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Comparative Distribution and Taxonomic Value of Cellular Fatty Acids in Thirty-Three Genera of Anaerobic Gram-Negative Bacilli

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Cellular fatty acid profiles were determined for species in 33 genera of anaerobic gram-negative bacilli and were confirmed to be a useful taxonomic tool. Most of the genera could be differentiated by visual inspection of their profiles. The three genus pairs that were most difficult to distinguish visually (*Bacteroides* and *Prevotella*, *Pectinatus* and *Megamonas*, and *Serpulina* and *Bilophila*) and the species of these genera were differentiated by the MIDI (Microbial ID, Inc.) identification system. Similarities in cellular fatty acid profiles may be correlated with similarities in other phenotypic characteristics, but more often there is no other obvious phenotypic relationship. Although medium components may not change the constituents detected or the ratios among the constituents detected for some species, identical medium changes may result in vast differences in the profiles obtained with other species. Thus, if a worker wishes to compare profiles of various taxa, it is essential that the same cultural and analytical conditions be used.

In 1968 Ifkovits and Ragheb (23) analyzed the cellular fatty acid (FA) compositions of anaerobic gram-negative rods isolated from rumen contents and concluded that similarities and differences in cellular FA composition could aid in identification, but these authors did not differentiate between families and genera. Since the original study of Ifkovits and Ragheb, there have been numerous reports detailing the cellular FAs of anaerobic gram-negative bacilli (2, 4, 10, 23, 32, 35, 36, 38, 40, 46, 53, 62, 65). Most of these later reports focused on description or taxonomy of single species or the species of a single genus (2, 10, 32, 35, 38, 46) or on species restricted to certain ecological niches (4, 10, 23, 38, 40, 65), particularly the human oral cavity and the rumen.

Since the original investigation of Ifkovits and Ragheb in 1968, the taxonomy and nomenclature of anaerobic gram-negative rods have changed considerably, particularly with regard to species formerly included in the genus *Bacteroides* (20). There are now 11 genera that have been described for the species formerly included in the genus *Bacteroides*, and 16 species are designated species incertae sedis (51). In the light of the newer taxonomy, Ifkovits and Ragheb probably would have interpreted their data differently.

Even though various growth conditions and analytical methods have been used by previous investigators, there is relatively good agreement among investigators for some taxa; however, researchers have obtained discrepant results for other taxa. Although the major cellular FAs of most genera that have been studied are distinct, there is general agreement that all species of the genus *Bacteroides* (as recently redefined) (51) and the genus *Prevotella* that have been tested produce the same major cellular FAs (4, 35, 36, 46).

For tested *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Fusobacterium* species isolated from humans (4, 32, 35, 36, 46), the results of previous investigators are relatively uniform qualitatively with regard to the major cellular constituents, although there are differences in the amounts of the major constituents detected, as well as qualitative differences in minor components. The results of previous investigators are less consistent for the genera *Fibrobacter* and *Ruminobacter* (23).

No information regarding cellular FA composition is available for several genera and for some species belonging to well-studied genera of anaerobic gram-negative rods. Indeed, the results of previous individual and combined reports do not encompass all species currently included in any genus that comprises more than one species.

In this study we analyzed the cellular FA compositions of genera of anaerobic gram-negative rods by using the same growth and analytical conditions so that the taxonomic value of cellular FA composition could be determined for this wide spectrum of organisms.

MATERIALS AND METHODS

Bacterial strains. The organisms that we tested are listed in Table 1. All of the test strains of a species were from different individuals or sources. *Campylobacter* species were included because this genus now includes species of obligate anaerobes. *Capnocytophaga* and *Eikenella* species were included because strains of species belonging to these genera may be obligately anaerobic upon initial isolation.

Characterization of strains. Fermentation, enzymatic, and antimicrobial agent susceptibility tests in prereduced media and polyacrylamide gel electrophoresis of soluble proteins were performed as described previously (17, 41).

Cellular FA analyses. Unless otherwise specified, for analysis of cellular FAs actively growing cultures were inoculated into tubes containing 10 ml of prereduced anaerobically sterilized peptone-yeast extract-glucose (PYG) broth (17). The tubes were inoculated under oxygen-free carbon dioxide or under an oxygen-free gas mixture containing 10% carbon dioxide and 90% nitrogen. The tubes were closed with neoprene stoppers and were incubated at 37°C. All subsequent sample preparation steps were done by using the same tubes closed with Teflon-lined screw caps in an aerobic atmosphere. The caps were removed for centrifugation.

Sedimented cells from cultures that had been incubated at 37°C for 18 to 24 h were used immediately or were frozen and

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TABLE 1. Strains used in analyses of cellular FAs

Taxon	Type strain[a]	No. of other strains used in this study	
		Homology strains (reference[s])	Other strains
Anaerorhabdus furcosus	3253 → ATCC 25662	0	0
Anaerovibrio lipolytica	ATCC 33276	0	0
Bacteroides fragilis	2553 → ATCC 25285	36 (25)	59
Bacteroides caccae	ATCC 43185	16 (30)	3
Bacteroides distasonis	ATCC 8503	11 (25)	3
Bacteroides eggerthii	ATCC 27754	9 (25)	0
Bacteroides merdae	T4-1 → ATCC 43184	7 (30)	1
Bacteroides ovatus	ATCC 8483	18 (25)	7
Bacteroides stercoris	B5-21 → ATCC 43183	17 (30)	3
Bacteroides thetaiotaomicron	5482 → ATCC 29148	12 (25)	22
Bacteroides uniformis	ATCC 8492	7 (25)	37
Bacteroides vulgatus	ATCC 8482	10 (25)	15
Bilophila wadsworthia	14946 = ATCC 49260	0	6
Butyrivibrio fibrisolvens	077 ← Bryant D1 = ATCC 19171	0	0
Campylobacter fetus subsp. *fetus*	ATCC 33248	0	14
Campylobacter fetus subsp. *venerealis*	ATCC 19438	0	4
Campylobacter coli	ATCC 33559	0	15
Campylobacter concisus	ATCC 33237	0	99
Campylobacter curvus	ATCC 35224	1 (60)	19
Campylobacter hyointestinalis	ATCC 35217	0	10
Campylobacter jejuni	ATCC 33560	0	18
Campylobacter lari	NCTC 11352	0	0
Campylobacter mucosalis	NCTC 11000	0	14
Campylobacter rectus	ATCC 33238	0	69
Campylobacter sputorum subsp. *bubulus*	ATCC 33562	0	8
Campylobacter sputorum subsp. *sputorum*	S17 → ATCC 35980	0	3
Campylobacter upsaliensis	ATCC 43954	0	1
Capnocytophaga ochracea	ATCC 27872	0	82
Capnocytophaga canimorsus	ATCC 35979	0	0
Capnocytophaga cynodegmi	ATCC 49044	0	0
Capnocytophaga gingivalis	ATCC 33624	0	80
Capnocytophaga sputigena	ATCC 33612	0	64
Catonella morbi	ATCC 51271	0	7
Centipeda periodontii	ATCC 35019	0	0
Desulfomonas pigra	ATCC 29098	0	0
Dialister pneumosintes	ATCC 33048	0	52
Dichelobacter nodosus	ATCC 25549	0	1
Eikenella corrodens	8499 ← Jackson 333 = ATCC 23834	0	71
Fusobacterium nucleatum subsp. *nucleatum*	4355 → ATCC 25586	5 (26)	87
Fusobacterium nucleatum subsp. *polymorphum*	ATCC 10953	5 (26)	90
Fusobacterium nucleatum subsp. *vincentii*	ATCC 49256	14 (26)	88
Fusobacterium alocis	D40B-5 → ATCC 35896	0	50
Fusobacterium gonidiaformans	0482A → ATCC 25563	0	26
Fusobacterium mortiferum	ATCC 25557	0	18
Fusobacterium naviforme	5658 → ATCC 25832	0	23
Fusobacterium necrogenes	2368 → ATCC 25556	0	1
Fusobacterium necrophorum subsp. *necrophorum*	2891 → ATCC 25286	0	61
Fusobacterium necrophorum subsp. *funduliforme*	6161 → JCM 3724	0	2
Fusobacterium perfoetens	ATCC 29250	0	0
Fusobacterium periodonticum	ATCC 33963	0	17
Fusobacterium russii	0307 → ATCC 25533	0	9
Fusobacterium ulcerans		0	6[b]
Fibrobacter succinogenes	ATCC 19169	0	0
Hallella seregens	ATCC 51272	5	0
Johnsonella ignava	ATCC 51276	0	10
Leptotrichia buccalis	ATCC 14201	0	56
Megamonas hypermegas	ATCC 25560	0	8
Mitsuokella multiacida	8951 = ATCC 27723	0	0
Oribaculum catoniae	ATCC 51270	0	58
Porphyromonas asaccharolytica	4198 → ATCC 25260	0	14
Porphyromonas endodontalis	13912 = ATCC 35406	0	5
Porphyromonas gingivalis	ATCC 33277	0	40
Porphyromonas salivosa	NCTC 11632	0	0
Prevotella melaninogenica	2381 → ATCC 25845	8 (19, 65)	49
Prevotella bivia	6822 → ATCC 29303	12 (18)	6

Continued on following page

TABLE 1—*Continued*

Taxon	Type strain[a]	No. of other strains used in this study	
		Homology strains (reference[s])	Other strains
Prevotella buccae	D3A-6 → ATCC 33574	13 (21)	15
Prevotella buccalis	NCFB 2354	0	3
Prevotella corporis	9342 → ATCC 33547	6 (28)	0
Prevotella denticola	ATCC 35308	15 (19, 65)	35
Prevotella disiens	8057 → ATCC 29426	9 (18)	2
Prevotella heparinolytica	ATCC 35895	0	9
Prevotella intermedia	4197 → ATCC 25611	12 (28)	23
Prevotella loescheii	ATCC 15930	6 (19)	49
Prevotella nigrescens	8944 → ATCC 33563	10 (28, 54)	72
Prevotella oralis	D27B-24 → ATCC 33269	1 (64)	18
Prevotella oris	ATCC 33573	7 (21)	44
Prevotella oulora	ATCC 43324	0	0
Prevotella ruminocola subsp. *ruminicola*	0051B → ATCC 19189	0	0
Prevotella veroralis	D22A-7 → ATCC 33779	4 (64, 65)	12
Prevotella zoogleoformans	ATCC 33285	0	4
Rikenella microfusus	ATCC 29728	0	0
Ruminobacter amylophilus	ATCC 29744	0	0
Selenomonas sputigena	D19B-28 (sic) → ATCC 35185	3 (29)	81
Selenomonas artemidis	D22B-14 → ATCC 43528	5 (38)	32
Selenomonas dianae	D19A-11 → ATCC 43527	2 (38)	20
Selenomonas flueggeii	E4M-28B → ATCC 43531	13 (38)	49
Selenomonas infelix	D75B-30 → ATCC 43532	6 (38)	79
Selenomonas noxia	ATCC 43541	12 (38)	78
Selenomonas ruminantium subsp. *ruminantium*	ATCC 12561	0	1
Selenomonas ruminantium subsp. *lactilytica*	ATCC 19205	0	1
Serpulina hyodysenteriae	V-78 = ATCC 27164	0	2
Serpulina innocens	B256 = ATCC 29796	0	0
Serpulina species "J"		0	12[c]
Succinimonas amylolytica	ATCC 19206	0	0
Succinivibrio dextrinosolvens	0554 → ATCC 19716	0	1
Tissierella praeacuta	ATCC 25539	0	4
Treponema denticola		0	15
Treponema pectinovorum	D36DR2 → ATCC 33768	0	0
Treponema socranskii subsp. *buccale*	D2B8 → ATCC 35534	0	0
Treponema socranskii subsp. *paredis*	D46CPE1 → ATCC 35535	0	0
Treponema socranskii subsp. *socranskii*	DR56BR1116 → ATCC 35536	0	0
"*Treponema vincentii*"		0	1

[a] Strains were obtained from the Moores' Virginia Polytechnic Institute and State University Culture Collection unless designated otherwise. ATCC, American Type Culture Collection, Rockville, Md.; JCM, Japanese Collection of Microorganisms, Saitama, Japan; NCFB, National Collection of Food Bacteria, Reading, United Kingdom (formerly National Collection of Dairy Organisms); NCTC, National Collection of Type Cultures, London, England. →, deposited in; ←, received from.

[b] Strains were received from B. Adriaans, who described the species. One strain probably is the type strain, but no culture collection catalog gives Adriaan's number for the type strain, so we cannot be sure.

[c] Isolated from feces of AIDS patients.

later thawed. The cells were lysed and saponified with 1.0 ml of basic methanol (45 g of NaOH, 150 ml of methanol, 150 ml of deionized water), heated in a boiling water bath for 5 min, mixed with a vortex mixer, and heated in the boiling water bath for an additional 25 min. To methylate cell constituents, 1 ml of HCl-methanol (325 ml of 6.0 N HCl, 275 ml of methanol [certified grade]) and 1 ml of sulfuric acid-methanol (162.5 ml of H_2SO_4 [American Chemical Society reagent grade] added to 162.5 ml of deionized water, 275 ml of methanol [certified grade]) were added, and the solution was heated at 80°C for 10 min. After rapid cooling, the methylated components were extracted by adding 1.25 ml of hexane-ether (200 ml of hexane [high-performance liquid chromatography grade], 200 ml of methyl-tert butyl ether [high-performance liquid chromatography grade]) and turning the tubes end over end for 10 min. Each extract was washed once with 3 ml of a solution containing 5.4 g of NaOH in 450 ml of deionized distilled water saturated with NaCl. A 2-μl portion of the washed extract was chromatographed on a fused-silica capillary column with a model HP-5890A chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detector and a model HP-3392A integrator (Hewlett-Packard). The gas flow rates were ca. 400 ml/min for air, 30 ml/min for hydrogen, and 30 ml/min for nitrogen. The temperatures used were 250°C for the injection port and 300°C for the detector. After injection, the oven temperature was increased from 170 to 270°C at a rate of 5°C/min and then from 270 to 310°C at a rate of 30°C/min, held at 310°C for 2 min, and then returned to 170°C before the next sample was injected. A standard mixture containing known FAs (C_9 through C_{20} straight-chain FAs and 2-OH C_{10}, 3-OH C_{10}, 2-OH C_{14}, 3-OH C_{14}, and 2-OH C_{16} FAs) was chromatographed at the beginning of each day on which samples were analyzed and after each set of 10 samples.

We used the MIS software package (Microbial ID, Inc., Newark, Del.) to identify the peaks (by retention time) and to determine the area, the ratio of area to height, the equivalent chain length (ECL), the total area, and the total area for named or listed compounds. The MIS software package also

was used to calculate the percentage of area for each named or listed compound compared with the total area of the compounds detected. Compounds were identified by using the Moore Broth Library, version 3.8 (released in February 1994 by Microbial ID, Inc.).

For organisms that produced low turbidity in broth cultures, multiple 10-ml cultures were pooled to obtain a larger cell pellet, and the extract was concentrated before analysis by evaporation under a stream of grade 5.0 nitrogen. Serum was not added to cultures because the FAs that occur in serum could not be removed from the cells by any wash reagent that we tested.

For strains of most species, one 10-ml PYG culture incubated overnight produced a sufficient pellet of sedimented cells to give a satisfactory extract for analysis. If a cell pellet larger than a flattened bead about 4 mm in diameter and 2 mm deep was produced (e.g., the pellets produced by *Bacteroides fragilis* cultures), some of the culture was discarded. The following conditions were used for strains that produced low turbidity in PYG cultures: for *Bilophila* strains, two to four 10-ml PYG cultures were incubated for 3 to 5 days, and the extract was concentrated; for *Catonella* strains, two to three PYG cultures were incubated for 3 to 4 days, and the extract was concentrated; for *Dialister* strains, four to six PYG cultures were incubated for 1 to 4 days, and the extract was concentrated; for *Dichelobacter* strains, four to six PYG cultures were incubated for 1 to 2 days, and the extract was concentrated; and for *Johnsonella* strains, two to four PYG cultures were incubated for 1 to 3 days, and the extract was concentrated. The cultures used for the *Succinimonas* library entry were grown in PYG medium with volatile FAs added (17), and the cultures used for the *Desulfomonas* entry were grown in pyruvate medium (17). For *Butyrivibrio fibrisolvens*, the results obtained with cells grown in media that did and did not contain volatile FAs (17) were combined for the reference library entry and for the information shown in Table 3. Only the results obtained with cells grown in PYG medium supplemented with volatile FAs were used for the data for *Fibrobacter succinogenes* shown in Table 2, but the results obtained with cells grown in media both with and without volatile FAs are given in Table 3 for *Fibrobacter succinogenes.*

To determine the cellular constituents that were representative of a genus, the amounts of constituents found in the different species of the genus (see above) were averaged. The amounts found in subspecies were averaged to obtain species values. The cellular constituent data for each species were derived from at least 20 analyses of the available strain(s) belonging to the species. Ideally, multiple strains were represented, but if only one strain was available, the results of multiple analyses of that strain, determined on different days and derived from different subcultures, were used to calculate the library entry.

RESULTS AND DISCUSSION

The analytical results are presented in Table 2. In Table 2, the taxa are arranged on the basis of similarity of cellular composition and are given reference numbers. All 33 genera studied could be differentiated by the MIDI identification system, and most could be differentiated visually by qualitative and quantitative differences in their cellular FA profiles. An example of the effects of different medium components (e.g., volatile FAs) on cellular FA composition is shown in Table 3. Some major phenotypic characteristics of the genera are shown in Table 4.

Despite the different culture conditions and analytical methods used in various previous investigations and the diversity of strains studied, there was reasonably good agreement between our results and those of other authors for the genera *Bacteroides* (2, 4, 35, 36, 46), *Prevotella* (4, 35, 36, 46), *Porphyromonas* (4, 32, 35, 36), *Fusobacterium* (10, 36), and *Bilophila* (2).

Our results for all 10 described *Bacteroides* species (Table 2, taxon 1) and 17 described *Prevotella* species (taxon 2) confirmed the previous findings (2, 35, 36, 46) that were based on up to five different *Bacteroides* species and up to six different *Prevotella* species namely, that anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-3-OH $C_{17:0}$ and $C_{16:0}$ FAs are the major cellular FAs in these two genera. Among all anaerobic gram-negative rods, species belonging to the genera *Bacteroides* and *Prevotella* are the most similar in other phenotypic characteristics (17, 20, 39). These two genera currently are differentiated by the inability of *Prevotella* strains to grow in the presence of 20% bile and by the production of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by *Bacteroides* species (52). The genus *Prevotella* corresponds to the second phylogenetic cluster delineated by Johnson and Harich (27), who observed 16 to 43% rRNA homology between *Bacteroides* species and species now recognized as members of the genus *Prevotella.*

Our results for 12 *Fusobacterium* species (taxon 14) are in general agreement with those of Calhoon et al. (10) and Miyagawa et al. (36), who studied representatives of eight and four *Fusobacterium* species, respectively. Major amounts of $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$-*cis*9 FAs distinguish the fusobacteria from the other genera tested. We did not include values for *Fusobacterium prausnitzii* and *Fusobacterium sulci* in our calculations for the genus *Fusobacterium* because these species probably should not be classified in this genus. The DNA of *Fusobacterium prausnitzii* has a much higher G+C content (52 to 57 mol%) than the DNA of the type species, *Fusobacterium nucleatum* (27 to 28 mol%) (43). *Fusobacterium sulci* tends to stain gram positive, and 14% of its cellular FA is $C_{18:1}$-*cis*11 dimethyl acetal (DMA), in contrast to other species in this genus.

Our results for the genus *Porphyromonas* (taxon 5) were in general agreement with those of Mayberry et al. (35), Lambe et al. (32), Miyagawa et al. (36), Shah and Collins (46), and Brondz and Olsen (4). There is consensus that iso-$C_{15:0}$ FA is the major FA in *Porphyromonas* strains, accounting for 33 to 58% of the FAs present. The next most common acid, which is present at a level of 10 to 20% and which we identified as either iso-3-OH C_{17} FA or $C_{18:2}$ DMA (ECLs, 18.163 and 18.178, respectively) is probably the compound reported to be iso-OH C_{17} FA by Lambe et al. (32), iso-C_{17} FA by Miyagawa et al. (36), and iso-3-OH C_{17} FA by Brondz and Olsen (4).

Species belonging to the genera *Porphyromonas* (taxon 5), *Capnocytophaga* (taxon 4), and *Rikenella* (taxon 6) have similar FA profiles. The most distinctive difference among the cellular FAs of these three genera is in the relative amount of iso-$C_{15:0}$ FA produced, which ranges from 36 to 55% (Table 2). The three genera are quite distinct on the basis of their phenotypic characteristics (Table 4). *Porphyromonas* species are nonsaccharolytic and produce acetate, propionate, isovalerate, isobutyrate, and succinate from peptone. *Capnocytophaga* and *Rikenella* species are saccharolytic and produce major amounts of acetic and succinic acids. *Rikenella microfusus* is obligately anaerobic and has a DNA G+C content of 60 to 61 mol%. Most *Capnocytophaga* strains are facultatively anaerobic (22) and have DNA G+C contents of 33 to 41 mol%.

Our results and those of Miyagawa et al. (36) for *Anaerorhabdus* (*Bacteroides*) *furcosus* (taxon 13) are in excellent agreement if the compounds that we differentiated as $C_{18:1}$-

TABLE 2. Cellular FA compositions of genera of anaerobic gram-negative rods

Taxon		FA composition (%)[a]										
Reference no.	Genus	Unknown with ECL of 9.74	$C_{11:0}$ FA	$C_{12:0}$ FA	iso-$C_{13:0}$ FA	$C_{13:0}$ FA	iso-$C_{14:0}$ FA	$C_{14:0}$ FA	$C_{14:0}$ DMA	iso-$C_{15:0}$ FA	anteiso-$C_{15:0}$ FA	$C_{15:0}$ FA
1	*Bacteroides*	—	—	—	1	—	1	1	—	6	36	6
					(0–2)		(0–4)	(1–2)		(6–13)	(32–40)	(2–14)
2	*Prevotella*	—	—	—	1	—	5	2	—	10	35	1
					(0–5)		(0–13)	(0–9)		(4–18)	(22–45)	(0–3)
3	*Oribaculum*	—	—	1	15	—	—	7	—	26	23	2
4	*Capnocytophaga*	—	—	—	1	—	—	1	—	55	4	1
					(0–3)			(0–2)		(45–70)	(1–8)	(0–3)
5	*Porphyromonas*	—	—	—	4	—	—	5	—	45	7	—
					(1–6)			(3–6)		(33–58)	(2–15)	
6	*Rikenella*	—	—	—	1	—	—	1	—	36	—	9
7	*Tissierella*	—	—	2	4	—	—	14	2	24	5	1
8	*Serpulina*	2	—	—	—	—	—	2	2	14	2	2
		(1–2)						(2–2)	(0–3)	(11–16)	(1–2)	(1–3)
9	*Bilophila*	—	—	—	—	—	—	6	—	17	—	—
10	*Desulfomonas*	—	—	—	—	—	—	1	—	21	—	—
11	*Butyrivibrio*	—	—	1	—	—	—	4	2	—	—	—
12	*Dialister*	—	—	8	—	—	—	5	—	—	—	—
13	*Anaerorhabdus*	—	—	1	—	—	—	—	—	—	—	—
14	*Fusobacterium*	—	—	3	—	—	—	22	3	—	—	—
				(1–10)				(11–44)	(0–11)			
15	*Treponema*	—	—	2	—	1	4	19	7	—	4	9
				(0–5)		(0–2)	(0–6)	(12–31)	(3–12)		(0–8)	(3–15)
16	*Succinimonas*	—	—	16	—	—	—	11	—	—	—	1
17	*Succinivibrio*	—	—	1	—	—	—	20	—	—	—	—
18	*Leptotrichia*	—	—	6	—	—	—	10	—	—	—	—
19	*Campylobacter*	—	—	3	—	—	—	9	—	—	—	1
				(0–10)				(5–14)				(0–19)
20	*Ruminobacter*	—	—	4	—	—	—	13	—	—	—	—
21	*Dichelobacter*	—	—	5	—	—	—	3	—	—	—	—
22	*Eikenella*	—	—	7	—	—	—	2	—	—	—	—
23	*Anaerobiospirillum*	—	—	3	—	—	—	3	—	—	—	—
24	*Hallella*	—	—	—	—	—	15	11	—	—	5	1
25	*Fibrobacter*	—	—	—	—	—	3	19	8	—	2	2
26	*Catonella*	—	—	3	—	—	—	42	14	—	—	—
27	*Johnsonella*	45	—	—	—	—	—	8	—	—	—	—
28	*Selenomonas*	—	5	3	—	11	—	2	17	—	—	13
			(4–7)	(2–4)		(3–17)		(1–4)	(10–22)			(9–17)
29	*Centipeda*	—	4	1	—	14	—	1	17	—	—	21
30	*Pectinatus*	—	11	—	—	4	—	1	18	—	—	21
31	*Megamonas*	—	11	—	—	—	—	—	21	—	—	18
32	*Anaerovibrio*	—	5	3	—	4	—	—	11	—	—	8
33	*Mitsuokella*	—	—	12	—	—	—	7	—	—	—	—

[a] Mean percentages of the total acids. — or 0, less than 0.5% of total acids. The values in parentheses are ranges of values for species in the genera. The compounds included are those compounds that occurred at levels of 10% or more in one or more of the species. ECL, equivalent chain length.

*cis*9 FA and $C_{18:1}$-*cis*11/t9/t6 FA or an unknown having an ECL of 17.824 (combined percentage, 46% [Table 2]) are equivalent to the 50% of $C_{18:1}$-*cis*9 FA reported by Miyagawa et al.

Our results agreed only moderately well with those previously reported for the genera *Ruminobacter* (taxon 20) (23, 36), *Fibrobacter* (taxon 25) (23, 36), *Butyrivibrio* (taxon 11) (23), and *Desulfomonas* (taxon 10) (62). We agree with Ifkovits and Ragheb (23) and Miyagawa et al. (36) that $C_{16:0}$ (31%), $C_{18:1}$ (14 and 19%), and $C_{14:0}$ (13%) are the major FAs found in cells of *Ruminobacter* (*Bacteroides*) *amylophilus*. In addition to differences in the percentages detected, there also are differences concerning the precise identity of the $C_{18:1}$ compound found by the three different sets of investigators. Ifkovits and Ragheb (23) reported that $C_{18:1}$ FA was present at a concentration 22.5%, Miyagawa et al. (36) reported that $C_{18:1}$-*cis*7 FA was present at a concentration of 27%, and we found that $C_{18:1}$-*cis*9 FA was present at a concentration of 14% and either $C_{18:1}$-*cis*11/t9/t6 FA or an unidentified compound with an ECL of 17.834 was present at a concentration of 19%. Although these differences might be attributed to the different growth media used (VL-carbohydrate medium was used by the other two groups of workers; PYG medium was used in this study), it is more logical to presume that the differences are due to improved technology that enables more precise separation and identification of eluted methylated compounds. The 14% $C_{18:1}$-*cis*9 FA helps differentiate the genus *Ruminobacter* from other genera that contain major amounts of $C_{16:0}$ and $C_{14:0}$ FAs.

For *Fibrobacter* (*Bacteroides*) *succinogenes* (taxon 25), we confirmed the finding of Miyagawa et al. (36) that $C_{16:0}$ and $C_{14:0}$ FAs are major cell constituents. However, we did not detect the major amount of $C_{15:0}$ FA reported by both

TABLE 2—*Continued*

FA composition (%)[a]														
iso-$C_{16:0}$ FA	$C_{16:1}$-*cis*7 FA	$C_{16:1}$-*cis*9 FA	$C_{16:0}$ FA	$C_{16:0}$ DMA	iso-$C_{17:0}$ FA	$C_{18:1}$ DMA (ECL, 17.25)	3-OH $C_{16:0}$ FA	$C_{18:1}$-*cis*9 FA	$C_{18:0}$ FA	$C_{18:1}$ *cis*11 DMA	$C_{15:0}$ DMA or 3-OH $C_{14:0}$ FA	$C_{17:1}$-*cis*9 FA or $C_{17:2}$ FA (ECL, 16.8)	$C_{18:1}$-*cis*11/ t9/t6 FA or unknown with ECL of 17.834	iso-3-OH $C_{17:0}$ FA or $C_{18:2}$ DMA
1	—	1	9	—	2	—	4	—	—	—	—	—	—	15
(0–2)		(0–4)	(6–12)		(0–6)		(2–6)							(11–19)
3	—	—	9	—	3	—	5	1	—	—	—	—	—	11
(0–9)			(2–24)		(0–9)		(1–13)	(0–3)						(5–18)
—	—	1	5	—	2	—	1	1	—	—	—	—	—	5
—	—	1	6	—	3	—	5	2	—	—	—	—	—	13
		(0–1)	(3–8)		(1–8)		(4–5)	(0–7)						(10–18)
—	—	1	9	—	1	—	3	1	1	—	—	—	—	15
		(0–1)	(4–13)		(1–1)		(1–5)	(1–2)	(0–1)					(10–20)
—	—	1	7	—	1	—	1	8	4	—	—	—	1	15
—	—	4	19	8	2	—	—	2	3	—	—	—	—	—
—	—	1	31	3	2	—	1	6	8	—	—	—	—	—
		(1–2)	(26–37)	(2–4)	(1–3)		(0–2)	(2–9)	(5–12)					
—	—	2	26	7	1	—	2	8	7	—	—	—	2	—
—	—	13	30	3	21	—	—	1	3	—	—	—	1	—
1	—	1	45	11	—	—	—	2	3	15	—	3	4	—
—	2	11	20	—	—	—	—	21	12	—	8	—	2	—
—	1	2	13	3	—	—	—	28	25	—	—	—	18	—
—	3	15	22	7	—	—	1	2	1	1	6	—	4	—
	(0–6)	(2–19)	(10–38)	(0–16)			(0–4)	(1–5)	(0–3)	(0–14)	(0–10)		(0–16)	
4	—	1	21	—	—	—	—	4	4	—	—	—	—	—
(0–6)		(0–1)	(14–29)					(0–7)	(0–7)					
—	—	3	37	—	—	—	8	3	2	—	3	—	13	—
—	—	6	32	—	—	—	3	1	1	—	8	—	28	—
—	—	2	42	—	—	—	—	1	1	—	8	—	28	—
—	—	9	29	1	—	—	—	2	2	—	5	—	29	—
		(5–16)	(24–40)	(0–5)				(0–7)	(0–4)		(4–6)		(12–52)	
—	—	5	31	1	—	—	2	14	5	—	2	—	19	—
—	—	21	18	—	—	—	—	4	3	—	1	1	34	—
—	—	15	24	—	—	—	1	7	6	—	—	—	27	—
—	—	4	27	—	—	—	—	2	2	—	4	—	53	—
15	—	2	23	1	—	—	16	3	2	—	—	—	—	—
7	—	3	20	—	—	—	—	10	5	—	—	—	1	—
—	—	2	12	1	—	—	—	5	4	—	—	—	—	—
—	—	2	15	5	—	—	—	5	4	2	—	—	3	1
—	6	—	1	—	—	5	—	3	—	—	5	5	—	—
	(3–16)		(0–4)			(2–8)		(1–6)			(3–10)	(2–12)		
—	3	—	—	—	—	4	—	1	—	—	3	7	—	—
—	3	—	2	—	—	12	—	2	1	—	1	9	—	—
—	2	—	2	—	—	8	—	1	1	—	1	17	—	—
—	6	—	1	—	—	8	—	3	—	—	4	18	—	—
—	20	3	11	2	—	—	—	5	2	—	19	—	—	—

Miyagawa et al. and Ifkovits and Ragheb (23) or the major amount of $C_{13:0}$ FA reported by Ifkovits and Ragheb. Miyagawa et al. reported the presence of 20% $C_{18:0}$ FA; this compound accounted for only 5% of the acids that we detected and was not detected at all by Ifkovits and Ragheb. Ifkovits and Ragheb examined three strains obtained from Bryant that we have not examined, which might explain the different results. However, both Miyagawa et al. and we analyzed ATCC strain 19169^T (T = type strain), so the large differences between the results of Miyagawa et al. and our observations cannot be attributed to a misidentified strain and we are not able to explain them.

The only major difference between our results and those of Ifkovits and Ragheb (23) for *Butyrivibrio fibrisolvens* (taxon 11) is that Ifkovits and Ragheb found 12% $C_{18:1}$ FA and we found 2% $C_{18:1}$-*cis*9 FA and 16% $C_{18:1}$-*cis*11 DMA, which were not found by Ifkovits and Ragheb. Since these two compounds have ECLs of 17.770 and 18.285, respectively, it is unlikely that there was a problem in identification of the compounds. Both Ifkovits and Ragheb and we tested cultures derived from Bryant strain D1^T. The culture medium used by Ifkovits and Ragheb contained volatile FAs, but we did not detect major differences when our strains of *Butyrivibrio fibrosolvens* were analyzed by using cells grown in media with and without added volatile FAs (Table 3).

For the type strain of *Desulfomonas pigra* (taxon 10), we agree with Vainshtein et al. (62) that about 20% of the acids is iso-$C_{15:0}$ FA. However, we found quantitative differences in the percentages of $C_{16:0}$ FA (30% in our study, 11% in the study of Vainshtein et al.), iso-$C_{17:0}$ FA (21% in our study, 12% in the study of Vainshtein et al.), and $C_{16:1}$-*cis*9 FA (13% in our study, 6.5% in the study of Vainshtein et al.). We did not detect iso-$C_{17:0}$-*cis*7 FA, which accounted for 26% of the cell constituents reported by Vainshtein et al. Although the ana-

TABLE 3. Effect of the presence of volatile FAs in the growth medium on cellular FAs

Compound	% in[a]:			
	Butyrivibrio fibrosolvens		*Fibrobacter succinogenes*	
	With volatile FAs	Without volatile FAs	With volatile FAs	Without volatile FAs
$C_{14:0}$ FA	2	4	6	16
$C_{15:0}$ FA	0	0	47	1
$C_{16:0}$ FA	38	45	3	21
$C_{16:0}$ DMA	9	11	0	1
$C_{18:1}$ *cis*9 FA	1	2	1	14
$C_{18:1}$ *cis*11 DMA	19	15	0	0

[a] Percentage of the total chromatogram area for each compound. The values in each column do not add up to 100% because only acids that were present at levels at >10% in either of the species are listed.

lytical method used by Vainshtein et al. was similar to our method in that the MIDI identification system was used, Vainshtein et al. used a chemically defined growth medium with an oxidized sulfur compound as the electron acceptor, which probably explains the rather large difference in the cellular FAs detected. Our results agreed with those of Baron et al. (2), who also used growth in a complex medium for analysis.

For the genera *Megamonas*, *Mitsuokella*, *Succinimonas*, and *Succinivibrio*, agreement was relatively poor between our results and the results reported by other workers. In cells of *Megamonas hypermegas* (taxon 31), we detected 21% $C_{14:0}$ DMA, which was not reported by Miyagawa et al. (36), and did not detect a compound reported to have an ECL of 19.21 that was present at a concentration of 17% in the study of Miyagawa et al. The cellular constituents of the genus *Pectinatus* (taxon 30) were similar to those of the genus *Megamonas* qualitatively, but there were quantitative differences in the major components (Table 2). Although species belonging to both genera produce major amounts of propionic acid, *Pectinatus* strains are motile by means of lateral flagella and *Megamonas* strains are not motile (Table 4).

For *Mitsuokella multiacidus* (taxon 33), the major differences between our results and those of Miyagawa et al. were as follows: we detected 12% $C_{12:0}$ FA, a compound not detected by Miyagawa et al. (36), and we detected only 3% $C_{16:1}$-*cis*9 FA, compared with the 32% reported by Miyagawa et al. *Mitsuokella multiacidus* was the only species in this study in which we detected major amounts of $C_{16:1}$-*cis*7 FA (20%) and $C_{15:0}$ DMA or 3-OH $C_{14:0}$ FA (19%).

In strain N6 of *Succinimonas amylolytica* (taxon 16), Ifkovits and Ragheb (23) detected 53% iso-$C_{15:0}$ FA and 11% iso-$C_{17:0}$ FA; we detected neither of these compounds in cells of ATCC 19206^T (= Bryant strain B_24^T). Compared with the relatively good correlations for major compounds that we observed between our results and those of other authors for some other genera, we are at a loss to explain these major discrepancies.

Although both we and Ifkovits and Ragheb (23) found about 20% $C_{14:0}$ FA in the type strain of *Succinivibrio dextrinosolvens* (taxon 17), there were major differences in the other acids detected. Ifkovits and Ragheb detected 22% anteiso-$C_{15:0}$ FA, 11% $C_{15:0}$ FA, and iso-$C_{16:0}$ FA, compounds that were not detected by us, whereas we detected 28% $C_{18:1}$-*cis*11/t9/t6 FA or an unknown compound with an ECL of 17.834. Also, we detected 32% $C_{16:0}$ FA, compared with the 7% $C_{16:0}$ FA detected by Ifkovits and Ragheb. The genus *Anaerobiospirillum* (taxon 23), another taxon that comprises motile gram-negative anaerobes that produce major amounts of succinic acid, has a distinct cell composition. Cells of the type strain of *Anaerobiospirillum succiniciproducens* also contain a compound identified as either $C_{18:1}$-*cis*11/t9/t6 FA or an unknown compound with an ECL of 17.8, but at a much higher percentage (53%). Among the genera included in this study, the presence of this compound at a concentration greater than 35% is unique to the genus *Anaerobiospirillum*.

Although the major acids of the genera *Leptotrichia* (taxon 18) and *Campylobacter* (taxon 19) are similar to those of the genus *Succinivibrio* qualitatively, there are quantitative differences that distinguish these taxa (Table 2). Phenotypically, these genera are differentiated by metabolic acid products, motility, and aerotolerance (Table 4).

The cellular composition of the genus *Centipeda* (taxon 29) is similar to the composition of the genus *Selenomonas* (taxon 28) that we reported previously (38); the two taxa differ principally in the relative amounts of $C_{15:0}$ FA. Members of these two genera and of the genus *Pectinatus* (taxon 30) also are similar phenotypically, differing in the location of flagella. The FA profile of the genus *Anaerovibrio* (taxon 32), another genus that comprises motile anaerobic gram-negative bacilli that produce major amounts of propionic acid, is similar to but distinct from the FA profiles of the genera *Centipeda*, *Selenomonas*, and *Pectinatus* (Table 2).

Three recently proposed (40) fermentative genera of obligately anaerobic gram-negative rods, the genera *Oribaculum*, *Hallella*, and *Catonella*, have unique fermentation profiles. Although the cellular FAs of the genus *Oribaculum* (taxon 3) are somewhat similar to those of the genera *Bacteroides* and *Prevotella* (Table 2), *Oribaculum* strains differ significantly in the amount of iso-$C_{13:0}$ FA (15 versus 1%), and there are smaller differences in the amounts of iso-$C_{15:0}$ FA, anteiso-$C_{15:0}$ FA, and iso-3-OH $C_{17:0}$ FA or $C_{18:2}$ DMA produced. The genus *Oribaculum* is the only taxon included in this study that has a major amount of iso-$C_{13:0}$ FA. In addition to its different cellular FA profile, *Oribaculum catoniae* produces substantially more propionic acid and less succinic acid in PYG cultures than bacteroides or prevotella strains do (Table 4). Strains of the genus *Hallella* (taxon 24) contain $C_{16:0}$ and $C_{14:0}$ FAs, as do several other genera of anaerobic gram-negative bacilli. However, the major amounts of iso-$C_{14:0}$ FA (15%) and iso-$C_{16:0}$ FA (15%) produced by members of the genus *Hallella* are unique to this taxon. The major cellular FA of the genus *Catonella* (taxon 26) is $C_{14:0}$; the percentage of this acid is 42%, which is the highest percentage of this acid found in any anaerobic, gram-negative, rod-shaped organism tested.

The two taxa of spirochetes analyzed, the genus *Treponema* (taxon 15) and the genus *Serpulina* (taxon 8), were less like each other than we expected. The genus *Treponema* (taxon 15) and the genus *Fusobacterium* (taxon 14) contain major amounts of $C_{14:0}$ and $C_{16:0}$ FAs, but *Treponema* cells do not contain the major amount of $C_{16:1}$-*cis*9 FA (15%) found in *Fusobacterium* cells. The cellular FA profile of the genus *Serpulina* (taxon 8) was most like that of the nonfermentative species *Bilophila wadsworthia* (taxon 9). Species of both genera produce major amounts of iso-$C_{15:0}$ and $C_{16:0}$ FAs; trace amounts of other constituents in the genus *Serpulina* differentiate the two taxa, which are quite distinct phenotypically (Table 4). A major amount (14%) of $C_{14:0}$ FA produced by cells of the genus *Tissierella* (taxon 7) helps differentiate this taxon from the genera *Serpulina* and *Bilophila*.

The major cellular constituents of two genera of nonfermentative bacteria, the genera *Dichelobacter* (taxon 21) and *Eikenella* (taxon 22), are $C_{16:1}$-*cis*9 FA, $C_{16:0}$ FA, and a compound that is either $C_{18:1}$-*cis*11/t9/t6 FA or an unidentified

TABLE 4. Some differential properties of taxa of anaerobic gram-negative bacilli

Taxon		G+C content (mol%)	Saccharolytic[a]	Major product(s)[b]	Motility[a]	O_2 tolerance[c]	Reference(s)
Reference no.	Genus						
1	*Bacteroides*	40–48	+	S,A	−	An	20, 30
2	*Prevotella*	36–52	+	S,A	−	An	39, 52, 53, 54
3	*Oribaculum*	49	+	P,a,s,l	−	An	40
4	*Capnocytophaga*	33–41	+	S,A	−	F-An	22
5	*Porphyromonas*	46–51	−	A,B,iV	−	An	20, 34, 50
6	*Rikenella*	60–61	w	A,S,p	−	An	13, 20
7	*Tissierella*	28	−	A,B,iV	−	An	12, 20
8	*Serpulina*	26	w	a,b	+	An	56, 59
9	*Bilophila*	39–40	−	A	−	An	2
10	*Desulfomonas*	66–67	−	a	−	An	42
11	*Butyrivibrio*	36–41	+	B(L)	+	An	5
12	*Dialister*	NR[d]	−	a(l,p,s)	−	An	40
13	*Anaerorhabdus*	34	w	L,a	−	An	20, 49
14	*Fusobacterium*	26–34[e]	−, w	B,a(p,f,l)	−	An	1, 11, 43, 55
15	*Treponema*[f]	36–51	v	A(p,b)	+	An	56
16	*Succinimonas*	NR	+	S,A	+	An	6
17	*Succinivibrio*	NR	+	S,A,F	+	An	7
18	*Leptotrichia*	25	+	L	−	An	16
19	*Campylobacter*	30–38	−	None	+	M, An	3, 15, 45, 57, 61, 63
20	*Ruminobacter*	40–42	+	S,A,F	−	An	20, 58
21	*Dichelobacter*	45	−	a,s,p	−	An	14, 20
22	*Eikenella*	56–58	−	a	−	F-An	24
23	*Anaerobiospirillum*	44	+	S,A	+	An	8
24	*Hallella*	58	+	S,a,l	−	An	40
25	*Fibrobacter*	47–49	+	A,S	−	An	20, 37
26	*Catonella*	34	+	A	−	An	40
27	*Johnsonella*	32	−	a,iv,l,s,ib,i	−	An	40
28	*Selenomonas*	54–61	+	P,A	+	An	9, 38
29	*Centipeda*	53	+	P,A	+	An	31
30	*Pectinatus*	40	+	P,A	+	An	33
31	*Megamonas*	35	+	P,a,l,s	−	An	20, 47
32	*Anaerovibrio*	NR	+	P,A	+	An	44
33	*Mitsuokella*	56–58	+	L,S,A	−	An	20, 48

[a] +, positive reaction; −, negative reaction; w, weak reaction; v, variable among species.

[b] A and a, acetic acid; B and b, butyric acid; F and f, formic acid; ib, isobutyric acid; iV and iv, isovaleric acid; L and l, lactic acid; P and p, propionic acid; S and s, succinic acid; uppercase letters, >100 meq/100 ml of culture; lowercase letters, <100 meq/100 ml of culture. The compounds in parentheses are compounds that are detected irregularly.

[c] An, anaerobic; F-An, facultatively anaerobic; M, microaerophilic.

[d] NR, not reported.

[e] *Fusobacterium prausnitzii* and *Fusobacterium sulci* not included.

[f] Cultivable strains.

compound with an ECL of 17.8. The FA profiles of the two taxa differ in the relative amounts of these constituents and in other constituents detected in trace to moderate amounts. The FAs of a third genus of non-fermentative organisms, the genus *Dialister* (taxon 12) (40), are unique (Table 2).

Of all of the genera of gram-negative rods that we have studied, the genus *Johnsonella* (40) has the most distinctive cellular FAs; 45% of the total chromatogram area for the products that were listed and detected represents an unidentified acid with an ECL of 9.740 (Table 2), and 15% is $C_{16:0}$ FA. The metabolic end products of members of the genus *Johnsonella* are different from those of members of the genera *Porphyromonas*, *Tissierella*, *Desulfomonas*, and *Bilophila*, which are other genera that contain nonfermentative organisms (Table 4). Of these, only the genus *Tissierella* contains organisms with a low G+C content (28 mol%) (8) similar to the G+C content of *Johnsonella* strains (32 mol%).

Within genera that have multiple species, visual differentiation of cellular FA patterns among species often is difficult, if not impossible (data not shown), but the species can be distinguished by the MIDI identification system. If a worker wishes to use the MIDI identification system, it is essential that the media, incubation temperatures, and chromatography conditions used for the unknown cultures be the same as those used to develop the library entries. The effect of different media on cellular constituents described above (Table 3) is only one of many possible effects and was included to emphasize the importance of strict adherence to standard operating conditions if reproducible results and valid comparisons are to be made.

Because the major cellular constituents of species in a genus are relatively uniform (see range of variation in Table 2), they can be useful in indicating the genus to which organisms with characteristics unlike those of any described species might be assigned or in excluding unknown strains from particular genera.

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