APPLIED MICROBIOLOGY, Mar., 1967, p. 390-397
Vol. 15, No. 2

Printed in U.S.A.

Characterization of Clostridia by Gas Chromatography

I. Differentiation of Species by Cellular Fatty Acids

C. WAYNE MOSS AND VESTER J. LEWIS
Communicable Disease Center, Atlanta, Georgia

Received for publication 8 November 1966

Fatty acids of 41 strains representing 13 species of *Clostridium* were extracted directly from whole cells and examined as methyl esters by gas-liquid chromatography. Both visual and quantitative comparisons of the resulting chromatograms for the presence and relative amounts of large major peaks allowed rapid differentiation of *C. perfringens*, *C. sporogenes*, and *C. bifermentans* from each other and from 10 other species. Each of the three former species possessed a different characteristic fatty acid methyl ester profile that was exhibited by all strains tested within the respective species. Culture age and growth media influenced the relative proportions of certain of the acids, but such differences did not limit species differentiation.

In recent years, there has been increased interest in the possibility of utilizing gas-liquid chromatography (GLC) for rapid characterization of microorganisms. Several different GLC approaches to attain this goal have been made. These include analysis of pyrolysis products of whole bacterial cells (6, 13), of media extracts or head-space gas for metabolic products synthesized by the microorganisms during their growth (2, 3, 8), and of specific chemical compounds extracted directly from cells (1, 4, 15). Abel, DeSchmertzing, and Peterson (1), using the latter approach, were the first to employ GLC for classification of bacteria. These workers were able to distinguish among families of *Bacillaceae*, *Enterobacteriaceae*, *Parvobacteriaceae*, and *Micrococcaceae* by GLC analysis of cellular fatty acids. In a similar study, Brown and Cosenya (4) were able to distinguish between the genera *Gaffkya* and *Micrococcus* of the family *Micrococcaceae*, and, in a recent study, Yamakawa and Ueta (15) demonstrated differentiation of bacteria at the species level. These workers (15) were able to differentiate *Neisseria haemolysans* from other *Neisseria* species by GLC analysis of cellular fatty acids and carbohydrate.

Only limited studies of the fatty acid composition of organisms comprising the biochemically diverse genus *Clostridium* have been reported (7, 11, 14). A species of this genus, *C. perfringens*, is of much interest by virtue of its infectivity for man and animals and of its role in outbreaks of food poisoning. This species consists of a group of related organisms which vary in source, pathogenicity, cellular antigens, and in toxin production, yet are indistinguishable by present morphological and biochemical criteria. In the present study, a comparison of cellular fatty acid composition of the toxigenic types and of food-poisoning strains of *C. perfringens* was made by GLC to evaluate this technique as a possible tool for rapid identification of these organisms. The fatty acid composition of other *Clostridium* species also was investigated.

MATERIALS AND METHODS

Cultures. All cultures used in this study were obtained from the General Bacteriology Unit, Communicable Disease Center, Atlanta, Ga. The following 41 strains, representing 13 species of the genus *Clostridium* were studied: 1 strain each of *C. capitovale*, *C. chauvoei*, *C. difficile*, *C. histolyticum*, *C. putrefaciens*, *C. septicum*, *C. sordelli*, *C. subterminale*, *C. tetani*, and *C. tertium*; 3 strains of *C. sporogenes;* 4 strains of *C. bifermentans;* and 24 strains of *C. perfringens*. Included among the *C. perfringens* were representative strains of each toxigenic type (A to F) as well as typical food-poisoning strains. All cultures were identified by standard microscopic, cultural, biochemical, and toxigenic tests.

Growth media and cultural procedures. Cultures were grown in either tryptose phosphate broth (TPB) or peptone broth (PB). The same lot of individual ingredients of these two media was available in bulk supply and was used throughout all the experiments. TPB (*p*H 7.2) contained 2% tryptose (Difco), 0.5% glucose (Difco), 0.5% NaCl (Fisher Scientific Co., Fair Lawn, N.J.), 0.25% Na_2HPO_4 (Merck & Co., Inc., Rahway, N.J.), and 0.05% cysteine (Eastman Organic Chemicals, Rahway, N.J.). PB (*p*H 7.2)

contained 3% peptone (Difco), 0.5% glucose (Difco), and 0.45% NaCl (Fisher Scientific Co.). Stock cultures were maintained at 4 C in Cooked Meat Medium (Difco). Cell crops for fatty acid studies were produced by two successive transfers of cultures in TPB (or PB) prior to final transfer into 250 ml of the same medium. All media were boiled for 5 to 10 min and rapidly cooled immediately before inoculation. Cultures were incubated at 37 C under a hydrogen-carbon dioxide-nitrogen (10:10:80) atmosphere in a Brewer anaerobic jar. Unless otherwise noted, cultures were incubated for 15 hr. Cells from the third transfer (250-ml medium) were harvested by centrifugation at 4 C and analyzed without washing. Preliminary studies indicated the presence of fatty acids in the growth medium but not in amounts sufficient to affect the results reported here.

Extraction procedures. Packed cells from centrifugation were transferred to a 30-ml ground-glass flask, which then was attached to a small Soxhlet extraction assembly. Fatty acids were extracted by direct saponification of the cells under nitrogen for 4 hr at 70 C with 15% KOH in 50% methanol. Nonsaponifiable material was extracted with two portions of an ether-hexane mixture (1:1) and discarded. The residue (aqueous layer) was acidified to *p*H 2 by dropwise addition of concentrated HCl, and the fatty acids were extracted with three portions of the ether-hexane mixture. The extracts were combined in a 50-ml beaker and the solvent was evaporated at room temperature under a gentle stream of nitrogen. The residues obtained were transferred to a test tube (20 by 150 mm) by two successive rinsings with 2-ml samples of Boron-Trichloride-Methanol Reagent (Applied Science Laboratories, Inc., State College, Pa.). The mixture was heated at 80 C for 5 min, cooled, and transferred to a separatory funnel containing 30 ml of the ether-hexane mixture (1:1) and 30 ml of distilled water. This was shaken vigorously and the ether-hexane layer was removed and evaporated to dryness as described above. The methyl esters were reconstituted to a final volume of approximately 0.5 ml with hexane and transferred to a 12 by 75 mm test tube fitted with a Teflon-lined screw cap. The methyl esters were stored at −20 C.

GLC analysis. The methyl esters, prepared as indicated above, were analyzed by use of a Barber-Colman model 5000 gas chromatograph (Barber-Colman Co., Rockford, Ill.), equipped with a hydrogen-flame ionization detector and a disc integrator recorder (Series 8000). Operating parameters of the instrument were: injection temperature, 220 C; detector temperature, 230 C; column temperature 110 C for 5 min then temperature programmed to 195 C at 5 C per min; carrier gas, nitrogen. All samples were analyzed with both polar and nonpolar liquid-phase coated supports in 6-ft (1.8 meter) U-tube glass columns. The polar phase was 12% ethylene-glycol adipate on Chromosorb P (80 to 100 mesh), and the nonpolar phase was 2% SE30 methyl silicone rubber gum (SE30), also coated on Chromosorb P (80 to 100 mesh). Both of the column-packing materials were prepared commercially (Applied Science Laboratories, Inc.).

For routine analysis, 3 to 5 μliters of the methyl esters was analyzed for 60 min after injection onto both the EGA and SE30 columns under the above-stated conditions. Fatty acid methyl ester peaks were identified by comparison of retention times on both columns with those of highly purified methyl ester standards (Applied Science Laboratories and National Institutes of Health). Relative retention times for unidentified fatty acids indicated in the text were calculated by use of methyl myristate as reference. Peak areas were determined from disc integrator data, and the percentage of each acid was calculated from the ratio of the area of its peak to the total area of all peaks. In this study, only those acids which were present at levels greater than 1% are considered, even though numerous smaller peaks were noted in some cases.

RESULTS

Grouping of Clostridium by cellular fatty acids analysis. Visual comparisons of gas chromatographic methyl ester profiles of fatty acids extracted from 41 strains of *Clostridium* permitted rapid separation into four general groups (Fig. 1). Strains were grouped without difficulty by observing the absence or presence and relative sizes of major peaks. Each group had certain major distinguishing fatty acid methyl ester peaks. Group I had large percentages of both lauric and myristic acid with smaller percentages of palmitic and stearic acid. However, the most obvious distinguishing feature of this group compared to the other groups was the large percentage of lauric acid. Group II was distinguished from other groups by the presence of a large unidentified peak with a retention time of 5.5 min. In addition, this group differed from Group I by having large percentages of palmitic acid, a feature also shared with Group IV, and small percentages of lauric acid, a characteristic also common with Groups III and IV. The distinguishing feature of Group III, in contrast to other groups, was the lack of any one or two major peaks relative to other peaks on the chromatograms. Instead, this group is characterized by the presence of several peaks which have approximately the same relative proportions. These peaks represent C14- through C17-saturated straight-chained acids, C15-saturated branched-chain acid, palmitoleic acid, and an unidentified peak with a retention time of 23.25 min. Characteristic of Group IV are large percentages of myristic, palmitic, and stearic acids with smaller percentages of C16- and C18-monounsaturated straight-chained acids. Quantitative comparison of the percentages of the principal fatty acids from representative strains of each group supports the grouping made by visual observation (Table 1).

Each of the 41 strains used in the study was grown and analyzed two or more times under

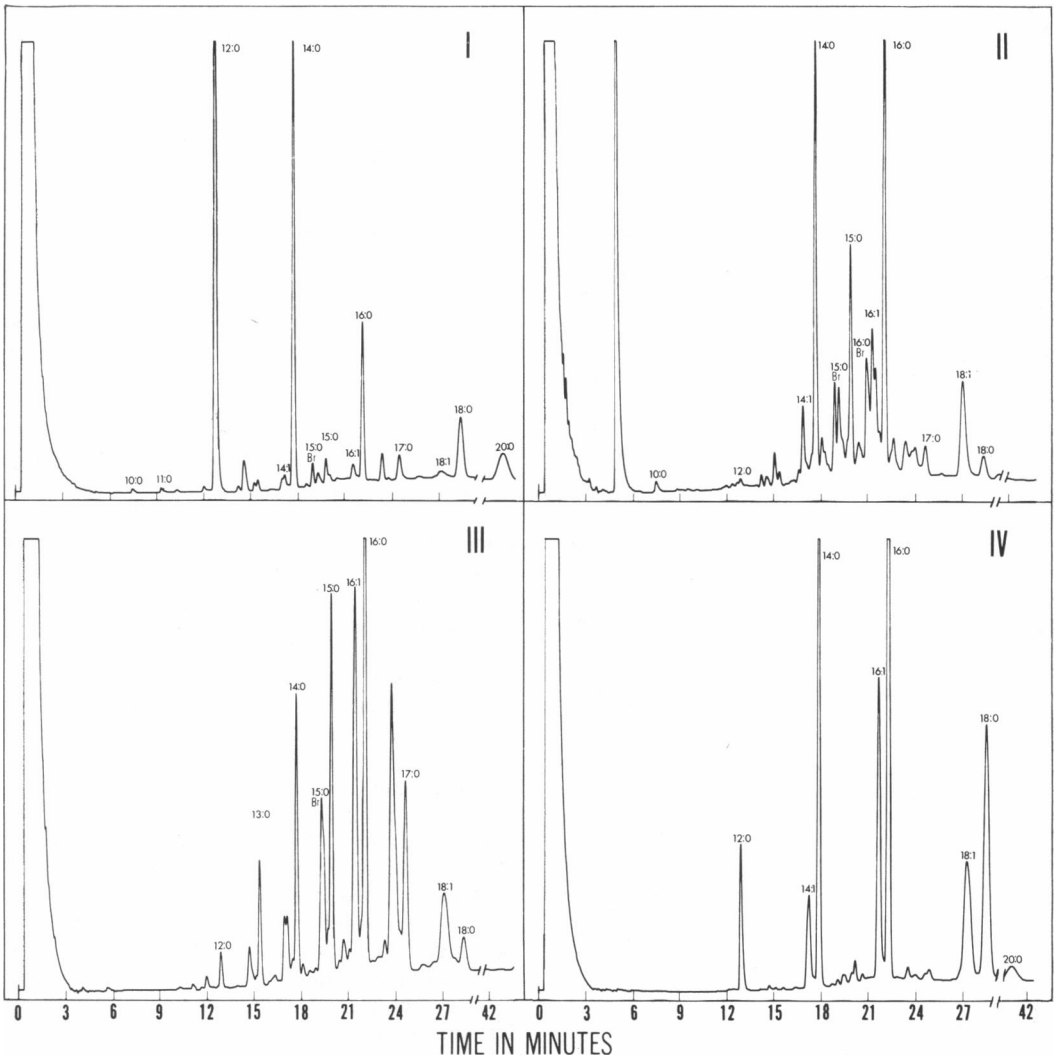

FIG. 1. *Grouping of Clostridium species by comparisons of characteristic gas chromatographic methyl ester profiles of fatty acids extracted from whole cells. Number to left of colon refers to number of carbon atoms; number to right refers to number of double bonds; Br denotes branched chain acid.*

identical experimental conditions to determine whether the fatty acid methyl ester profile produced by each strain was reproducible. In most instances, the profiles were practically identical to those observed in the initial experiment with only minor differences in relative sizes of certain peaks. In no case were differences of such magnitude that group separation was altered. The degree of reproducibility obtained from duplicate experiments with two strains of *C. perfringens* is indicated in Table 2. Differences in the principal fatty acid composition between duplicate analysis of strain E98 did not exceed 2 percentage points; the largest variation in strain KA137 was a difference of 4 percentage points in palmitic acid.

All *C. perfringens* strains studied had similar fatty acid methyl ester profiles (Fig. 1, Group I) which permitted them to be exclusively and homogeneously grouped. None of the 24 strains studied was of doubtful status in regard to grouping since each contained a high percentage of both lauric and myristic acid. Moreover, the absence or presence of only small percentages of lauric acid in the remaining 17 strains, representing several other species, allowed for unmistakable exclusion of each of these strains from the *C. perfringens* group. Differences in the relative proportions of lauric and myristic acids among the *C. perfringens* strains were noted. An indication of the range of differences between these two acids is represented

TABLE 1. *Percentage composition of the principal fatty acids of representative strains of Clostridium species*[a]

Fatty acid[b]	*C. perfringens* (Group I)[c]			*C. sporogenes* (Group II), strain 392	*C. bifermentans* (Group III), strain 409	*C. histolyticum* (Group IV), strain KA84
	Strain Hobbs 3	Strain KA137	Strain E98			
Unidentified[e]	0[d]	0	0	22	Tr	0
10:0	0	Tr	3	Tr	0	Tr
11:0	0	Tr	Tr	0	Tr	0
12:0	24	31	33	Tr	2	6
Unidentified[f]	4	3	Tr	1	2	1
13:0	Tr	Tr	Tr	Tr	7	0
14:1	3	1	4	3	6	8
14:0	24	19	20	17	8	33
15:0Br	3	2	3	5	10	6
15:0	4	2	2	7	12	8
16:0Br	2	Tr	4	5	Tr	0
16:1	5	1	4	5	11	10
16:0	8	8	8	24	13	20
Unidentified[g]	2	5	3	1	14	1
17:0	4	5	2	1	8	1
18:1	4	2	2	7	5	4
18:0	8	7	9	2	2	2
Unidentified[h]	Tr	6	Tr	0	Tr	0
20:0	5	8	3	0	Tr	Tr

[a] Strains representing each species were processed under identical experimental conditions: 15 hr of incubation in tryptose phosphate broth, extraction, methylation, and gas-liquid chromatography analysis.

[b] Number to left of the colon refers to number of carbon atoms; number to right refers to number of double bonds; Br denotes branched chain acid.

[c] Group designation as in Fig. 1.

[d] Numbers refer to percentages of total acids; Tr indicates less than 1%.

[e–h] Relative retention time (SE30 column) of unidentified peaks: *e*, 0.25; *f*, 0.82; *g*, 1.34; *h*, 1.89.

TABLE 2. *Results of duplicate analyses of the principal fatty acid composition of Clostridium perfringens*

Fatty acid[a]	Retention time (min) on SE 30 column		Strain KA137 (*C. perfringens* type C)		Strain E98 (*C. perfringens* type E)	
	Actual	Relative	Trial I	Trial II[b]	Trial I	Trial II
10:0	7.50	0.38	Tr[c]	Tr	3	3
12:0	12.75	0.72	31	30	33	31
Unidentified	14.50	0.82	3	6	Tr	Tr
14:1	17.00	0.94	1	1	4	6
14:0	17.75	1.00	19	17	20	20
15:0Br	19.12	1.06	2	1	3	2
15:0	20.00	1.12	2	3	2	4
16:1	21.75	1.22	1	1	4	2
16:0	22.25	1.26	8	12	12[d]	12[d]
Unidentified	23.25	1.34	5	3	3	3
17:0	24.75	1.40	5	4	2	2
18:1	27.12	1.54	2	Tr	2	2
18:0	28.12	1.62	7	9	9	8
Unidentified	33.00	1.89	6	3	Tr	Tr
20:0	41.00	2.36	8	10	3	5

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

[b] Experimental conditions identical to Trial I: incubation for 15 hr in tryptose phosphate broth; extraction, methylation, and GLC analysis.

[c] Numbers refer to percentages of total acids; Tr indicates less than 1%.

[d] Value includes approximately 20% 16:0Br.

in Table 1 for three *C. perfringens* strains. Strain Hobbs 3 contained equal percentages (24%) of lauric and myristic acid; strain E98 contained 33% of lauric acid and 20% of myristic acid. Quantitative data for five additional *C. perfringens* strains showed differing percentages of lauric and myristic acid, but in no instance were differences in the relative proportions of these two acids greater than that shown in Table 1 for strain E98. It should be noted that there were no major differences in the fatty acid methyl ester profiles among the different toxigenic types of *C. perfringens* (A to F) tested, nor were there obvious differences between type A and atypical type A (food poisoning) strains.

Cultures comprising Group II were the three *C. sporogenes* strains and were easily grouped together on the basis of an unidentified peak which eluted from the SE30 column at 5.5 min. This peak, representing 22% of the total fatty acid composition of *C. sporogenes* strain 392 (Table 1), was equally abundant in a second strain, but was present in approximately one-half this concentration in a third strain. The smaller concentration in the third strain did not prohibit grouping these strains together since this peak was unique among the 41 strains tested with the exception of trace amounts in a limited number of other strains. At this time, no attempts have been made to identify this peak other than GLC comparisons of retention times relative to known standards.

The four *C. bifermentans* strains which were studied comprise Group III. Grouping of these strains by visual observations of chromatograms was difficult since there were no unique or major distinguishing peaks. The basis for grouping of the *C. bifermentans* strains was the comparison of relative sizes of several of the major peaks. *C. bifermentans* 409 (Fig. 1, Group III; Table 1) was easily separated from all other strains in the study by the above criterion. However, two other *C. bifermentans* strains contained greater amounts of myristic and palmitic acid relative to other major peaks, resulting in an increase of the percentage of these two acids with a concomitant decrease in the percentage of other acids. As can be seen from Table 1, such a substantial increase in the percentage of these two acids could result in difficulty of placement into Group III or Group IV.

Group IV contains 10 strains which represent the following 10 species of *Clostridium*: *C. capitovale*, *C. chauvoei*, *C. difficile*, *C. histolyticum*, *C. putrefaciens*, *C. septicum*, *C. sordelli*, *C. subterminale*, *C. tetani*, and *C. tertium*. As noted above, strains in this group are characterized by large percentages of myristic, palmitic, and stearic acids with smaller percentages of C16- and C18-monounsaturated straight-chained acids. There were characteristic differences among some species of this group in the relative amounts of certain of the fatty acids (primarily C16:1, C18:0, C18:1). However, the differences noted were not large. Since this study was concerned with gross similarities or differences, no attempt was made to secure other strains to determine the feasibility of further separation of the species in this group.

Effects of age of culture and growth media on fatty acid composition. Inasmuch as the fatty acid composition of bacteria has been shown to be influenced by culture age, experiments were carried out to determine whether age effects would modify the grouping of strains by gross comparisons of GLC fatty acid methyl ester profiles. In this experiment, the fatty acid composition of four *Clostridium* strains (representing each of the four groups) was compared after 5, 15, and 48 hr of growth in TPB. The results (Table 3) show differences in the level of some of the acids at the three stages of growth, but there were no additional peaks in either the 5- or 48-hr cultures. Generally, the largest differences were noted between young cultures (5 hr) and 15-hr cultures, with only minor differences between 15- and 48-hr cultures. The greatest difference, generally, was larger percentages of unsaturated acid (C16:1 and C18:1) in the young, actively growing cultures (5 hr). However, with the exception of *C. sporogenes* 392 (Group II), the differences observed in the levels of some acids, even in 5-hr cultures, would not limit grouping. Nevertheless, our results indicate the most desirable culture age for fatty acid comparison to be 15 or 48 hr. The large unidentified peak with a retention time of 5.5 min, which is characteristic of *C. sporogenes*, was absent in chromatograms from the 5-hr culture of *C. sporogenes* 392, but was present in large quantities in older cultures (15 and 48 hr). The absence of this peak in young, actively growing cells (5 hr) suggests that this unidentified compound may be directly involved in fatty acid synthesis and may accumulate in older cells.

Another factor which has been shown to influence fatty acid composition in other microorganisms is the growth medium (1, 12). This factor was tested in an experiment in which the fatty acid composition of two *C. perfringens* strains was compared after growth in TPB and PB. The results (Table 4) show that growth medium does influence the relative amounts of some of the principal acids. However, with the exception of a difference of 11 percentage points in lauric acid in strain Hobbs 3 and a difference of 12 percentage points in arachidic acid in strain KA137, large differences were not apparent. Further, it should be noted that, even with these dif-

TABLE 3. *Effect of age of culture on fatty acid composition of Clostridium species*

Fatty acid[a]	Species and strain designation											
	C. perfringens (E98)			*C. sporogenes* (392)			*C. bifermentans* (409)			*C. histolyticum* (KA84)		
	5 hr[b]	15 hr	48 hr	5 hr	15 hr	48 hr	5 hr	15 hr	48 hr	5 hr	15 hr	48 hr
Unidentified[d]	0[c]	0	0	0	22	30	0	Tr	Tr	0	0	0
10:0	Tr	3	1	Tr	Tr	Tr	0	0	Tr	Tr	Tr	Tr
11:0	0	Tr	Tr	0	0	0	Tr	Tr	Tr	Tr	0	0
12:0	27	33	33	Tr	Tr	Tr	2	2	1	9	6	1
Unidentified[e]	Tr	Tr	1	1	1	7	3	2	3	1	1	1
13:0	Tr	Tr	Tr	Tr	Tr	Tr	5	7	4	0	0	Tr
14:1	3	4	5	Tr	3	3	6	6	4	8	8	4
14:0	15	20	25	17	17	16	7	8	8	28	33	33
15:0Br	1	3	3	4	5	2	11	10	8	5	6	8
15:0	1	2	3	4	7	8	12	12	8	8	8	8
16:0Br	Tr	3	2	Tr	5	5	Tr	Tr	Tr	0	0	Tr
16:1	9	4	5	8	5	2	12	11	14	15	10	8
16:0	19	9	8	22	24	16	13	13	18	20	20	27
Unidentified[f]	1	3	2	4	1	7	14	14	15	Tr	1	1
17:0	1	2	2	6	1	1	6	8	8	Tr	1	1
18:1	8	2	2	22	7	1	7	5	5	5	4	6
18:0	12	9	4	12	2	2	2	2	3	1	2	2
Unidentified[g]	1	Tr	Tr	0	0	0	Tr	Tr	1	0	0	0
20:0	2	3	4	0	0	0	Tr	Tr	Tr	Tr	Tr	Tr

[a] See footnote *b*, Table 1.
[b] Time of harvest.
[c] Numbers refer to percentages of total acids; Tr indicates less than 1%.
[d–g] Relative retention times of unidentified peaks: *d*, 0.25; *e*, 0.82; *f*, 1.34; *g*, 1.89.

ferences in lauric and arachidic acid, grouping of these strains would not be limited since the major distinguishing fatty acids of *C. perfringens* (lauric and myristic) were present in relatively large amounts in cells grown in either medium.

DISCUSSION

The results of this study have demonstrated the feasibility of differentiating certain species of the genus *Clostridium* by GLC of the methyl esters of their cellular fatty acids. Visual comparisons of GLC fatty acid methyl ester profiles permitted rapid differentiation of *C. perfringens*, *C. sporogenes*, and *C. bifermentans* from each other and from 10 other species of the genus *Clostridium*. Each of these three species possessed characteristic fatty acid methyl ester profiles, and all strains within the species gave similar profiles. These three species were easily differentiated by the absence or presence or the relative amounts of certain large major peaks. Ten other *Clostridium* species could not be separated by the gross comparisons used in this study and were grouped together on the basis of several major peaks. However, smaller but characteristic differences in certain of the major peaks among some of these 10 species were noted and suggested that further species separation may become feasible when smaller differences are considered. Such smaller differences must, however, be greater than the apparent variability inherent in GLC fatty acid analysis, which in this study ranged from 0 to 4 percentage points in relative proportions of certain of the fatty acids in replicate experiments (Table 2).

Further indication that other species of *Clostridium* may be amenable to differentiation by analysis of cellular fatty acid is apparent from the data reported by Goldfine and Block (7), who found that palmitic acid constituted 49% of the cellular fatty acids of *C. butyricum*. In recent studies, we confirmed this observation with a strain of *C. butyricum* obtained through the courtesy of Elizabeth McCoy in which palmitic acid accounted for more than 50% of the total fatty acids (*unpublished data*). Clearly, if this high percentage of palmitic acid is characteristic of *C. butyricum*, this species could also be differentiated by the gross comparison used in this study, since no other species tested contained such high levels of this acid relative to other acids (Table 1).

Changes in fatty acid composition at different stages of growth were noted in each *Clostridium* strain tested, but the degree of change varied

TABLE 4. *Effect of growth media on the fatty acid composition of Clostridium perfringens*

Fatty acid[a]	*C. perfringens* (Hobbs 3)[b]		*C. perfringens* (KA137)[b]	
	Tryptose phosphate broth	peptone broth	Tryptose phosphate broth	Peptone broth
10:0	0[c]	0	Tr	Tr
11:0	0	0	Tr	Tr
12:0	25	36	31	34
Unidentified[d]	4	3	3	3
13:0	Tr	Tr	Tr	0
14:1	3	5	1	3
14:0	24	20	19	16
15:0Br	3	2	2	2
15:0	3	2	2	3
16:0Br	Tr	Tr	Tr	Tr
16:1	5	5	1	4
16:0	10	10	8	7
Unidentified[e]	2	1	5	1
17:0	4	1	5	3
18:1	4	5	2	2
18:0	8	5	7	2
Unidentified[f]	Tr	Tr	6	Tr
20:0	5	5	8	20

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

[b] Incubation time, 15 hr.

[c] Numbers refer to percentages of total acids; Tr indicates less than 1%.

[d–f] Relative retention time (SE 30 column) of unidentified peaks: *d*, 0.82; *e*, 1.34; *f*, 1.89.

among strains (Table 3). Greatest differences occurred in young cultures (5 hr), in which, in general, higher levels of unsaturated acids (C16:1 and C18:1) were found. The level of these acids decreased with increased incubation time, and only minor differences were noted between 15- and 48-hr cultures. Similar age effects have been reported with other bacterial species (1, 5, 9, 10, 12), for which it was found that the decrease in unsaturated acids with increased culture age was due to their conversion to cyclopropane acids. Since cyclopropane acids have been found in other *Clostridium* species (7, 14), this finding may explain the age effects in this study and also account for some of the unidentified peaks. The importance of proper consideration of age effects in comparative studies of fatty acids is dramatically illustrated by the complete absence of the distinguishing characteristic peak from young cells of *C. sporogenes* 392 (Table 3). This peak (retention time = 5.5 min) has not been identified and will be the subject of future studies.

The results of this study of the major fatty acids in *C. perfringens* are in general agreement with the work of MacFarlane (11), except that our strains possessed smaller percentages of arachidic acid. However, when one of our strains was grown in the medium used by MacFarlane (PB), there was an increase in arachidic acid (Table 4) which strongly suggests that this difference may be due to growth medium. In this connection, other workers (1, 12) have noted that the fatty acid composition of other microorganisms is directly influenced by the composition of the growth medium. Even though our limited comparison of media effects indicates no serious restriction to grouping of *C. perfringens* according to the criteria in this study, the results (Table 4) emphasized that this variable must be considered in comparing the fatty acid composition of these species.

The advantages of GLC for the detection and identification of bacteria have been discussed by Abel, DeSchmertzing, and Peterson (1) and more recently by Henis, Gould, and Alexander (8). Our results support their view that this technique may prove to be a powerful tool for bacterial classification. Further, our results with cellular fatty acids, which are amenable to rapid preparation (approximately 15 min) from whole cells by another technique (1), suggest that GLC may be equally valuable in diagnostic laboratories in which speed of identification is a prime consideration. On the other hand, our results indicate that comparisons of cellular fatty acids in certain species may serve only to augment classification by other recognized procedures. This is clearly the case with *C. perfringens*, for which there were no major differences in the fatty acid methyl ester profiles among different toxigenic types (A to F); neither were there differences between type A and atypical type A (food poisoning) strains.

The necessity for further investigation with other *Clostridium* strains for comparison to the results obtained in this study is evident. Among various reasons, these further studies also would help to determine more clearly whether fatty acid composition is a feature of taxonomic significance for the species of the genus *Clostridium*.

ACKNOWLEDGMENTS

We are grateful to Wallis L. Jones for technical assistance. Appreciation is expressed to William B. Cherry for his helpful suggestions during this study.

LITERATURE CITED

1. ABEL, K., H. DESCHMERTZING, AND J. I. PETERSON. 1963. Classification of microorganisms by analysis of chemical composition. I. Feasibility of utilizing gas chromatography. J. Bacteriol. **85**: 1039–1044.
2. BASSETTE, R., AND T. J. CLAYDON. 1965. Characterization of some bacteria by gas chromato-

graphic analysis of head-space vapors from milk. J. Dairy Sci. **48**:775.

3. BAWDEN, R. E., AND R. BASSETTE. 1966. Differentiation of *Escherichia coli* and *Aerobacter aerogenes* by gas liquid chromatography. J. Dairy Sci. **49**:624–627.
4. BROWN, J. P., AND B. J. COSENYA. 1964. Fatty acid composition of lipids extracted from three spherical bacteria. Nature **204**:802–803.
5. CROOM, J. A., J. J. MCNEILL, AND S. B. TOVE. 1964. Biotin deficiency and the fatty acids of certain biotin-requiring bacteria. J. Bacteriol. **88**:389–394.
6. GARNER, W., AND R. M. GENNARO. 1965. Related bacteria differentiated by gas chromatography. Chem. Eng. News **43**:69.
7. GOLDFINE, H., AND K. BLOCK. 1961. On the origin of unsaturated fatty acids in clostridia. J. Biol. Chem. **236**:2596–2601.
8. HENIS, Y., J. R. GOULD, AND M. ALEXANDER. 1966. Detection and identification of bacteria by gas chromatography. Appl. Microbiol. **14**: 513–524.
9. KATES, M., G. A. ADAMS, AND S. M. MARTIN. 1964. Lipids of *Serratia marcescens*. Can. J. Biochem. Physiol. **42**:461–479.
10. LENNARY, W. J., G. SCHEUERBRANDT, AND K. BLOCK. 1962. The biosynthesis of oleic and 10-methylstearic acid in *Mycobacterium phlei*. J. Biol. Chem. **237**:664–671.
11. MACFARLANE, M. G. 1962. Characterization of lipoamino-acids as O-amine acid esters of phosphatidyl-glycerol. Nature **196**:136–138.
12. MARR, A. G., AND J. L. INGRAHAM. 1962. Effects of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260–1267.
13. REINER, E. 1965. Identification of bacterial strains by pyrolysis gas liquid chromatography. Nature **206**:1272–1274.
14. SCHEUERBRANDT, G., AND K. BLOCK. 1962. Unsaturated fatty acids in microorganisms. J. Biol. Chem. **237**:2064–2068.
15. YAMAKAWA, T., AND N. UETA. 1964. Gas chromatographic studies of microbial components. I. Carbohydrate and fatty acid constitution of *Neisseria*. Japan. J. Exptl. Med. **34**:361–374.