wolinella recta, Wolinella curva,* and *Campylobacter concisus* (Tanner *et al.*, 1981; Tanner *et al.*, 1984). Initially, the name *Bacteroides corrodens* was assigned to both anaerobic and facultative, gram-negative bacilli that formed "corroding" colonies on agar surfaces (Eiken, 1958; Hill *et al.*, 1969). Jackson and Goodman (1972) renamed the facultative and the anaerobes as *Eikenella corrodens* and *Bacteroides corrodens,* respectively. Subsequently, Jackson and Goodman (1978) characterized and renamed this *Bacteroides corrodens* group as *Bacteroides ureolyticus.* The species *B. ureolyticus* and *B. gracilis* were separated from one another primarily on the basis of urease production.

Bacteroides ureolyticus. According to Jackson and Goodman (1978), *B. ureolyticus* strains are gram-negative, urease-positive, agar-corroding, anaerobic rods that lack flagella. Conventional fermentation test are negative. The organisms are oxidase-positive, catalase-negative, and assacharolytic. They possess cytochrome *b* and *c.* The urease activity of *B. ureolyticus* separates this species from other nitrate-positive and nonfermentative species (e.g. *Bacteroides gracilis, Campylobacter concisus,* and *Wolinella recta*) that use hydrogen (or formate) as an electron donor and fumarate as an electron acceptor. The mol% G+C of the DNA is in the range of 28 to 30. Strains have been isolated from face and limb lesions and from the genital tract of humans, but their pathogenicities are unknown.

Bacteroides gracilis. According to Tanner *et al.* (1981), *B. gracilis* cells are small, straight, gram-negative, nonsporeforming, unbranched cells, approximately 0.4 by 4 to 6 μm. No flagella have been observed (Lai *et al.*, 1981). Anaerobic, but some strains can grow in the presence of 5% oxygen but not in air enriched with 10% CO_2. Chemoorganotrophs; carbohydrates are neither fermented nor oxidized. Hydrogen and formate are utilized as an electron donor. Fumarate, nitrates, and nitrites are reduced. Oxidase- and catalase-negative. The G+C content of the DNA is 44 to 46 mol% (*Tm*). The organisms are found in the gingival crevice of humans. Although their pathogenicities

Table 1. Names and clinical significance of *Campylobacter, Wolinella,* and *Bacteroides* species.

Species	Pathogenicity
C. fetus	
subsp. *fetus*	Sporadic abortion in cattle and sheep; blood infections in humans; orally transmitted
subsp. *venerealis*	Sexually transmitted abortion in cattle
C. hyointestinalis	Proliferative ileitis in pigs; diarrhea in calves; occasionally causes blood infections in humans
C. jejuni	
subsp. *jejuni*	Gastroenteritis in humans; normal intestinal flora of cattle, sheep, dogs, cats and poultry
subsp. *doylei*	Isolated from gastric biopsies of adults and from the feces of children with diarrhea; its pathogenicity has not been established.
C. coli	Gastroenteritis in humans (orally transmitted); normal intestinal flora of pigs and poultry
C. lari	Intestines of sea gulls, humans, dogs, horses; occasionally causes blood infections in humans
C. mucosalis	Isolated from lesions of porcine intestinal adenomatosis; from mouths of pigs
C. concisus	Isolated from humans with periodontal disease
C. sputorum	
biovar sputorum	Normal flora of human mouth
biovar bubulus	Normal flora of bovine genitalia
biovar fecalis	Normal flora of sheep feces and bovine genitalia
C. upsaliensis	Feces of dogs, cats, and humans; from blood cultures of pediatric patients
C. pylori(H. pylori)	Gastric and duodenal ulcers in humans; from the stomach of monkeys and baboons
C. mustelae(H. mustelae)	Isolated from the stomach of ferrets; pathogenicity unknown
C. cinaedi	Found in proctitis, proctocolitis, enteritis, and bacteremia in homosexual men
C. fennelliae	Found in proctitis, proctocolitis, enteritis, and bacteremia in homosexual men
C. cryaerophila	Abortion in pigs, cattle, horses and sheep; occasionally causes blood infections in humans
C. nitrofigilis	Found in association with roots of salt marsh grasses
W. succinogenes	Isolated from bovine rumen; from humans with gingivitis, periodontitis, and lesions in alveolar bone
W. recta	Found in gingival crevice of humans
W. curva	Isolated from lesions in the human oral cavity and from blood cultures
B. ureolyticus	Isolated from face and limb lesions in humans and from the human genital tract
B. gracilis	Isolated from the gingival crevices of humans

are unknown, the organisms are associated with serious visceral or head or neck infections and anaerobic pleuropulmonary infections. Although the role of *B. gracilis* in the pathogenicity remains somewhat uncertain, the frequency of association of this species with serious anaerobic infections implies virulence (Johnson *et al.*, 1985).

Diseases associated with *Campylobacter, Woline-*

lla, and *Bacteroides* species are listed in Table 1. Phenotypic characteristics that differentiate the species are summarized in Table 2.

PHYLOGENETIC STUDIES OF THE GENUS *CAMPYLOBACTER* AND ITS RELATIVES

In the last 30 years, the development of techniques for comparing the entire genome of one organism with that of another have provided taxonomists with a powerful tool for determining the relatedness of bacteria at the species level of classification. Various methods have been used to determine the level of DNA homology, such as the direct binding method, the membrane-filter competition method, and free-solution hybridization methods (the S-1 nuclease procedure, hydroxyapatite procedure, and optical method) (Johnson, 1981; Schleifer and Stackebrandt, 1983). Homology studies have delineated the various *Campylobacter* species (Belland and Trust, 1982; Benjamin *et al.*, 1983; Harvey and Greenwood, 1983; He'bert *et al.*, 1983; Leaper and Owen, 1982; Owen, 1983; Owen and Leaper, 1981; Roop *et al.*, 1984, 1985b; Ursing *et al.*, 1983). Since these studies, new species have been designated: *Campylobacter pylori* (Marshall *et al.*, 1984, reclassified as *Helicobacter pylori* (Goodwin *et al.*, 1989); *Campylobacter mustelae,* reclassified as *Helicobacter mustelae* (Goodwin *et al.*, 1989), and *Campylobacter cinaedi* and *Campylobacter fennelliae* (Totten *et al.*, 1985). All of the *Campylobacter* and *Helicobacter* species can be differentiated by tests for various phenotypic features (Table 2); also, DNA probes have been developed for the identification of several species; for examples, see Bradbury *et al.* (1984), and Langenberg *et al.* (1986).

DNA Homology analyses, while extremely useful at the species level of classification, can not answer the question of whether these species are sufficiently related to justify classification within a single genus. This is because DNA homology values reflect the similarities between the entire genome of organisms, and only bacteria with a high degree of base sequences similarity (ca 90% or more) will show any significant level of DNA homology. Since frequent mutations occur in the bulk of the genome, DNA homology values are only meaningful when determining the relatedness between two organisms which have only recently diverged from one another (Johnson, 1984; Schleifer and Stackebrandt, 1983).

To deduce phylogenetic relationships above the level of species, one can measure the similarities and differences between certain genes whose nucleotide sequence is highly conserved compared to that for the bulk of the genome (Johnson, 1984; Schleifer and Stackebrandt, 1983). These genes, or the proteins whose amino acid sequence they code for, are called molecular chronometer. Initially, the amino acid sequence of cytochrome *c* was used as a molecular chronometer. However, because cytochrome *c* undergoes rapid evolution and because cytochrome *c* dose not occur in all bacteria, its usefulness is limited in the study of bacterial phylogeny (Woese, 1985). In contrast, ribosomal ribonucleic acid (rRNA) molecules offer many advantages as molecular chronometers: (i) rRNA molecules are essential elements of the cellular transitional apparatus and are therefore functionally and evolutionarily homologous in all organisms; (ii) their nucleotide sequence is moderately to highly conserved across kingdoms; (iii) rRNA constitutes a significant fraction of the cellular mass and is easily isolated; (iv) rRNA can provide sufficient data to be statistically significant in phylogenetic analysis; and (v) there appears to be no lateral transfer of rRNA genes between contemporaneous organisms (Woese, 1985; Olsen *et al.*, 1986). All bacterial cells contain 5S, 16S, and 23S rRNA. Since 5S rRNA is a relatively small molecule (approximately 120 nucleotides, most of which are highly conserved), 16S rRNA has been widely used instead. It can provide a large nucleotide sequence data base (approx. 1600 nucleotides), and base changes within the 16S rRNA molecule vary in frequency in different positions wi-

Table 2. Differential characteristics of *Campylobacter, Wolinella,* and *Bacteroides species*[a].

Species, subspecies, biovar	Oxidase	Catalase	Flagella present	Curved cells	Growth in air atmosphere on complex media	Growth at 15℃	Growth at 25℃	Growth at 42℃	Hippurate	Nitrate reduced to nitrite	Nitrite reduced	Suceptible to nalidixic acid[b]	Suceptible to cephalothin[b]	Urease
C. fetus														
subsp. *fetus*	+	+	+	+	−	−	+	d	−	+	−	−	+	−
subsp. *venerealis*	+	+	+	+	−	−	+	−	−	+	−	−	+	−
C. hyointestinalis	+	+	+	+	−	−	+	+	−	+	−	−	+	
C. jejuni														
subsp. *jejuni*	+	+	+	+	−	−	−	+	+	+	−	+	−	−
subsp. *doylei*	+	d	+	+	−	−	−	W	+	−		+	+	
C. coli	+	+	+	+	−	−	−	+	−	+	−	+	d	−
C. lari	+	+	+	+	−	−	−	+	−	+	−	−	−	
C. mucosalis	+	−	+	+	−	−	+	+	−	+	d	d	+	−
C. concisus	+	−	+	+	−	−	−	+	−	+	+	−	−	−
C. sputorum														
biovar sputorum	+	−	+	+	−	−	−	+	−	+	d	d	+	−
biovar bubulus	+	−	+	+	−	−	−	+	−	+	d	d	+	−
biovar fecalis	+	+	+	+	−	−	−	+	−	+	+	−	+	−
C. upsaliensis	+	Wor-	+	+	−	−	−	+	−	+		+	+	−
C. pylori(H. pylori)	+	+	+	+	−	−	−	d	−	−		d	+	+
C. mustelae(H. mustelae)	+	+	+	+	−	−	−	+	−	+		+	−	+
C. cinaedi	+	+	+	+	−	−	−	−	−	+	−	+	+	
C. fennelliae	+	+	+	+	−	−	−	−	−	−	−	+	+	
C. cryaerophila	+	+	+	+	+	+	+	−	−	+	+	d		
C. nitrofigilis	+	+	+	+	+	+	+	−	−	d	−	+	+	d
W. succinogenes	+	−	+	+	−	−	−	+ or-		+	+			−
W. recta	+	−	+	−	−					+	+			−
W. curva	+	−	+	+	−					+	+			-
B. ureolyticus	+	−	−	−	−					+	-			+
B. gracilis	−	−	−	−	−					+	+			−

Table 2 continued

Species, subspecies, biovar	Growth in presence of:									H_2 or formate required as electron donor	Indoxyl acetate hydrolysis	Alkaline phosphatase	Anaerobic growth with TMAO, 0.1%
	Glycine, 1%	Oxgall, 1%	NaCl, 3.5%	Crystal violet, 0.0005%	Indulin scarlet, 0.05%	Basic fuchsin, 0.005%	Sodium deoxycholate, 0.1%	Methyl orange, 0.025%	Alizarine Red S, 0.032%				
C. fetus													
subsp. *fetus*	+	+	−							−	−	−	−
subsp. *venerealsi*	−	−	−							−	−	−	−
C. hyointestinalis	+		−							−	−	−	+
C. jejuni													
subsp. *jejuni*	+	+	−							−	+	+	−
subsp. *doylei*	+	+								−	+	+	−
C. coli	+	+	−							−	+	d	−
C. lari	+		−							−	−	−	+
C. mucosalis	+	+	−							+	−	−	−
C. concisus	+	+	−	+		+	+	+	+	+	−	−	−
C. sputorum													
biovar sputorum	+	+	−	−		+	+	+	+	−	−	−	d
biovar bubulus	+	−								−	−	−	+
biovar fecalis	+	+	−							−	−	−	+
C. upsaliensis	d	d	−							−	+	+	−
C. pylori(*H. pylori*)	−										−		
C. mustelae(*H. mustelae*)	d										+	+	
C. cinaedi	+		−							+	−		
C. fennelliae	+		−							+	+		
C. cryaerophila	−		−				−			−	+	−	−
C. nitrofigilis	−	d	+							−	−		
W. succinogenes	−	+	−	+	−	+	+	+	+	+	−	−	
W. recta	+	−		−	−	−	−	−	−	+	+		
W. curva	+	+	−	−	+	+	+	+	+	+	+		
B. ureolyticus		d		−		−	d	−	+	+			
B. gracilis		d		d		+	+	+	+	+			

Table 2 continued

Species, subspecies, biovar	H_2S production		Nitrogenase activity, microaerobic conditions	Lipase (C_8)	Mol% G+C of DNA
	SIM medium[c]	TSI slants[c]			
C. fetus					
subsp. *fetus*	−	−	--		33-34
subsp. *venerealis*	−	−	--		33-34
C. hyointestinalis	−	+	--		35-36
C. jejuni					
subsp. *jejuni*	−	−	--		30-32
subsp. *doylei*		−	--		29
C. coli	−	+	--		31-33
C. lari	−	−	--		31-33
C. mucosalis	+	+	--		38-39
C. concisus	+	+	--	−	34-38
C. sputorum					
biovar sputorum	d	d	--		31-32
biovar bubulus	+	+	--		31-32
biovar fecalis	+	+	--		32-33
C. upsaliensis		−	--		35-36
C. pylori(*H. pylori*)		−	--	+	36-37
C. mustelae(*H. mustelae*)		−	--	+	36-41
C. cinaedi		−	--		37-38
C. fennelliae		−	--		37-38
C. cryaerophila	−	−	--		29-30
C. nitrofigilis		+	+		28-29
W. succinogenes	+		--	−	46-49
W. recta	+		--	−	42-46
W. curva	+		--	−	43-47
B. ureolyticus			--		28-30
B. gracilis	+		--	−	44-46

[a]Symbolis: +, 90% or more strains positive; −, 10% or fewer positive; d, 11-89% of strains positive; W = weakly positive.

[b]30 μg disk.

[c]Abbreviations: TMAO, trimethylamine oxide; SIM, sulfide-indole-motility medium; TSI, triple sugar iron agar (in water of syneresis at base of slant).

thin the molecule (Olsen *et al.*, 1986; Woese, 1985). According to Lane *et al.* (1985), determination of the entire sequence of 16S rRNA is not necessary in phylogenetic analysis because a partial sequence can reflect the evolutionary information contained within the entire sequence. Comparison of 16S rRNA sequences for deduction of phylogenetic relationships has proven to be a powerful tool for the accurate classification of microorganisms above the level of species (Fox *et al.*, 1980; Olsen *et al.*, 1986; Stackebrandt, 1985; Stackebrandt and Woese, 1984; Woese, 1985; Woese, 1987). However, it must be remembered that the phylogenetic relationships that have been deduced for bacteria are based mainly on this single molecular chronometer. Before the present evolutionary schemes

can be considered completely reliable, it is necessary to confirm that code for the subunits of RNA polymerases or ATPases might be good candidates.

With regard to rRNA, comparisons between organisms have been done by rRNA/DNA hybridization experiments or by determining and comparing actual nucleotide sequences of rRNA. The latter method is particularly useful because it allows the establishment of an extensive data base to which new information can be added. The development of a technique which facilitated the rapid generation of partial 16S rRNA sequences has been widely used to determine the phylogenetic relationships among bacteria (Lane *et al.*, 1985). This method is based on the use of reverse transcriptase to synthesize DNA complementary to the rRNA, followed by routine DNA sequencing. More recently, the development of the polymerase chain reaction (PCR) technique allows generation of large amounts of DNA directly from rRNA cistrons in an organism's DNA, and it is no longer necessary to use reverse transcriptase to obtain the DNA (Weisburg *et al.*, 1991).

Using the nucleotide sequencing procedure described by Lane *et al.* (1985), Romaniuk *et al.* (1987) derived partial 16S rRNA sequences from *C. jejuni, C. coli, C. lari, C. fetus* subsp. *fetus, C. sputorum* biovar sputorum, and *C. pylori.* These sequences were then compared to several others which had been previously published. The results showed that *C. pylori,* although related to other campylobacters, is related closely enough to be included in the genus. *C. jejuni, C. coli, C. fetus* subsp. *fetus, C. lari* or *C. sputorum* biovar sputorum, and these latter species represented the "true" genus *Campylobacter,* whereas *C. pylori* was more closely related to *Wolinella succinogenes* than to the true campylobacters.

Based on comparison of partial 16S rRNA sequences, Lau *et al.* (1987) reported that *C. jejuni, C. coli,* and *C. lari* were very closely related, that the *C. jejuni-C. coli-C. lari* group, *C. fetus, C. sputorum* biovar fecalis, and *C. pylori* were related, *C. pylori* was only distantly related to the other campylobacters, and *W. succinogenes* was even more distantly related, with its nearest neighbor being *C. pylori.*

Paster and Dewhirst (1988) and Paster *et al.* 1991) similarly found that *C. jejuni, C. coli,* and *C. lari* were very closely related and that this group was in turn related to *C. fetus, C. concisus,* and *C. sputorum. C. pylori* and *W. succinogenes* formed a second group that was only distantly related to other *Campylobacter* species. The most interesting finding in this study, however, was that the anaerobes *Wolinella recta, Wolinella curva, Bacteroides gracilis,* and *Bacteroides ureolyticus* belonged to the same cluster as the true campylobacters. *W. succinogenes* was related more closely to *Helicobacter* than to the true *Campylobacter* group.

Thompson *et al.* (1988) performed the most comprehensive phylogenetic study of the genus *Campylobacter* based on comparison of partial 16S ribosomal RNA sequences. They included all of the *Campylobacter* species, including *C. cryaerophila* and *C. nitrofigilis, C. hyointestinalis, C. mucosalis, C. upsaliensis, C. cinaedi,* and *C. fennelliae,* none of which had been studied previously. *W. succinogenes* was also included. The results indicated that the species previously classified in the genus *Campylobacter* make up three separate rRNA sequence homology groups. Group I contains the true *Campylobacter* species, viz., *C. fetus, C. jejuni, C. coli, C. lari, C. hyointestinalis, C. concisus, C. mucosalis, C. sputorum,* and *C. upsaliensis.* On the other hand, *C. cinaedi, C. fennelliae, C. pylori,* and *W. succinogenes* constitute group II. Group III contains *C. cryaerophila* and *C. nitrofigilis.* The three groups were considered by Thompson *et al.* (1988) to represent separate genera.

Vandamme *et al.* (1991) performed rRNA/DNA hybridizations using the 23S rRNA of various campylobacters and related organisms concluded that the organisms belonged to three rRNA clusters. These clusters were similar to the rRNA groups previously delineated by other authors

from 16S rRNA sequence analyses.

PROBLEMS ARISING FROM THE PHYLOGENETIC CLASSIFICATION OF THE GENUS *CAMPYLOBACTER* AND ITS RELATIVES

According to the results of Paster and Dewhirst (1988) combined with those of Thompson *et al.* (1988). rRNA goup I contains the true campylobacters (*C. fetus, C. hyointestinalis, C. jejuni, C. coli, C. lari, C. upsaliensis, C. sputorum, C. mucosalis,* and *C. concisus*), as well as *W. recta, W. curva, B. ureolyticus,* and *B. gracilis.* Group II contains *W. succinogenes, C. pylori, C. cinaedi,* and *C. fennelliae. C. cryaerophila* and *C. nitrofigilis* constitute rRNA group III.

Despite the clear phylogenetic separation of these three groups, it is not yet possible to define them on the basis of easily determinable phenotypic characteristics. As indicated by Krieg (1988), fundamental questions about phylogeny vs. practicality have arisen in bacterial taxonomy because many groups based on rRNA analysis have not been easily definable in terms of phenotypic similarities. These difficulties could lead to the unattractive idea of having two separate classification systems, a practical one based on phenotypic characteristics and a more esoteric one based on phylogeny. Yet at least some of these difficulties may be due to the fact that organisms which have been found to be related to one another by rRNA analysis may not have been characterized phenotypically by comparable methods or tests.

The phylogenetic relationship of *W. recta, W. curva, B. gracilis,* and *B. ureolyticus* to the true campylobacters is especially puzzling. These four species do resemble campylobacters in some respects: (i) like campylobacters, *W. recta* and *W. curva* are motile by polar flagella; (ii) *W. recta, W. curva,* and *B. ureolyticus* are oxidase-positive; (iii) *W. curva* has a vibroid shape; and (iv) like some campylobacters (*C. concisus, C. mucosalis, C. cinaedi,* and *C. fennelliae*), *W. recta, W. curva, B. gracilis,* and *B. ureolyticus* all require H_2 or formate as an electron donor. However, there are some important differences: (i) *W. recta, B. ureolyticus,* and *B. gracilis* are straight rods; (ii) *B. ureolyticus* and *B. gracilis* are nonmotile; and (iii) *B. gracilis* is oxidase-negative. The most important difference, however, is that, unlike the true campylobacters, *W. curva, W. recta, B. ureolyticus,* and *B. gracilis* are all considered to be anaerobes. However, their phylogenetic placement with the campylobacters, as well as the fact that three of the species (*W. recta, W. curva,* and *B. ureolyticus*) are oxidase-positive, suggests that they might actually be microaerophiles like the campylobacters. This would make their inclusion with the true campylobacters much more satisfying in phenotypic terms. Some previous reports suggest that this may be the case. Tanner *et al.* (1981) reported that some strains of *W. recta, W. curva,* and *B. gracilis* could grow under microaerobic conditions and it has cytochrome *b* and cytochrome *c.* Han *et al.* (1991, 1992) concluded that *W. recta, W. curva, B. ureolyticus,* and *B. gracilis* all are microaerophiles which respire with O_2 as the electron acceptor and which also possess membrane-bound cytochrome *b* and *c*, and soluble cytochrome *c.*

As mentioned previously, Group II contains *C. pylori, C. cinaedi, C. fennelliae,* and *W. succinogenes. W. succinogenes* is the type species of the genus *Wolinella,* and since *W. recta* and *W. curva* are not closely related to it and must be removed from the genus, *W. succinogenes* remains as the sole member of the genus *Wolinella.* Although Thompson *et al.* (1988) indicated that if Group IV is considered to be a distinct genus, it would have to bear the name *Wolinella,* with *C. pylori, C. cinaedi,* and *C. fennelliae* becoming *W. pylori, W. cinaedi, W. fennelliae.* However, mainly because of a desire to group the gastric organisms *C. pylori* and *C. mustelae* together in a separate genus, the proposal to consider Group IV as a single genus met with resistance, and in 1989, Goodwin *et al.* created a new genus, *Helicobacter,* for *C. pylori* and *C. mustelae.* Thus a number of taxonomic pro-

Table 3. The validated or proposed genus and species names in the genus *Campylobacter* and its relatives by rRNA sequence homology and rRNA/DNA hybridization studies.

Genus	Species	Remarks
Campylobacter	*C. fetus* subsp. *fetus*	
	subsp. *venerealis*	
	C. hyointestinalis	
	C. jenuni subsp. *jejuni*	
	subsp. *doylei*	new subspecies
	C. coli	
	C. lari	*C. laridis*[sic]
	C. mucosalis	*C. sputorum* subsp. *mucosalis*
	C. concisus	
	C. sputorum	
	C. upsaliensis	CNW strains
	C. rectus	*W. recta*
	C. curvus	*W. curva*
Wolinella	*W. succinogenes*	
Helicobacter	*H. pylori*	*C. pylori, C. pyloridis*[sic]
	H. mustelae	*C. mustelae*
	H. cinaedi	*C. cinaedi*
	H. fennelliae	*C. fennelliae*
	H. felis	new species
	H. nemestrinae	new species
Arcobacter	*A. cryaerophilus*	*C. cryaerophila*[sic]
	A. nitrofigilis	*C. nitrofigilis*
	A. butzleri	*C. butzleri,* new species

blems remain to be solved: *C. cinaedi* and *C. fennelliae* still bear the name Campylobacter even though they do not belong to that genus; *W. succinogenes* remains the sole species in the genus *Wolinella,* since it is unrelated to *W. recta, W. curva*; and *W. recta* and *W. curva* continue to bear the name *Wolinella,* even though they should be removed from that genus.

The relatedness of *W. succinogenes, C. pylori, C. cinaedi,* and *C. fennelliae* is puzzling because, according to *Bergey's Manual* of *Systematic Bacterology, W. succinogenes* is an anaerobe (Tanner and Socransky, 1984). However, Wolin *et al.* (1961) clearly showed that *W. succinogenes* (then called "*Vibrio succinogenes*") was oxidase-positive and was capable of using O_2 as a terminal electron acceptor under microaerobic conditions (approx. 2% O_2), but not under atmospheric levels of O_2. These findings, along with additional evidence on the electron transport system (Jacobs and Wolin, 1963a, 1963b), indicate that *W. succinogenes* is not an anaerobe but instead a H_2-requiring microaerophile. *C. pylori, C. cinaedi,* and *C. fennelliae* are microaerophilic and grow best in microaerobic atmospheres containing H_2; they are catalase-positive, H_2S-negative, and have a mol% G+C of 37 to 38.

Group III consists of *C. cryaerophila* and *C. nitrofigilis,* which are related by a rRNA sequence homology value of 86.9%. In view of the fact that *C. cryaerophila* is an aerobe that causes abortion in pigs and other animals and occasionally causes blood infections in humans, whereas *C. nitrofigilis* is a microaerophilic, NaCl-requiring nitrogen-fixer

associated with the roots of marsh grasses, it is difficult to arrive at a phenotypic definition of a genus represented by these two species. However, now that the relationship between the two species is known, further characterization studies might reveal unifying phenotypic similarities. For instance, both species can grow at temperatures as low as 6℃, unlike other campylobacters (McClung and Patriquinn, 1980; McClung *et al.*, 1983). Moreover, it is interesting that 80% of the *C. nitrofigilis* strains characterized by McClung *et al.* (1983) exhibited urease activity, and 75% of the strains could grown in 1% bile. Since urea is a waste product of animals, this suggests that the habitat of this species might not be, or have been, limited only to marsh grass roots.

CONCLUDING REMARKS

In bacterial taxonomy, ribosomal ribonucleic acid (rRNA) analyses have been widely used for deducing phylogenetic relationships. However, these studies have frequently led to strange groups, i.e., groupings that include organisms which are seemingly very dissimilar in their phenotypic characteristics. Because of such groupings, bacterial genera and broader groups that have been delineated in phylogenetic terms have often been difficult to describe in phenotypic terms. This has raised questions about the practical usefulness of the phylogenetic groups. No where has this been more true than in the case of the proteobacteria--a large group that includes diverse gram-negative eubacteria. For instance, phototrophic organisms such as purple bacteria are mixed in with various nonphototrophic organisms. Such arrangements suggest that classification of bacteria based on phylogenetic relationships might be mainly an esoteric exercise that has little relevance to ordinary benchtop microbiology. It is more likely, however, that underlying phenotypic similarities may not have been recognized within a group of apparently dissimilar organisms. This might be due to the fact that apparently dissimilar organisms often have not been compared on the same basis. The problem of reconciling practicality with phylogeny is certainly one of extreme importance to bacterial taxonomy.

As shown in Table 3, all the species of the genus *Campylobacter* and its relatives might be reclassified as three separate rRNA homology groups (or four genera in phenotypic terms) by rRNA sequence analyses (Lau *et al.*, 1987; Paster and Dewhirst, 1988; Paster *et al.*, 1991; Romaniuk *et al.*, 1987; Thompason *et al.*, 1988), rRNA/DNA hybridization experiment (Vandamme *et al.*, 1991), and inclusions of new species of *Helicobacter felis, Helicobacter nemestrinae,* and *Arcobacter butzleri* (Bronsdon *et al.*, 1991; Kiehlbauch *et al.*, 1991; Paster *et al.*, 1991).

At present, easily determinable phenotypic characteristics needed to clearly differentiate the four genera are not apparent, although the genus *Helicobacter* can be differentiated from other genera on the basis of the occurrence of sheathed flagella (Han *et al.*, 1989) and the genus *Arcobacter* does on the basis of growth at low temperature or under aerobic condifions (other genera showed microaerophilic growth; Han *et al.*, 1991, 1992). So far, *B. ureolyticus* and *B. gracilis* have not been included into the genus *Campylobacter,* because of the difficulty in phenotypic terms; their straight-rod cell shape and no flagellation are different to those of the genus *Campylobacter.* The difficulty in arriving at suitable, mutually exclusive phenotypic descriptions of the genera and some species represented by four genera can be attributed to the fact that all the organisms have not been compared by the same tests and methods. Now that the relationships among the genus *Campylobacter* and its relatives have been delineated, it is to be hoped that unique differential phenotypic features of each genus represented by three rRNA sequence homology groups or the four genera can be discovered.

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