

## Cellular Fatty Acid Composition of *Campylobacter pylori* from Primates and Ferrets Compared with Those of Other *Campylobacters*

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The cellular fatty acid profiles of newly described campylobacters were determined on a polar, capillary column. Six isolates of the gastric spiral organism, *Campylobacter pylori* subsp. *mustelae*, from ferrets from Australia, England, and the United States were all found to have a similar fatty acid profile which was different from that of *C. pylori* from humans; *C. pylori* subsp. *mustelae* did not have 3-hydroxyoctadecanoic acid (3-OH C<sub>18:0</sub>) and had much less tetradecanoic acid (C<sub>14:0</sub>) and much more hexadecanoic acid (C<sub>16:0</sub>). Inasmuch as Lambert et al. (M. A. Lambert, C. M. Patton, T. J. Barrett, and C. W. Moss, J. Clin. Microbiol. 25:706-713, 1987) have proposed that campylobacters can be grouped by cellular fatty acid composition, we propose this organism should be in a new gas-liquid chromatography (GLC) group, group J. Seven isolates of gastric spiral organisms from macaque monkeys and baboons, including three from *Macaca nemestrina*, and one isolate from a pig were found to have fatty acid profiles very similar to that of *C. pylori*; but a second type of organism (type B) from *M. nemestrina* had a unique profile without 19-carbon cyclopropane fatty acid (C<sub>19:0 cyc</sub>) but with 3-hydroxy tetradecanoic acid (OH C<sub>14:0</sub>), which is not present in other gastric spiral bacteria. We propose that this organism (*nemestrina* type B) should be in a new GLC group, group K. The cellular fatty acid profile of seven isolates of *C. jejuni* subsp. *doylei* was found to be similar to that for *C. jejuni*, but with possibly significant differences in that the former did not have 3-OH C<sub>14:0</sub> but did have 3-hydroxyhexadecanoic acid (3-OH C<sub>16:0</sub>) and had more C<sub>14:0</sub> than did *C. jejuni*. Two strains of urease-positive thermophilic campylobacters were found to have a profile similar to that of "*C. cinaedi*" and thus should be included with them in GLC group D. We confirm that *C. sputorum* has a unique cellular fatty acid composition and suggest that it should be in a new group, group H.

The human stomach spiral organism *Campylobacter pylori*, first isolated in 1982 in Western Australia (12), has a distinct cellular fatty acid composition that is different from those of other campylobacters (7, 10). Since then, similar spiral bacteria have been isolated from the stomachs of ferrets (4), rhesus macaques (*Macaca mulatta*) (16), pig-tailed macaques (*Macaca nemestrina*) (1), a baboon (*Papio* sp.), and a pig (8). In Perth, we have isolated a gastric spiral organism from a crab-eater macaque (*Macaca fascicularis*). One or more of these animals could become a useful model for human duodenal ulcer disease, and therefore these animal spiral bacteria should be studied carefully. Cellular fatty acids have taxonomic significance (9, 11), and so the fatty acid profiles of these animal stomach organisms could be useful in indicating how closely related they are to *C. pylori*.

From the stomachs of humans in Germany, another campylobacter-like organism has been isolated, which is termed gastric campylobacter-like organism 2 (G. Kasper and N. Dickgiesser, Lancet i:111, 1985) and which has now been named *Campylobacter jejuni* subsp. *doylei* (18). We have studied the fatty acid profiles of seven isolates of this organism.

In a major review of the cellular fatty acids of campylobacters, Lambert et al. (10) defined seven gas-liquid chromatography (GLC) groups of campylobacters (groups A

through G) by cellular fatty acid composition, but they did not analyze *C. jejuni* subsp. *doylei*, the gastric spiral bacteria from animals, the urease-positive thermophilic campylobacters (UPTC) which have been found in rivers by Bolton et al. (F. J. Bolton, A. V. Hold, and D. N. Hutchinson, Lancet i:1217-1218, 1985), or *C. sputorum*. We report here the cellular fatty acid compositions of all these bacteria and indicate that some of them constitute new GLC groups.

### MATERIALS AND METHODS

**Cultures.** *C. jejuni* NCTC 11351 and *C. jejuni* subsp. *doylei* NCTC 11847, 11848, and 11849 were obtained from the National Collection of Type Cultures, London, United Kingdom; the latter were cultured from endoscopic biopsy specimens of the human gastric mucosa in Germany by one of us (G.K.). We also analyzed four other strains of *C. jejuni* subsp. *doylei* also isolated in Germany. *C. pylori* NCTC 11637 was isolated by us in Royal Perth Hospital, Perth, Western Australia. The type stains of "*C. cinaedi*" and "*C. fennelliae*" were obtained from homosexuals in Seattle (20) and were supplied by C. L. Fennell (Seattle, Wash.). Two strains of UPTC were supplied by F. J. Bolton (Preston, United Kingdom). These organisms were identified by standard methods already described (14, 17). One isolate of the ferret gastric organism (FP1) was obtained by us in Western Australia, other isolates (FM1 and FM2) were supplied by D. M. Jones (Manchester, United Kingdom) and Diane Newell (Porton, Down, United Kingdom) (FPD1), and iso-

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late ATCC 47332 was supplied by Jim Fox (Boston, Mass.). These organisms were identified as described by Fox et al. (4). Two isolates of gastric spiral organisms from *M. mulatta* (RPD2, RPD3) were also supplied by D. Newell; she had identified them as *C. pylori* (16). Four isolates of gastric spiral bacteria were isolated from *M. nemestrina* (NAS1, NAS2, NAS3, NBS1) by one of us (M.A.B.) in Seattle, Wash., and identified as *C. pylori* (1). A spiral gastric organism from a baboon (BM1) and one from a pig (PM1) were kindly provided by D. M. Jones, and have been found to have an identical ultrastructure to that of *C. pylori* (8). In Perth, we have isolated spiral bacteria from endoscopic specimens of the gastric mucosa from *M. fascicularis* (CP1), *M. nemestrina*, and *Papio* sp. We have found these organisms to have similar biochemical reactions to those of *C. pylori* and an identical ultrastructure. All the gastric spiral bacteria except *C. jejuni* subsp. *doylei* were urease positive. The organisms were cultured on plates of brain heart infusion agar (Oxoid) with 7% lysed horse blood–1% IsoVitaleX (BBL Microbiology Systems), incubated at 35 to 37°C for 24 to 72 h in an atmosphere of approximately 5% O<sub>2</sub>–7% CO<sub>2</sub>–7% H<sub>2</sub>–81% N<sub>2</sub> (6), and identified by standard methods (14, 17).

**Preparation of FAMES.** The strains of bacteria were cultured for 2 to 4 days on lysed blood agar plates, but only if a heavy, healthy growth was obtained were the cells harvested and processed. Bacteria from old or poorly growing cultures tended to give variable results and so were discarded. Bacteria from three plates were harvested into 10 ml of 0.3 M phosphate buffer (pH 7.3) and centrifuged at 5,000 × *g* for 10 min; the pellet was then washed twice in phosphate buffer. The pellet was suspended in 1 ml of 1 M sodium hydroxide and heated at 85°C for 30 min. The fatty acids liberated by this saponification were then methylated by adding 3 ml of 4% sulfuric acid in methanol (pH <2) and heating at 78°C for 15 min. The samples containing the cellular fatty acid methyl esters (FAMES) were then cooled to room temperature, and 3 ml of saturated sodium chloride was added. The methyl esters were extracted with 3 ml of hexane, and the extract was concentrated to 0.2 ml under a stream of nitrogen. The samples were stored at –20°C until they were analyzed by gas chromatography.

The FAME samples were analyzed on a Hewlett-Packard 5890A gas chromatograph that was fitted with a flame ionization detector and a 3393A Hewlett-Packard integrator. Separation was achieved on a Superox-20M capillary column (0.53 mm by 10 m), using a column flow of 2.6 ml per min, with the injector at 250°C (to ensure efficient vaporization), the detector at 250°C, and the oven temperature at 60°C for 1 min and then increased by 5°C per min to 200°C, which was maintained for 10 min. Identification of major peaks was made by comparison with the retention time of known standards and by mass spectroscopy. The area under each peak was calculated by the integrator to determine the percentage of each fatty acid in the sample. Extractions from the various isolates were replicated three times to ensure that the results were reproducible. We used a long temperature program to ensure that all relevant cellular fatty acids were detected. It should be noted that our column was polar, and the resulting separation of the FAMES depended not only on molecular weight but also on the interaction between the column matrix and hydrophilic groups on the fatty acids. Our column differed from the fused silica column coated with Ov-101 used by Lambert et al. (10), which was a nonpolar column; FAMES on such a column are separated primarily by molecular weight.

## RESULTS

The cellular fatty acid compositions of seven isolates of *C. jejuni* subsp. *doylei* are shown in Table 1. It can be seen that the profiles do not contain 3-hydroxytetradecanoic acid (3-OH C<sub>14:0</sub>) which was a cardinal feature of the Lambert et al. GLC group A (10) which contained *C. jejuni* and *C. coli*. In six of seven strains of *C. jejuni* subsp. *doylei*, we found approximately 7% of linoleic acid (C<sub>18:2</sub>). In Tables 1 and 2, it can be seen that in all the campylobacters we found C<sub>18:2</sub> in amounts between 2 and 13%.

In Table 1, we also show the profiles of three isolates of *C. sputorum* which were not reported by Lambert et al. (10). We also show the profiles of five strains of “*C. cinaedi*” and three of “*C. fennelliae*.” In Table 1, the profiles of the two strains of UPTC are seen to be similar to those of “*C. cinaedi*”.

In Table 2, we show the profiles by our new method of four strains of *C. pylori*. We confirm the presence of 3-OH C<sub>18:0</sub> (Fig. 1).

The fatty acid profile of *Wolinella succinogenes* is shown in Table 2 to indicate how different it is from that of *C. pylori*. The profile of *W. succinogenes* has a very small quantity of C<sub>14:0</sub> and a high percentage of C<sub>16:1</sub>, with no 3-OH C<sub>18:0</sub> or C<sub>19:0</sub> cyc.

In Table 2, we show the profiles of gastric spiral bacteria from the ferret, three macaques (*M. mulatta*, *M. nemestrina*, *M. fascicularis*), baboons (*Papio* sp.), and a pig compared with that of *C. pylori*. The ferret organism, *C. pylori* subsp. *mustelae* (5) differs markedly from *C. pylori*, with a lower percentage of C<sub>14:0</sub> (14 to 17%), the presence of a small amount of C<sub>15:0</sub>, a large amount of C<sub>16:0</sub>, and without 3-OH C<sub>18:0</sub> (Fig. 2). We propose that this is a new GLC group, group J, because the organism has C<sub>19:0</sub> cyc, which is found only in *C. pylori* and *C. jejuni*, but unlike *C. jejuni* the ferret organism has C<sub>15:0</sub> and a greater amount of C<sub>14:0</sub>. It does not contain 3-OH C<sub>18:0</sub>, which is unique to *C. pylori*.

There is one unusual gastric spiral organism in Table 2, which we have designated as type B from *M. nemestrina*, which has no C<sub>19:0</sub> cyc but has 3-OH C<sub>14:0</sub> and 4% of C<sub>15:0</sub>. Although it is similar to “*C. cinaedi*,” it does not have any 3-OH C<sub>16:0</sub> and therefore we suggest that it is a new GLC group, group K. It also has different colonial appearances from other gastric spiral bacteria; irregular fingerlike projections are seen from the edges of the colonies. However, it is strongly urease-positive like other gastric bacteria.

The isolates from *Papio* sp. and the pig are very similar to *C. pylori* but have less C<sub>18:1</sub> than *C. pylori* and only a trace of 3-OH C<sub>18:0</sub>. If this is confirmed, it is possible they represent a new GLC group. Two isolates from *M. mulatta* and the nemestrina type A isolates from *M. nemestrina* (NAS1, NAS2, NAS3) have very similar profiles, with a somewhat lower percentage of C<sub>14:0</sub> than *C. pylori* (27 to 31%) and slightly more 3-OH C<sub>16:0</sub> (8 to 14%). We have also isolated this latter type of organism from two other *M. nemestrina* in Australia and the baboon organism from another *Papio* sp.

## DISCUSSION

Lambert et al. (10) stressed the usefulness of cellular fatty acids for the differentiation of *Campylobacter* strains, and we strongly support this conclusion. We have used a saponification step in our extraction protocol to ensure the detection of 3-OH C<sub>18:0</sub>; with a carrier gas flow of 2 to 3 ml per min we have found it easy to detect 3-OH C<sub>18:0</sub>. We compared

TABLE 1. Cellular fatty acid compositions of *Campylobacter* species

Species <sup>a</sup>	Amt <sup>b</sup> of indicated compound <sup>c</sup>											
	C <sub>12:0</sub>	C <sub>14:0</sub>	3-OH C <sub>14:0</sub>	C <sub>15:0</sub>	C <sub>16:0</sub>	C <sub>16:1</sub>	3-OH C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	3-OH C <sub>18:0</sub>	C <sub>19:0</sub> cyc
<i>C. jejuni</i> subsp. <i>doylei</i>												
NCTC 11847	T	10	—	—	33	T	2	T	32	3	—	9
NCTC 11848	T	10	—	—	32	T	2	T	32	7	—	9
NCTC 11849	T	15	—	—	35	T	T	T	29	7	—	8
180	T	15	—	—	32	T	2	2	29	8	—	10
251	T	15	—	—	33	T	T	T	24	8	—	10
262	T	16	—	—	31	T	2	2	26	7	—	7
345	T	10	—	—	36	T	T	T	31	6	—	4
<i>C. jejuni</i>												
NCTC 11351		5	6	T	30	4	—	4	29	3	—	15
<i>C. sputorum</i> subsp. <i>mucosalis</i>												
NCTC 11000	19	6	5	—	19	11	—	T	20	13	—	—
<i>C. sputorum</i> subsp. <i>bubulus</i>												
NCTC 11367	14	8	6	—	26	8	—	T	25	12	—	—
<i>C. sputorum</i> subsp. <i>sputorum</i>												
NCTC 11528	14	9	7	—	27	8	—	T	24	10	—	—
“ <i>C. cinaedi</i> ”												
NCTC 11611	T	9	—	T	37	—	T	T	42	3	—	—
77	T	9	—	T	32	—	T	2	43	5	—	—
117	T	9	—	T	35	—	T	T	44	3	—	—
588	T	8	—	T	35	—	T	T	46	T	—	—
593	T	6	—	T	34	—	T	T	45	4	—	—
“ <i>C. fennelliae</i> ”												
NCTC 11612	T	7	T	3	26	—	3	2	34	6	—	—
915	3	9	T	3	27	—	3	4	25	7	—	—
1138	T	8	T	5	28	—	4	T	32	7	—	—
UPTC												
UPTC 1	T	2	—	—	31	4	4	3	44	7	—	—
UPTC 2	T	3	—	—	32	3	—	2	45	3	—	—
<i>C. fetus</i> subsp. <i>fetus</i>	T	7	T	T	22	25	2	T	30	8	—	—

<sup>a</sup> ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

<sup>b</sup> Values are percentages of total fatty acids and are arithmetic means; —, not detected or less than 0.5%; T, 0.5 to 1.9%.

<sup>c</sup> Number before the colon is the number of carbon atoms; number after the colon is the number of double bonds; cyc, cyclopropane fatty acid; 3-OH, hydroxy group at carbon 3.

our extraction method with other methods, including that of Moss et al. (15); we found that our method achieved the highest concentrations of fatty acids. However, the major difference between our technique and that of Lambert et al. (10) is that we used a polar capillary column, so that the percentages of fatty acids in our profiles may be slightly different from those of Lambert et al. (10). For example, they found 8% of 3-OH C<sub>18:0</sub> in *C. pylori*, whereas we have not found more than 3% in the isolates studied by our new method.

The composition of *C. sputorum* has been reported by Curtis (2), and our profile is similar to his, except that we detected C<sub>18:2</sub>, as did Moss et al. (15). Lambert et al. (10) did not report the profile of *C. sputorum*, and we consider that this organism is a separate GLC group because it possesses a distinctively high percentage (14%) of dodecanoic acid (C<sub>12:0</sub>) and C<sub>18:2</sub> (10 to 12%); 3-OH C<sub>14:0</sub> is present, but 3-OH C<sub>16:0</sub> and 19-carbon cyclopropane fatty acid (C<sub>19:0</sub> cyc) are absent. Thus, we propose that this organism represents GLC group H. Moss et al. (15) reported the presence of 3-OH

C<sub>16:0</sub> in *C. sputorum*, but we did not detect this, even when we used his extraction method.

The gastric spiral bacteria isolated from ferrets in England, Australia, and the United States all showed very similar profiles and differ from those of any other campylobacters previously reported. *C. pylori* subsp. *mustelae* has major differences from *C. pylori*, the latter having 3-OH C<sub>18:0</sub>, a smaller amount of C<sub>16:0</sub>, and a larger amount of C<sub>14:0</sub>. The profile of *C. pylori* subsp. *mustelae* has been found to be different from that of *C. pylori* by other workers (D. S. Tompkins, J. I. Wyatt, B. J. Rathbone, and A. P. West, Epidemiol. Infect. Dis., in press), but they did not report the presence or absence of hydroxy fatty acids. One of our isolates, FM1 (Table 2), lost its urease activity, but the fatty acid profile of this urease-negative variant was the same as that of FM1. Thus, we suggest *C. pylori* subsp. *mustelae*, the ferret gastric spiral organism, is a new GLC group, group J, with the following features: absent 3-OH C<sub>14:0</sub> and 3-OH C<sub>18:0</sub> but C<sub>19:0</sub> cyc present, more than 2% C<sub>15:0</sub>, and more than 25% C<sub>16:0</sub>. Hydroxy fatty acids are probably of great

TABLE 2. Cellular fatty acids of spiral bacteria from the stomachs of humans (*C. pylori*), ferrets (*C. pylori* subsp. *mustelae*), monkeys, a pig, and a baboon

Species <sup>a</sup>	Amt <sup>b</sup> of indicated compound <sup>c</sup>											
	C <sub>12:0</sub>	C <sub>14:0</sub>	3-OH C <sub>14:0</sub>	C <sub>15:0</sub>	C <sub>16:0</sub>	C <sub>16:1</sub>	3-OH C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	3-OH C <sub>18:0</sub>	C <sub>19:0</sub> cyc
<i>C. pylori</i>												
NCTC 11637	T	41	—	—	5	T	5	5	16	4	2	17
NCTC 11638	T	41	—	—	7	—	5	7	9	2	3	16
Q188	T	37	—	T	5	T	5	8	17	3	2	17
72R	T	36	—	—	6	T	7	11	17	2	3	17
<i>C. pylori</i> subsp. <i>mustelae</i>												
NCTC 12032	T	15	—	2	32	—	2	T	23	7	—	15
ATCC 47332	—	13	—	2	30	—	2	T	17	5	—	16
FM1	T	17	—	3	28	—	4	T	17	9	—	13
FPD1	—	15	—	3	36	—	2	5	23	6	—	10
FM2	T	14	—	4	29	—	2	3	14	7	—	8
FP1	T	14	—	2	34	—	2	T	23	5	—	15
Rhesus monkey												
RPD2	T	29	—	—	10	—	10	3	14	5	3	14
RPD3	T	31	—	—	10	—	8	8	16	2	2	13
Nemestrina type A												
NAS1	T	28	—	—	10	—	12	10	14	2	2	10
NAS2	T	28	—	—	7	—	12	9	14	9	T	17
NAS3	T	27	—	—	11	—	14	11	15	4	2	10
Nemestrina type B												
NBS1	5	13	3	4	36	—	—	5	29	5	—	—
Fascicularis												
CP1	4	28	—	—	12	—	9	7	18	3	6	15
Baboon												
BM1	3	44	—	—	11	T	2	3	7	8	T	17
Pig												
PM1	T	42	—	—	8	—	6	6	8	3	T	16
<i>W. succinogenes</i>												
NCTC 11488	5	3	T	T	17	33	T	T	28	9	—	—

<sup>a</sup>, <sup>b</sup>, <sup>c</sup> See footnotes to Table 1.

importance taxonomically because of their presence in lipid A, which is a highly conserved region of bacterial lipopolysaccharides (13). However, we have found less than 6% DNA homology between *C. pylori* and *C. pylori* subsp.

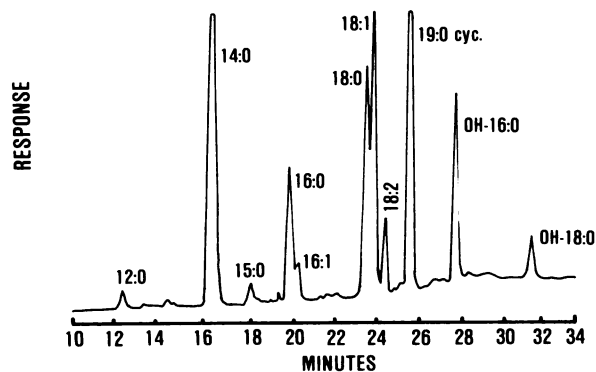


FIG. 1. Gas chromatogram of FAMES of *C. pylori* Q188 analyzed on a Superox-20M column (0.53 mm by 10 m). See the text for explanations of compound abbreviations and designations.

*mustelae*, so we suspect they are related more distantly than at the subspecies level.

We have designated one isolate from *M. nemestrina* as nemestrina type B (NBS1). Among gastric spiral bacteria, this isolate has the unusual feature of not having C<sub>19:0</sub> cyc or 3-OH C<sub>18:0</sub>, but it does have 3-OH C<sub>14:0</sub> and a small amount of C<sub>15:0</sub>; therefore, we propose it to be in a new GLC group, group K.

The other gastric spiral bacteria from *M. nemestrina* (nemestrina type A), *M. mulatta*, *Papio* sp., and the pig all appear very similar to *C. pylori*, although nemestrina type A and the *M. mulatta* isolate have slightly less C<sub>14:0</sub> than *C. pylori*, slightly more C<sub>16:0</sub>, and appreciably more 3-OH C<sub>16:0</sub>. Other studies by us, including a different G+C moles percent for nemestrina type A, and DNA-DNA hybridization experiments have shown that these organisms are somewhat different from *C. pylori*.

The fatty acid profile of *C. jejuni* subsp. *doylei* is similar to that of *C. jejuni* but does have some major differences, which are surprising in an organism so close to *C. jejuni* as to be considered a subspecies. Lambert et al. (10) identified the presence of 3-OH C<sub>14:0</sub> as a cardinal feature of *C. jejuni* and *C. coli* in GLC group A. However, *C. jejuni* subsp. *doylei*

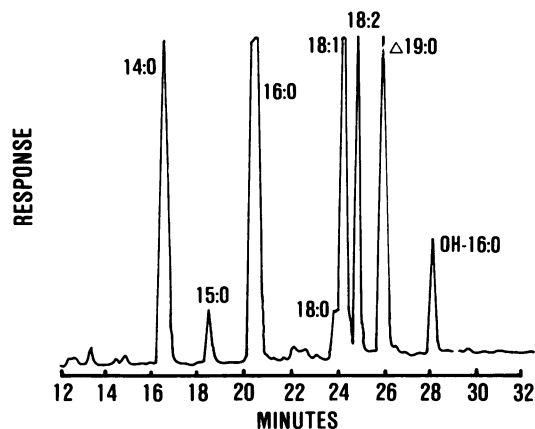


FIG. 2. Gas chromatogram of FAMES of *C. pylori* subsp. *mustelae* NCTC 12032 analyzed on a Superox-20M column (0.53 mm by 10 m). See the text for explanations of compound abbreviations and designations.

does not possess this hydroxy fatty acid but does have 3-OH  $C_{16:0}$ , which is absent in *C. jejuni*; it contains slightly more  $C_{14:0}$  than does *C. jejuni*. Thus, possibly *C. jejuni* subsp. *doylei* should not be included in Lambert group A; it also contains more  $C_{14:0}$  (10 to 16%) than does *C. jejuni* (5%) and contains 3-OH  $C_{16:0}$ , which is absent in *C. jejuni*. Wait and Hudson (21) reported the cellular fatty acid composition of one isolate of *C. jejuni* subsp. *doylei* but made no mention of the presence or absence of  $C_{12:0}$ , 3-OH  $C_{16:0}$ , or 3-OH  $C_{18:0}$ .

The UPTC had a simple cellular fatty acid composition, with large amounts of  $C_{16:0}$  and  $C_{18:1}$ . This is similar to that of "*C. cinaedi*," and we propose that these organisms be included in GLC group D the same as "*C. cinaedi*." Lambert et al. (10) studied one isolate of "*C. cinaedi*" and one of "*C. fennelliae*," whereas we studied five isolates of "*C. cinaedi*" and three of "*C. fennelliae*." In general, we confirm their findings, except that we found  $C_{15:0}$  in "*C. fennelliae*" (3 to 5%) and  $C_{18:2}$  in both organisms, with 7% in "*C. fennelliae*." These two organisms do not possess 3-OH  $C_{18:0}$  or  $C_{19:0}$  cyc, which clearly distinguishes them from all the gastric spiral bacteria except *nemestrina* type B. It has been proposed that *C. pylori* should be in the same genus as "*C. cinaedi*" and "*C. fennelliae*" (19), but their fatty acid profiles indicate that they should not be in the same genus.

The profile of *C. fetus* subsp. *fetus* in Table 1 is the same as that reported by Lambert et al. (10) except that we found 8% of  $C_{18:2}$ . We also grew the campylobacters in liquid medium but did not find any significant differences in their cellular fatty acid compositions compared with the profiles of bacteria grown on solid medium.

Lambert et al. (10) reported  $C_{18:2}$  in only one GLC group (*C. laridis*), but  $C_{18:2}$  was found in significant amounts in different species by Wait and Hudson (21) and by us in nearly all campylobacters, including *C. laridis*, but not by Wait and Hudson in this species (21). The seven major *Campylobacter* groups identified by GLC by Lambert et al. (10) have also been identified by immunotyping with rabbit antisera raised against each *Campylobacter* species (3). Immunotyping has identified an eighth group for *C. sputorum* and a ninth group for *C. nitrofigilis*.

By 16S rRNA analysis, *C. pylori* has been clearly separated from other campylobacters, and it has been suggested that it is closely related to the mouth organism *W. succinogenes* (19). However, we show that the fatty acid profile of

*W. succinogenes* is markedly different from that of *C. pylori*. Because cellular fatty acid profiles do have taxonomic significance (9, 11), this is one of many reasons that *W. succinogenes* should be in a different genus from *C. pylori*. For example, *C. pylori* is catalase and urease positive and possesses  $\gamma$ -glutamyl transpeptidase and alkaline phosphatase, but *W. succinogenes* is negative for all these enzymes; also, *C. pylori* will grow in air plus  $CO_2$ , but *W. succinogenes* will not.

*Campyocytophaga* sp. is another mouth organism as is *Cytophaga* sp., and we have found that the fatty acid profiles of these organisms are different from those of *C. pylori* and other campylobacters. *Campyocytophaga* sp. has a uniquely high percentage of  $C_{15:0}$  (35 to 57%) and does not have 3-OH  $C_{18:0}$ . *Cytophaga* sp. also does not have 3-OH  $C_{18:0}$  or  $C_{19:0}$ , but it has a very high percentage (56%) of octadecenoic acid ( $C_{18:1}$ ) and a high percentage of  $C_{16:0}$ .

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