APPLIED MICROBIOLOGY, Sept. 1968, p. 1406-1413
Vol. 16, No. 9

Cellular Fatty Acid Composition and Identification of Rumen Bacteria[1]

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Received for publication 14 May 1968

The fatty acid compositions of 21 pure cultures of rumen bacteria, representing 12 genera and 14 species, were compared as methyl esters. Each organism possessed a consistent and reproducible fatty acid profile. Overlapping similarities and differences in composition did not allow differentiation between families or genera. Although species differentiation was possible, fatty acid composition appeared to be only an aid in the identification of bacteria.

Many species of microorganisms are present in considerable concentrations in the rumen. These concentrations vary from time to time or from animal to animal. Examination of the causes of these variations in concentration has had to wait for adequate techniques for characterizing the microbial population. Methods of identifying rumen bacteria have previously been based on determination of morphological and biochemical characteristics (5, 8, 20). Such methods, however, are difficult and time-consuming. Recently, gas-liquid chromatography (GLC) has been used to characterize bacteria from environments other than the rumen (16, 17, 19, 22). Abel et al. (1) have noted similarities in the fatty acid composition of strains of the family *Enterobacteriaceae* and significant differences among the strains of selected families of the class *Schizomycetes*. Cellular lipid patterns of various strains of *Clostridium* were also investigated by Moss and Lewis (15). Although the age of the culture and type of growth medium influenced the relative proportions of certain fatty acids, such differences did not limit species differentiation.

In the present study, GLC was used to determine the fatty acid composition of pure cultures of some important rumen bacteria (5). The resulting chromatograms were compared to determine whether GLC is a sensitive and rapid method for identification and differentiation of these bacteria.

[1] Published with the approval of the Director of the Purdue University Agricultural Experiment Station as Journal Series Paper No. 3360.

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MATERIALS AND METHODS

Microorganisms. Twenty-two bacterial strains were examined. The majority of these organisms were obtained from M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, B. A. Dehority, Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, and D. R. Caldwell, U.S. Department of Agriculture, Beltsville, Md. These cultures comprised two groups. Group I: order *Pseudomonadales*, (i) family *Pseudomonadaceae*, *Succinimonas amylolytica* N6; (ii) family *Spirillaceae*, *Succinivibrio dextrinosolvens* 24, two strains of *Butyrivibrio fibrisolvens* (D1 and A38), *Selenomonas ruminantium* HD1, and *Lachnospira multiparus* 40. Group II: order *Eubacteriales*, (i) family *Bacteroidaceae*, *Bacteroides amylophilus* 78, three strains of *B. succinogenes* (S85, B21a, and A3c), *B. symbiosus* ATCC 14940 (a nonrumen organism), and a culture tentatively identified as *Fusobacterium* sp. 9-21 (4, 5); (ii) family *Lactobacillaceae*, *Peptostreptococcus elsdenii* B159, four strains of *Ruminococcus flavefaciens* (C1a, B34b, C94, and FD1), two strains of *R. albus* (D89 and 7), *Streptococcus bovis* (isolated from rumen in this laboratory), *Eubacterium ruminantium* B1C23, and *Lactobacillus* sp. (GA1). All rumen bacteria examined have been described by Bryant (4).

Preparation of cells. The cultures were grown in rumen glucose-cellobiose agar (6) at 39 C. The organisms were transferred three times on the general-purpose medium devised by Bryant (5). This medium contained 0.5% (w/v) cellobiose, 0.5% (w/v) glucose, minerals, casein hydrolysate (BBL; 0.5% w/v), volatile fatty acids (isovaleric, *n*-valeric, isobutyric, and 2-methyl butyric), a carbonate-bicarbonate buffer system, and cysteine. A 5-ml sample of the actively growing culture was transferred to a 2-liter round-bottom flask containing 1 liter of sterile general-purpose medium. The flask was fitted with a culture tube (16 by 150 mm) to allow for optical density (OD) determination. The flask

was thoroughly gassed with carbon dioxide, stoppered, and incubated at 39 C. Unless otherwise stated, the cells were harvested by centrifugation at 4 C in the logarithmic growth phase. At this time, the OD, as measured at 520 nm with a Beckman model B spectrophotometer, was approximately 1.0. The cells were then washed with isotonic saline solution, suspended in distilled water, and lyophilized.

Extraction of lipids. Quantities of cells (50 mg) were suspended in an ethyl alcohol–water (4:1) mixture and were sonically treated with a Branson Sonifier until complete disruption occurred. Saponification was carried out at room temperature in 95% ethyl alcohol containing 5% potassium hydroxide. The reaction flasks were flushed with nitrogen, stoppered, and allowed to stand at room temperature overnight. Distilled water was added, and the *p*H was adjusted to 2. Unsaponifiable matter was not removed. Fatty acids were extracted with purified petroleum ether–diethyl ether (4:1), washed with distilled water until the wash water was neutral, dried over sodium sulfate, and evaporated in a rotary vacuum evaporator to give the free fatty acids.

Preparation of methyl esters. The concentrated fatty acids were transferred to a 15-ml centrifuge tube with the aid of 10 ml of diethyl ether–methanol mixture (8:2), and esterification was carried out with diazomethane according to the method of Schlenk and Gellerman (18). In some cases, it was necessary to purify the methyl ester fraction by thin-layer chromatography.

Gas chromatography. The instrument employed for gas chromatography consisted of a model 600B Aerograph Hy-Fi (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) equipped with a hydrogen flame ionization detector.

Samples were analyzed on both polar and nonpolar liquid-phase coated supports in glass columns [60 by ⅛ inch (152.4 by 0.32 cm)]. The polar phase (A) was 15% HI EFF-1AP (diethylene glycol adipate) and the nonpolar phase (B) was 15% Apiezon L. Each phase was on 60/80 mesh Gas Chrom P. Both column-packing materials were prepared by Applied Science Laboratories, State College, Pa. The column temperatures were 170 and 200 C, and the flow rates of nitrogen carrier gas were 26 and 20 ml/min for A and B columns, respectively.

Identification of fatty acids. Fatty acids were identified by comparison with relative retention volumes of purified standard methyl esters of saturated, unsaturated, iso-, and anteiso fatty acids obtained from Hormel Institute, St. Paul, Minn., and Applied Science Laboratories, State College, Pa. Plots of carbon atoms versus $\log_{10}$ retention volumes of standard fatty acids were made for both columns. The relationship between the $\log_{10}$ relative retention volumes of each component was also compared on both columns. For further identification of unsaturated fatty acids, these acids were brominated and rechromatographed on both columns. The fatty acid pattern or chromatographic signature for each organism was obtained. The percentage of each fatty acid peak was calculated from the ratio of its peak area to the total area for all peaks. Only acids which were present at levels greater than 1% were considered.

To determine reproducibility of the fatty acid ester profile, duplicate experiments were done. Each culture was carried through the growth, harvest, extraction, methylation, and chromatography procedures at different times.

RESULTS

Results of duplicate experiments with several strains of rumen microorganisms are shown in Table 1. In general, identical chromatograms were obtained for duplicate analyses. In a few instances, additional small peaks appeared which were within experimental variation and did not significantly change the pattern. The largest variation observed between duplicates did not exceed 8%.

Several investigators (1, 13, 14) have observed

TABLE 1. *Comparison of duplicate analyses of percentage fatty acid composition of some strains of rumen microorganisms*

Fatty acid[a]	*Butyrivibrio fibrisolvens* D1		*Succinivibrio dextrinosolvens* 24		*Bacteroides amylophilus* 78	
	I	II	I	II	I	II
11:1			2.3	2.8		
12:0	3.2	1.8	3.1	3.3	7.1	6.8
13:0 ant			2.5	3.2		
13:0	2.4	1.9			1.2	1.1
14:0 iso	2.1	1.7	4.7	4.7		
14:0	2.7	2.3	22.5	22.2	18.8'	17.6
14:1			4.0	4.4	2.5	2.4
15:0 iso	2.8	2.4				
15:0 ant			21.4	22.2		
15:0	3.4	3.8	10.6	11.0	1.5	2.4
Unidentified[b]					2.8	2.5
16:0 iso			10.6	11.0		
16:0	33.5	34.5	7.5	7.0	31.4	32.0
16:1	7.5	8.4			5.8	5.3
17:0 iso	5.8	6.2				
17:0 ant			8.9	8.6		
17:0	4.8	5.0				
18:0 iso	5.1	5.4				
18:0 ant	3.2	3.5	2.0	2.6		
18:0	4.5	3.2				
18:1	11.7	11.8			22.4	22.6
19:0 iso	7.3	8.1				
19:0					4.1	5.4
19:cy					1.2	2.0

[a] Number to left of colon refers to number of carbon atoms; number to right of colon refers to number of double bonds; iso denotes branched chain, ant denotes anteiso, and cy denotes cyclopropane acid.

[b] Retention volume relative to methyl palmitate of unidentified peak is 0.79.

variation in fatty acid composition between young and old cultures, with young cultures sometimes showing larger percentages of unsaturated fatty acids. Nevertheless, most workers have recommended a culture age of approximately 18 hr (15, 19). In the present study, *R. flavefaciens* was harvested at different OD values corresponding to the middle of the logarithmic, stationary, and death phases. Except for C15:0 iso and C15:1 iso, the results in Table 2 showed no differences between the growth phases in the type of fatty acids present and only minor differences in concentration. However, the concentration of C15:0 iso was about 10% lower in the stationary phase than in both the logarithmic and death phases. Furthermore, the C15:1 iso which appeared in the logarithmic and stationary phases could not be detected in the death phase. Due to these differences, the logarithmic growth phase was used in all of the experiments in the present study.

Another factor which has been shown to influence fatty acid composition in microorganisms is the growth medium (1, 14, 15). This factor was tested in an experiment in which the fatty acid composition of *Fusobacterium* sp. 9-21 was compared on the general-purpose medium (5) and on Bragg and Reeves (3) medium. No significant differences in the relative amounts or types of the principal fatty acids were found (Table 3) between these two media.

TABLE 2. *Comparison of the percentage composition of the principal fatty acids of Ruminococcus flavefaciens B34b in different growth phases*

Fatty acid[a]	Growth phase		
	Logarithmic (OD, 0.95)	Stationary (OD, 1.24)	Death (OD, 1.07)
12:0	6.4[b]	5.9	4.9
Unidentified[c]	3.4	2.9	2.3
14:0 iso	3.0	2.8	1.8
Unidentified[d]	—	3.1	4.6
15:0 iso	37.6	25.4	35.6
15:1 iso	11.4	10.4	—
15:0	6.2	4.0	5.8
15:1	3.9	4.4	2.1
16:0 iso	3.6	4.3	3.4
16:1 iso	2.5	3.9	4.8
16:0	1.8	4.3	5.7
17:0 iso	6.6	5.8	6.6
17:1 iso	6.0	4.7	—
Unidentified[e]	Tr	3.3	Tr
18:0 ant	6.6	4.7	2.6
18:0	—	3.4	3.5
18:1	—	Tr	3.6
19:1	3.4	6.7	8.8
20:0 iso	Tr	Tr	4.0

[a] *See* footnote *a* of Table 1.

[b] Numbers refer to percentages of total acids; Tr indicates less than 1.0%; dash indicates acid was not detected.

[c–e] Retention volumes relative to methyl palmitate of unidentified peaks are: *c*, 0.35; *d*, 0.44; *e*, 1.66.

TABLE 3. *Comparison of cellular fatty acids of Fusobacterium sp. 9-21 on different media*

Fatty acid[a]	General-purpose medium	Bragg and Reeves medium
12:0	2.8	8.4
15:0 iso	58.4	58.8
15:0	6.8	5.5
16:0 iso	5.6	—[b]
16:0	10.0	4.8
17:0 iso	11.6	10.4
18:0 ant	—	3.2
18:0	4.0	2.6
18:1	3.7	2.8
19:cy	—	3.4

[a] *See* footnote *a* of Table 1.

[b] Not detected.

Figures 1 to 5 show the gas chromatographic profiles of fatty acid methyl esters isolated from bacteria representing 12 different genera and 15 species. In general, fatty acids ranging from C12 to C18 were observed. In all cultures, esters of C12 and C16 acids were found in various concentrations. The average percentages of the component fatty acid esters are presented in Table 4.

Differentiation between orders, families, and genera. When the lipid composition of organisms belonging to the order *Pseudomonadales* was compared with the lipid composition of organisms belonging to the order *Eubacteriales*, no consistent differences were observed. Furthermore, it was not possible to differentiate between families within an order or genera within one family due to overlapping similarities and differences in fatty acid composition (Table 4).

Differentiation between species. When lipid composition was considered as a basis for species differentiation, the results appeared to be more promising. Among the different species of *Bacteroides*, the most important feature of *B. succinogenes* was its high content of C13:0 and C15:0 acids (Table 4). Wegner and Foster (21) reported that 99% of the radioactivity of *n*-valerate-*l*-^{14}C was incorporated into *n*-C13:0 and *n*-C15:0 fatty acids, which comprised more than 50% of the total lipids of *B. succinogenes*. Both *B. amylophilus* and *B. symbiosus* contained high concentrations of palmitic acid; however, the former contained larger amounts of C14:0 and C18:1 acids than the latter. Heptadecanoic acids (straight chain,

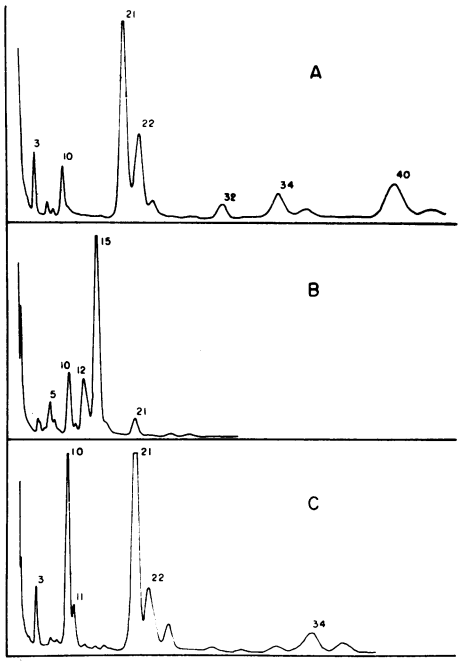

FIG. 1. *Gas chromatographic methyl ester profiles of fatty acids from (A) Lactobacillus sp. GA1, (B) Eubacterium ruminantium B1C23, and (C) Lachnospira multiparus 40. Numbers on fatty acid peaks refer to identity of acids as shown in Table 4.*

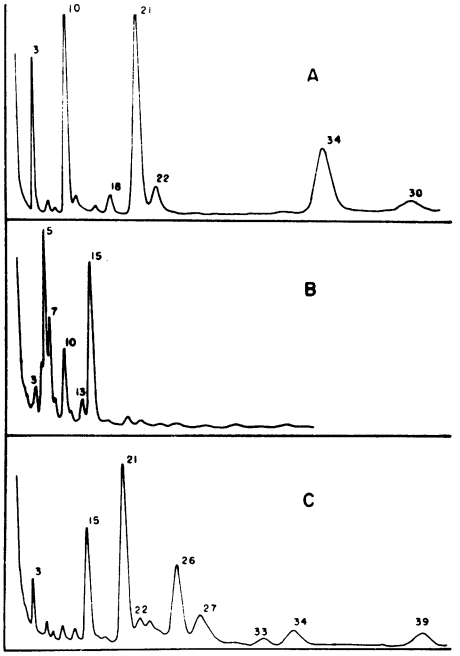

FIG. 2. *Gas chromatographic methyl ester profiles of fatty acids from (A) Bacteroides amylophilus 78, (B) B. succinogenes S85, and (C) B. symbiosus ATCC 14940. Numbers on fatty acid peaks refer to identity of acids as shown in Table 4.*

iso- and mono-unsaturated) were detected in *B. symbiosus*; these acids were not detected in the other two *Bacteroides* species. Of the ruminococci, iso-pentadecanoic acid was present in *R. flavefaciens* in concentrations above 25% of the total fatty acid content, whereas it was absent in *R. albus*. The opposite relationship was found for an unidentified fatty acid with a retention time of 0.44 relative to methyl palmitate. Similar findings were reported by Allison et al. (2) and Keeney et. al. (12). These workers found that *R. flavefaciens* incorporated most of the radioactivity from isovalerate-*l*-^{14}C into branched-chain 15-carbon fatty acid, whereas *R. albus* preferably incorporated isobutyrate-*l*-^{14}C into branched-chain 14- and 16-carbon fatty acids.

Differentiation between strains. Strain B21a of *B. succinogenes* differed from strains S85 and A3c by the absence of C13:1; this fatty acid was present in strains S85 and A3c in concentrations above 10%. Strains S85 and A3c were almost identical in their lipid composition and could not be differentiated.

The most distinguishing feature of *R. flavefaciens* strain C1a was the presence of C15:0 iso in a concentration above 50%. The only significant difference in composition between strains B34b and FD1 was the higher concentration of palmitate (12.5%) found in strain FD1, as compared to 1.8% for strain B34b. Strain C94 could be differentiated from the other three strains by the presence of C16:0 iso in a relatively high concentration (12.5%). This acid was not detected in strain FD1 and was present in much lower concentrations in strains C1a and B34b. It was also possible to differentiate between strains D89 and 7 of *R. albus*. Strain D89 contained a higher concentration of C14:0 iso than strain 7. On the other hand, strain 7 contained C18:0 Br, which could not be detected in strain D89. Examination of the fatty acid composition of strains A38 and D1 of *B. fibrisolvens* showed them to be identical; therefore, these strains could not be differentiated.

DISCUSSION

The above results demonstrate little relationship between cellular fatty acid composition of rumen bacteria and their classification except at the species level. As a result of overlapping similarities and differences in fatty acid composition, it was not possible to differentiate between orders, families, or genera. Abel et al. (1) were able to differentiate between bacteria belonging

TABLE 4. *Percentage composition of the fatty acid methyl esters of representative strains of rumen microorganisms*

Fatty acid[b]		Test organism[a]																					
		Sa-N6	Bf-A38	Bf-D1	Lm-40	Sd-24	Sr-HD1	Ba-78	B-sy	Bs-S85	Bs-B21a	Bs-A3c	F 9-21	Pe-B159	Rf-Cla	Rf-B34b	Rf-C94	Rf-FD1	Ra-D89	Ra-7	Sb	Er-B1C23	L-GA1
1. 11:0	(0.17)[c]						6.8																
2. 11:1	(0.18)					2.6																	
3. 12:0	(0.23)	4.8	4.1	2.5	4.5	3.2	3.2	7.0	2.6	4.4	8.2	2.5	2.8	17.2	7.6	6.4	6.8	2.7	3.4	3.0	3.2	2.3	3.8
4. 13:0 a t	(0.30)					2.8				8.9	6.6	2.6											
5. 13:0	(0.33)	1.6	2.6	2.2	1.6		6.2	1.2	1.6	19.8	14.2	19.5		4.0							1.4	5.4	1.6
6. Unidentified	(0.35)														4.4	3.4	2.9	1.6	1.4	1.4			
7. 13:1	(0.36)									12.5		13.7										2.9	
8. 14:0 iso	(0.40)	1.6	2.2	1.9	2.8	4.7	2.0		1.6	3.8	4.4	2.9		4.0	4.2	3.0	4.7	3.2	21.4	13.2	1.2		1.4
9. Unidentified	(0.44)																		13.2	10.2			
10. 14:0	(0.48)	4.4	2.8	2.5	15.5	22.4	2.2	18.2	2.0	9.0	6.0	6.5		4.8			4.7	3.6	7.9	2.9	8.0	10.4	4.6
11. 14:1	(0.52)				5.1	4.2		2.4		2.9		2.5							1.2	2.8	2.8	3.5	
12. 15:0 iso	(0.58)	52.6		2.6			3.2		2.2				58.4		53.5	37.6	29.5	28.6				17.7	
12a. Unidentified	(0.60)				2.0																		
13. 15:0 ant	(0.62)					21.8				4.9	7.7	3.0		6.0					9.6	6.4			
14. 15:1 iso	(0.64)															11.4		14.5					
15. 15:0	(0.69)	5.0	4.8	3.6	1.6	10.8	18.0	1.4	10.8	25.6	35.1	27.6	6.8			6.2		11.3	8.9	6.8	1.9	49.0	
16. 15:1	(0.76)		2.2				27.0									3.9		7.0					
17. Unidentified[d]	(0.76)				1.4																		
18. Unidentified	(0.79)							2.8						7.0									
19. 16:0 iso	(0.83)	5.3				10.8				3.0	7.2	1.8	5.6		6.3	3.6	14.5		7.0	12.4			
20. 16:1 iso	(0.94)															2.5	9.7						
21. 16:0	(1.00)	7.5	34.5	34.0	35.0	7.2	2.2	31.7	21.5	2.7	6.2	3.0	10.0	11.6	5.7	1.8	7.4	12.5	23.2	29.1	31.8	5.8	32.4
22. 16:1	(1.10)		8.8	8.0	13.5		3.5	5.6	8.2	2.3	2.4	1.3		8.6			5.8	3.0		1.6	12.3		17.8
23. 17:0 iso	(1.20)	10.8	5.4	6.0					7.3				11.6		8.6	6.6				2.2			
23a. Unidentified	(1.26)				5.6																		4.8
24. 17:0 ant	(1.29)					8.8									7.5					2.4		1.4	
25. 17:1 iso	(1.35)															6.0	5.7	1.8					
26. 17:0	(1.44)		5.4	4.9			2.7		15.4			1.2		3.8						2.4		1.5	
27. 17:1	(1.59)						11.5		12.3														
28. Unidentified[d]	(1.59)				2.0																		
29. Unidentified	(1.66)													4.5			4.3						
30. 18:0 iso	(1.74)		4.8	5.2																			
31. Unidentified	(1.82)				2.0		6.5																

32.	18:0 ant	(1.86)		2.2	2.8	3.3		2.3				2.8	1.8		5.2	4.7	6.6	8.2	2.6			8.1		2.9
33.	18:0	(2.09)			2.1	3.8	2.2		7.5		3.6			4.0	4.4				3.0	1.9	3.1	6.9		
34.	18:1	(2.30)		4.0	9.4	11.8	7.6			22.5	7.0			3.7	11.4				2.7			22.3		9.2
35.	19:0 iso	(2.51)			5.2	7.7																		
36.	Unidentified	(2.64)					3.6																	4.1
37.	19:0 ant	(2.69)													3.6									
38.	19:0	(3.02)								4.8							3.4							
39.	19:1	(3.31)									3.9													
40.	19:cy	(3.31)								1.6			8.1		3.9									14.0
41.	20:0 iso	(3.64)																						3.6

[a] Sa-N6, Bf-A38, Bf-D1, Lm-40, Sd-24, Sr-HD1, Ba-78, B-sy, Bs-S85, Bs-B21a, Bs-A3c, F 9-21, Pe-B159, Rf-C1a, Rf-B34b, Rf-C94, Rf-FD1, Ra-D89, Ra-7, Sb, Er-B1C23, and L-GA1 refer to *Succinimonas amylolytica* N6, *Butyrivibrio fibrisolvens* A38, *B. fibrisolvens* D1, *Lachnospira multiparus* 40, *Succinivibrio dextrinosolvens* 24, *Selenomonas ruminantium* HD1, *Bacteroides amylophilus* 78, *B. symbiosus*, ATCC 14940, *B. succinogenes* S85, *B. succinogenes* B21a, *B. succinogenes* A3c, *Fusobacterium* sp. 9-21, *Peptostreptococcus elsdenii* B159, *Ruminococcus flavefaciens* C1a, *R. flavefaciens* B34b, *R. flavefaciens* C94, *R. flavefaciens* FD1, *R. albus* D89, *R. albus* 7, *Streptrococcus bovis*, *Eubacterium ruminantium* B1C23, and *Lactobacillus* sp. GA1, respectively.

[b] *See* footnote *a* of Table 1.

[c] Numbers in parentheses refer to the retention volume relative to methyl palmitate.

[d] Appeared with same relative retention time as monounsaturated acid but by bromination showed no unsaturation; probably a cyclopropane acid.

FIG. 3. *Gas chromatographic methyl ester profiles of fatty acids from (A) Butyrivibrio fibrisolvens D1, (B) Succinivibrio dextrinosolvens 24, and (C) Selenomonas ruminantium HD1. Numbers on fatty acid peaks refer to identity of acids as shown in Table 4.*

to related families and genera. However, their findings were based on rather limited numbers of organisms examined for each family. To determine whether differentiation is possible, all known bacteria within one family (or genus) should not only show consistent similarity in composition of certain fatty acids, but should also be distinctly different from all members of other families.

It is known that biosynthesis of fatty acids in bacteria represents the activity of a variety of enzymes. In this study, it appears that many of these enzymes are commonly distributed among the organisms examined. This may account for similarities in lipid composition of unrelated genera and families.

Ikawa (9) and Kates (11) indicated a close phylogenetic relationship between the phosphatide content and classification of bacteria. In the present experiments, the whole-lipid contents of bacterial cells were examined. It would be of interest to learn whether certain lipid fractions or possible amino acid or carbohydrate contents could be used for classification purposes.

Successful differentiation among closely related species and among certain strains of bacteria was accomplished, as was the case in the

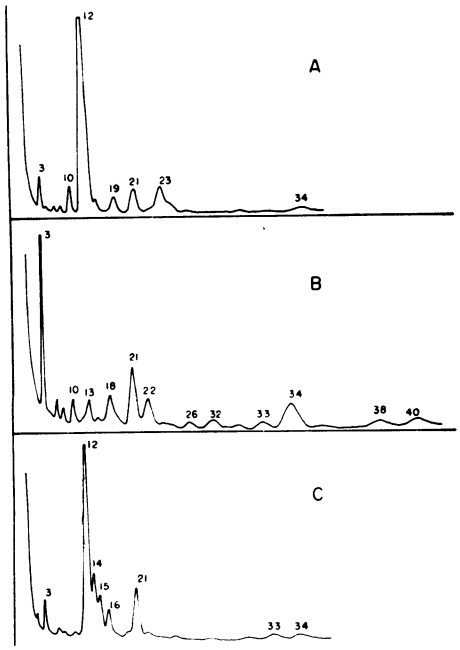

FIG. 4. *Gas chromatographic methyl ester profiles of fatty acids from (A) Succinomonas amylolytica N6, (B) Peptostreptococcus elsdenii B159, and (C) Ruminococcus flavefaciens FD1. Numbers on fatty acid peaks refer to identity of acids as shown in Table 4.*

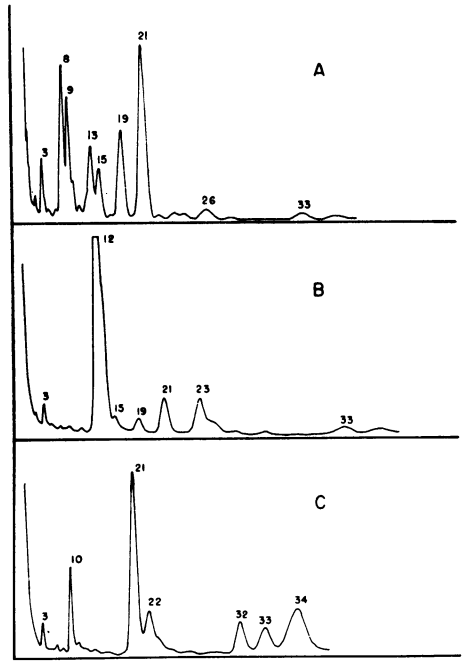

FIG. 5. *Gas chromatographic methyl ester profiles of fatty acids from (A) Ruminococcus albus 7, (B) Fusobacterium sp. 9-21, and (C) Streptococcus bovis. Numbers on fatty acid peaks refer to identity of acids as shown in Table 4.*

studies of Moss and Lewis (15). However, we could not differentiate between strains S85 and A3c of *B. succinogenes* or between strains A38 and D1 of *Butyrivibrio fibrisolvens*. It is possible that strain differentiation could be improved by further GLC studies. Furthermore, Bryant and Small (7), in their original publication on strains A38 and D1, showed very few differences in biochemical characteristics between these strains.

Visual examination of chromatograms of fatty acid methyl ester profiles showed that they were consistent and reproducible for each organism. The agreement between our results and those reported by other workers indicates that the fatty acid composition of bacteria under standard conditions is a stable characteristic. It appears, therefore, that fatty acid composition is only an aid in the rapid identification of bacteria. Thus, if an organism has been preliminarily identified by gross morphology and a few biochemical tests, its identity can be confirmed by its fatty acid composition. This is important in ruminant studies, because it is sometimes necessary to establish rapidly the identity of the predominating bacteria in the rumen.

The logarithmic growth phase appeared to be optimal in our studies. In fact, preliminary experiments in this laboratory showed no significant difference in fatty acid composition of rumen bacteria between early and late log phase. Obviously, the physiological age of the culture depends on the generation time. Experiments in this laboratory on lactobacilli showed that the cultures reach the logarithmic growth phase in about 4 hr. To be consistent, we chose an OD of 1.0 in our studies rather than time in hours. At this OD, all cultures examined were in late log phase. No explanation can be given for the decrease in the percentage of C15:0 iso in the stationary phase or the absence of C15:1 iso in the death phase. These fatty acids may have been converted to other components of the cell.

The growth media did not appear to change significantly the principal fatty acid content of the cells. This is probably due to the fact that the media used in these experiments, Bragg and Reeves medium (3) and the general-purpose medium of Bryant (5), are similar nutritionally. This does not imply that the growth medium, regardless of its composition, will have no effect on the fatty acid content of the organism. Kanegasaki and Takahashi (10) found that *Selemonas ruminantium* produces C12:0, C14:0, and C16:0

acids when grown with *n*-caproate rather than *n*-valerate. However, we did not think that it was necessary to examine other growth media, because rumen bacteria are rather fastidious in nutritional requirements and are difficult to grow. The general-purpose medium was very satisfactory for our studies.

ACKNOWLEDGMENTS

We are indebted to M. P. Bryant, K. A. Pittman, D. R. Caldwell for gifts of cultures of rumen bacteria.

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