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Differentiation of *Peptococcus* and *Peptostreptococcus* by Gas-Liquid Chromatography of Cellular Fatty Acids and Metabolic Products

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Gas-liquid chromatographic (GLC) profiles of cellular fatty acids and metabolic products were useful in identifying strains of *Peptococcus saccharolyticus*, *Peptococcus asaccharolyticus*, *Peptostreptococcus anaerobius*, *Peptostreptococcus micros*, and *Streptococcus intermedius*. The GLC results supported the recent taxonomic decision to transfer aerotolerant *Peptostreptococcus* species to the genus *Streptococcus*. Because inconsistencies in the results prevented our differentiating *Peptococcus prevotii*, *Peptococcus magnus*, and *Peptococcus variabilis* by GLC, additional strains will have to be examined. These GLC techniques are amenable to routine use; however, for interlaboratory results to be meaningful, the classification and nomenclature of the anaerobic gram-positive cocci should be standardized.

Interest in differentiating species of *Peptococcus* and *Peptostreptococcus* has increased recently because these gram-positive anaerobic cocci are frequently isolated from clinical materials (2, 5, 9, 11, 12, 18, 24). They have been identified in both mixed and pure cultures from a wide variety of diseases (2, 11, 12, 18) and probably account for 20 to 50% of all anaerobes seen in diagnostic laboratories (12, 18). However, despite their prevalence, some peptococci and peptostreptococci are difficult to identify to the species level because most isolates are virtually nonreactive biochemically (5, 9, 11, 12, 18, 21, 24). In addition, the existing classification for these anaerobic cocci is unsatisfactory because the nomenclature and the conventional criteria used to differentiate the various species are not consistently used among laboratories (5, 9, 18, 23, 24, 25).

Attempts to clarify the taxonomy or to improve the identification of the peptococci and peptostreptococci have involved using various techniques such as fluorescent antibody microscopy (21), inhibition with sodium polyanethol sulfonate (8), counterimmunoelectrophoresis (17), and gas-liquid chromatography (GLC) of volatile and nonvolatile metabolic products (5, 9, 10, 18, 23, 24) and cellular fatty acids (20, 25). In a preliminary report, we determined the cellular fatty acids of one strain each of *Peptococcus variabilis* and *Peptostreptococcus anaerobius* by GLC and showed that these two cultures could be differentiated with this technique (20). In a more detailed study, Wells and Field (25) examined the cellular fatty acid profiles of 12 species of the anaerobic gram-positive cocci and found they could be divided into four major groups. The fatty acid profile which we reported for one strain of *P. anaerobius* (CDC 17642, VPI 4329) was similar to that found by Wells and Field (25) for 15 strains of this species. However, the profile of the other culture we examined, *P. variabilis* 16284, was more like that which Wells and Field found for *Peptococcus saccharolyticus*. To determine whether this difference was related to experimental conditions or misclassification of strains, the cellular fatty acid compositions of additional isolates of five *Peptococcus*, two *Peptostreptococcus*, and four *Streptococcus* species were compared after they were grown in three different liquid media. In addition, the acidic and basic components of the spent growth media were extracted and analyzed by flame ionization-GLC to determine if we could detect additional metabolic products which would be useful in identifying these species.

MATERIALS AND METHODS

The cultures examined are listed in Table 1. They were obtained from the stock culture collection of the Center for Disease Control Anaerobe Section and included most of the species of gram-positive cocci which are commonly isolated from clinical materials. The facultative streptococci were included because they are often isolated initially under anaerobic conditions. In addition, we wanted to compare the GLC results from these cultures with those of *Streptococcus*

TABLE 1. *Peptococcus, Peptostreptococcus, and Streptococcus cultures analyzed by GLC*

Culture	CDC reference no.	Source no.	Comment
Peptococcus saccharolyticus	15685	WAL 2559[a]	
	16283	ATCC 14953	Type strain
	16284-77	Unknown	
P. asaccharolyticus	17137	VPI[b]	No source no.
	18749	None	Peritoneal isolate
	19108	WAL 3218	Reference strain
P. prevotii	15684	ATCC 9321	Type strain
	19115	None	Clinical isolate
P. magnus	17791	WAL 2508	Reference strain
	18633	None	Blood isolate
P. variabilis	16284	ATCC 14955	
	17785	ATCC 14956[c]	Cotype strain
Peptostreptococcus anaerobius	18623	None	Wound isolate
	18662	None	Abscess isolate
	17642[d]	VPI 4329	Reference strain
P. micros[e]	19001	None	Spinal fluid isolate
Streptococcus intermedius	16294	ATCC 27332	
	18449	ATCC 27335	Neotype strain
S. faecalis	18149	None	Blood isolate
	18440	SS-275[f]	Reference strain
S. durans (S. faecium)	18443	SS-661	Reference strain
S. mutans	17802	None	Blood isolate
	18430	SS-980	Reference strain

TABLE 1—*Continued*

[a] WAL, Wadsworth Anaerobe Laboratory, Los Angeles, Calif.

[b] VPI, Virginia Polytechnic Institute and State University, Blacksburg, Va.

[c] ATCC 14956 is currently listed by VPI as a reference strain of *P. magnus.*

[d] 17642 was analyzed previously (20).

[e] To our knowledge, there is no reference strain of *P. micros.*

[f] SS designates Center for Disease Control reference strain.

intermedius, a species of the aerotolerant gram-positive cocci which was recently included in the genus *Streptococcus* (10).

The cultures were maintained in chopped-meat medium (6) and checked for purity, morphology, and cultural and biochemical characteristics by the procedures described by Dowell and Hawkins (5) and by Holdeman et al. (9). They were transferred to tubes of thioglycolate broth (135-C, BBL Microbiology Systems) and incubated anaerobically for 36 to 48 h. One-milliliter volumes from each thioglycolate culture were used to inoculate three flasks, each of which contained 125 ml of peptone-yeast extract-glucose broth (6), Schaedler broth (BBL), and Lombard-Dowell glucose broth (6). The cultures were incubated in an anaerobic glove box for 48 to 60 h at 35°C and then centrifuged at 6,500 rpm in a refrigerated centrifuge for approximately 20 min. The spent medium was removed from the packed cells, and 25 ml was dispensed in 5-ml volumes to screw-capped test tubes (16 by 125 mm). The cells from each culture were washed once with distilled water and divided into approximately equal amounts. Both the broth volumes and the cells were stored at −20°C until analyzed by GLC.

Analysis of cellular fatty acids. After the cells were thawed, they were saponified, and the fatty acids were methylated and extracted by the procedures described previously (15). The methyl esters were analyzed on a Perkin-Elmer model 990 gas chromatograph (Perkin-Elmer, Norwalk, Conn.) equipped with flame ionization detectors and a disk integrator recorder. Samples were analyzed on a coiled glass column (3.66 m by 0.635-cm outer diameter) packed with 3% OV-101 coated on 100/120 mesh Gas Chrom Q (Applied Science, State College, Pa.). After the samples were injected, the temperature of the column was programmed from 160 to 265°C at 6°C/min and then maintained at 265°C for 6 min. The other instrument conditions were the same as those described previously (15). Fatty acid methyl esters were identified by comparing retention times on the OV-101 column with retention times of highly purified methyl ester standards (19). Identification was verified by analysis on a polar column, hydrogenation of unsaturated acids, and GLC-mass spectrometry (19).

Analysis of metabolic products. The frozen 5-ml volumes of broth from each culture were thawed and allowed to equilibrate to room temperature. The broth was acidified to pH 2.0 with 0.2 ml of 25% H_2SO_4, 0.5 to 1.0 g of NaCl crystals was added, and the broth was extracted with two 5-ml volumes of diethyl ether. The

ether phases were combined in a small beaker and allowed to evaporate in a chemical fume hood to 0.3 to 0.4 ml. Anhydrous Na_2SO_4 crystals were added to the beaker, and the ether layer was transferred to a small screw-capped test tube. The butyl ester or trifluoroacetyl butyl esters of the short-chain acids were prepared and analyzed by GLC as described previously (13). The only exception was that hexane or a mixture of equal parts of diethyl ether and hexane was used instead of chloroform ($CHCl_3$). In addition, the ester derivatives were always concentrated in an ice bath. The short-chain acids were identified by comparing retention times of the esterified acids in the samples with those of highly purified acid standards (Chem Service, Inc., Media, Pa.; Eastman Organic Chemicals, Rochester, N. Y.).

To test for basic metabolic products, the pH of the acidified broth was adjusted to pH 10.0 with 5 N NaOH. The broth was mixed with 5 ml of $CHCl_3$ or 5 ml of ethyl acetate and then centrifuged briefly to separate the two phases. The organic phase was removed to a small beaker and allowed to evaporate under a gentle stream of dry nitrogen gas to 0.3 ml. After anhydrous Na_2SO_4 was added, the organic phase was transferred to a small screw-capped test tube. The volume was adjusted to 0.1 to 0.2 ml, and 0.1 ml of trifluoroacetic anhydride (Pierce Chemical Co., Rockford, Ill.) was added. The tube was sealed and heated at 100°C for 5 min. After it was cooled to room temperature, the sample was evaporated just to dryness, and 0.1 ml of ethyl acetate was added. The acetylated samples were analyzed on the 3% OV-101 column by holding the initial column temperature at 120°C for 4 min and then temperature programming the column at 8°C/min to 250°C. Amines in the samples were tentatively identified by comparing retention times with those of known amine standards. Identities were confirmed by mass spectrometry.

Only six or nine samples were processed at one time so that the samples could be analyzed on the same day they were extracted and derivatized. Appropriate broth and reagent controls were analyzed in the same manner as the bacterial samples. The derivatives were stored at −20°C until all GLC and mass spectrometry studies were completed.

RESULTS

The major cellular fatty acids and the metabolic products found for each of the cultures are summarized in Table 2. To illustrate the overall similarities among cultures, most of the fatty acids are grouped together in broad classifications rather than listed individually. The values listed in the table for each acid category are average percentages from at least two different analyses of cells grown in either peptone-yeast extract-glucose broth or Schaedler broth. Even though the best growth occurred in Schaedler broth, the cellular fatty acid profiles were generally the same for each culture, regardless of the medium used to grow the cells. A black granular material observed in the packed cells from Schaedler broth and Lombard-Dowell glucose broth did not interfere with the analysis.

The acidic metabolites detected included the volatile fatty acids from one to seven carbon atoms in chain length; the basic metabolites included amines produced when amino acids present in the medium were decarboxylated (14). The acidic and basic metabolites produced by each culture were the same in the three media; however, greater amounts of volatile acids and amines per volume of medium were found from cultures grown in peptone-yeast extract-glucose broth or Schaedler broth. Unless otherwise noted, the cultural and biochemical characteristics for each species were the same as those described by the Center for Disease Control (5; G. L. Lombard, personal communication) as differential characteristics of the anaerobic gram-positive cocci.

Representative cellular fatty acid profiles for *P. saccharolyticus* 16283, *Peptococcus asaccharolyticus* 19108, *P. variabilis* 17785, *P. anaerobius* 18623, *Peptostreptococcus micros* 19001, and *Streptococcus faecalis* 18840 are shown in Fig. 1 to 6, respectively. The cellular fatty acids of the culture listed in Tables 1 and 2 as *P. saccharolyticus* 16284-77 had been examined in an earlier study (20). At that time, the culture was labeled *P. variabilis*, CDC 16284. Cells and spent medium from this culture had been stored at −20°C. When they were thawed and reanalyzed by GLC, the fatty acid composition was essentially the same as that determined previously (20; Table 2); the metabolic products detected were formic and acetic acids. Comparison of these results with those of Wells and Field (25) and with those of the present study led us to conclude that culture 16284-77 had been mislabeled and was a strain of *P. saccharolyticus*. When the original records were checked, this conclusion was confirmed.

The fatty acid profiles of the two *Peptococcus prevotii* cultures resembled those of *P. asaccharolyticus* (Table 2, Fig. 2), except that the strains of *P. prevotii* contained higher percentages of branched-chain acids. The profiles of the *P. prevotii* strains differed from each other because 19115 contained small amounts of four unidentified acids (total 15%) which were not found in 15684. Moreover, strain 15684 produced acetic, propionic, and butyric acids and was urease positive, whereas strain 19115 did not produce propionic acid and was urease negative.

In most respects, the fatty acid composition of *Peptococcus magnus* was similar to that of *P. asaccharolyticus* and *P. prevotii*. However, higher percentages of saturated acids with less than 14 carbon atoms were found in the two *P.*

TABLE 2. *Cellular fatty acid composition and metabolic products of Peptococcus, Peptostreptococcus, and Streptococcus species*

Culture	Cellular fatty acids[a]												Metabolic product	
	C_{14}		C_{14}, C_{16}, C_{18}			C_{15}, C_{17}, C_{19}			C_{20}		C_{19} cyc	Un	VFA[b]	Amines
	Br	:0	Br	:1	:0	Br	:1	:0	:1	:0				
Peptococcus saccharolyticus														
15685	–	T	6[c]	2	13	59	–	1	5	13	–	–	F, A	–
16283	–	T	6	2	12	64	–	1	4	10	–	–	F, A	–
16284-77	–	T	4	2	14	73	–	T	T	6	–	–	F, A	–
P. asaccharolyticus														
17137	–	7	–	31	39	4	10	7	–	–	–	2	A, B	–
18749	–	10	–	27	38	4	11	8	–	–	–	2	A, B	–
19108	–	9	–	32	40	3	9	6	–	–	–	T	A, B	–
P. prevotii														
15684	–	9	–	38	26	20	5	2	–	–	–	T	A, P, B	–
19115	–	8	–	30	31	12	4	T	–	–	–	15	A, B	–
P. magnus														
17791	–	28	–	33	22	13	–	4	–	–	–	–	A	–
18633	–	18	–	30	25	17	T	8	–	–	–	2	A	–
P. variabilis														
16284-78	–	35	–	22	18	12	T	5	–	–	–	7	A	–
17785	–	6	6	7	11	58	–	12	–	–	–	–	A, P	–
Peptostreptococcus anaerobius														
18623	20	10	29	2	26	9	–	4	–	–	–	T	A, (IB), (IV), (V), IC, S	–
18662	19	5	29	1	23	17	–	6	–	–	–	T	A, (IB), (IV), (V), IC, S	–
P. micros														
19001	–	4	–	32	60	–	–	T	2	T	–	–	A	–
Streptococcus intermedius														
16294	–	3	–	32	38	2	–	T	–	–	23	T	(F), A, L, Un	β-PEA, Tyr[d]
18449	–	2	–	33	39	T	–	T	–	–	24	T	(F), A, L, Un	β-PEA, Tyr
S. faecalis														
18149	–	2	–	33	39	2	–	T	–	–	23	T	(F), A, L, Un	β-PEA, Tyr
18440	–	T	–	28	42	–	–	–	–	–	29	T	(F), A, L, Un	β-PEA, Tyr
S. durans (*S. faecium*)														
18443	–	T	–	40	33	2	–	–	–	–	20	4	(F), A, L, Un	β-PEA, Tyr

TABLE 2—*Continued*

Culture	Cellular fatty acids[a]												Metabolic product	
	C_{14}		C_{14}, C_{16}, C_{18}			C_{15}, C_{17}, C_{19}			C_{20}		C_{19} cyc	Un	VFA[b]	Amines
	Br	:0	Br	:1	:0	Br	:1	:0	:1	:0				
S. mutans														
17802	–	2	–	29	39	T	–	3	22	2	T	–	(F), A, L, Un	–
18430	–	T	–	31	37	T	–	2	24	3	T	–	(F), A, L, Un	–

[a] C, Number of carbon atoms; <C_{14}, includes fatty acids with 10 to 14 carbon atoms; Br, includes iso and antiso branched-chain acids; colon 1 (:1), unsaturated acid; colon 0 (:0), saturated acid; cyc, cyclopropane acid; Un, unidentified acid(s).

[b] VFA, Volatile fatty acids; F, formic acid; A, acetic acid; P, propionic acid; IB, isobutyric acid; B, butyric acid; IV, isovaleric acid; V, valeric acid; IC, isocaproic acid; L, lactic acid; ( ), indicates minor or variable production of acid.

[c] Numbers refer to percentage of total acids; T, trace = less than 2%; –, not detected; values are an average of at least two different determinations.

[d] β-PEA, β-Phenethylamine; Tyr, tyramine.

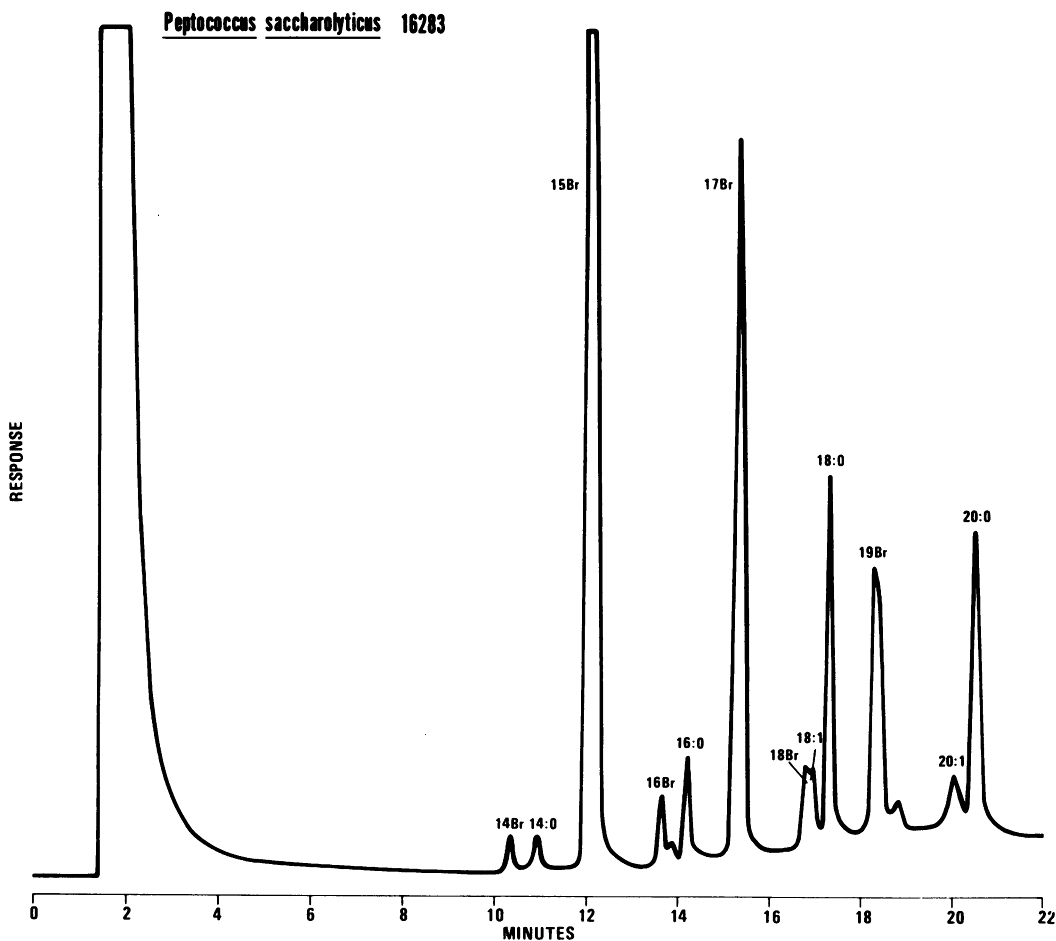

FIG. 1. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column.*

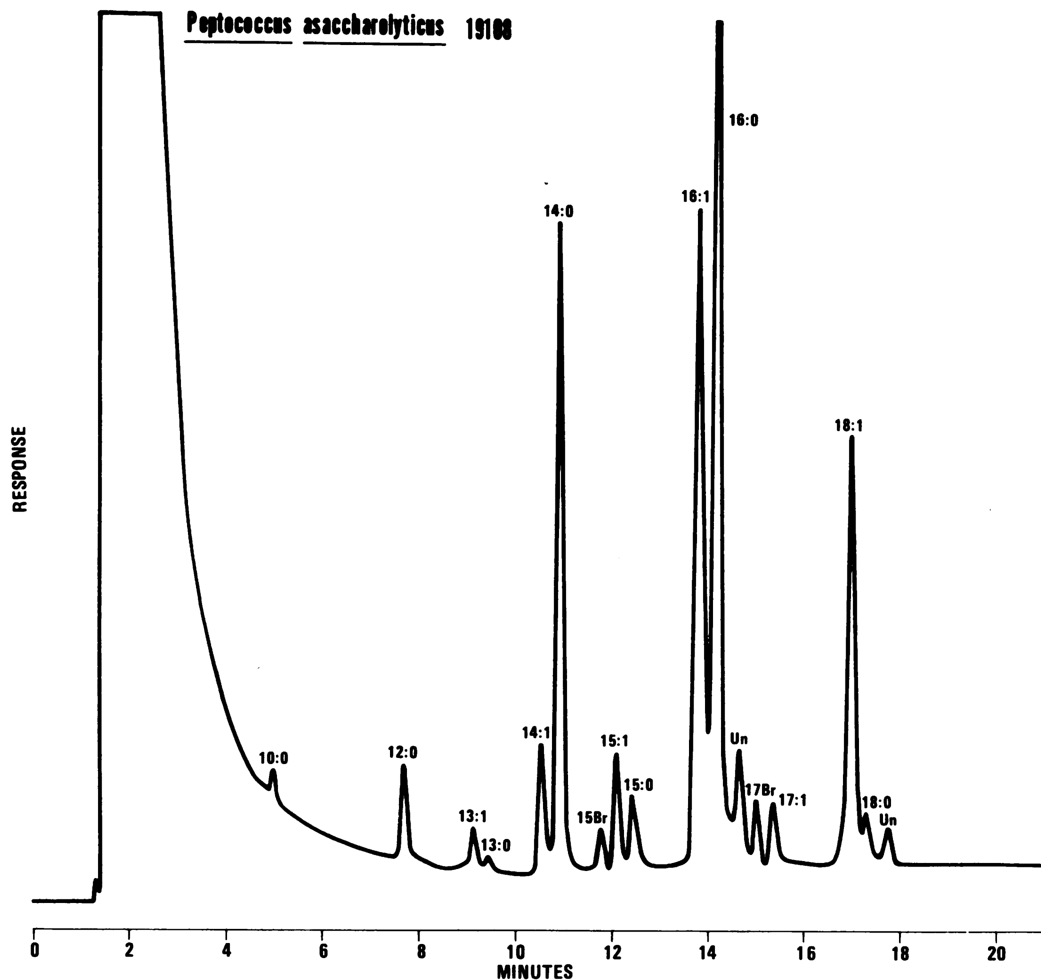

FIG. 2. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column.*

magnus cultures. The volatile acid profiles were also different because acetic acid was the only metabolic product detected.

The GLC profiles of the two *P. variabilis* cultures were different from each other (Table 2). Strain 16284-78 had fatty acid and metabolic profiles which were very similar to those of the *P. magnus* cultures, whereas strain 17785 had GLC profiles which did not resemble any of the other cultures tested. These differences are evident when the chromatogram of fatty acids from *P. variabilis* 17785 (Fig. 3) is compared with the chromatograms from the other species (Fig. 1, 2, 4, 5, and 6). This culture is further distinguished by its volatile acid production and biochemical reactions (5, 18, 24).

Both the cellular fatty acids and volatile acid profile of *P. anaerobius* (Fig. 4, Table 2) were unique and could be used to distinguish this species from any of the other species tested. Several peaks in Fig. 4 are labeled "unidentified" because mass spectrometric results showed that they were not fatty acid methyl esters. Although *P. anaerobius* has been reported to produce amines (22), we did not detect any during this study.

The fatty acid profiles of *Streptococcus intermedius, S. faecalis*, and *Streptococcus durans* (*Streptococcus faecium*) were similar to that of *P. micros* (Fig. 5). However, when a representative chromatogram of the fatty acids from *S. faecalis* 18440 (Fig. 6) and the data in Table 2 are compared, it is apparent that the presence of large amounts of lactobacillic acid (19 Cyc) distinguished *S. intermedius, S. faecalis*, and *S. durans* from *P. micros*. In addition, acetic acid

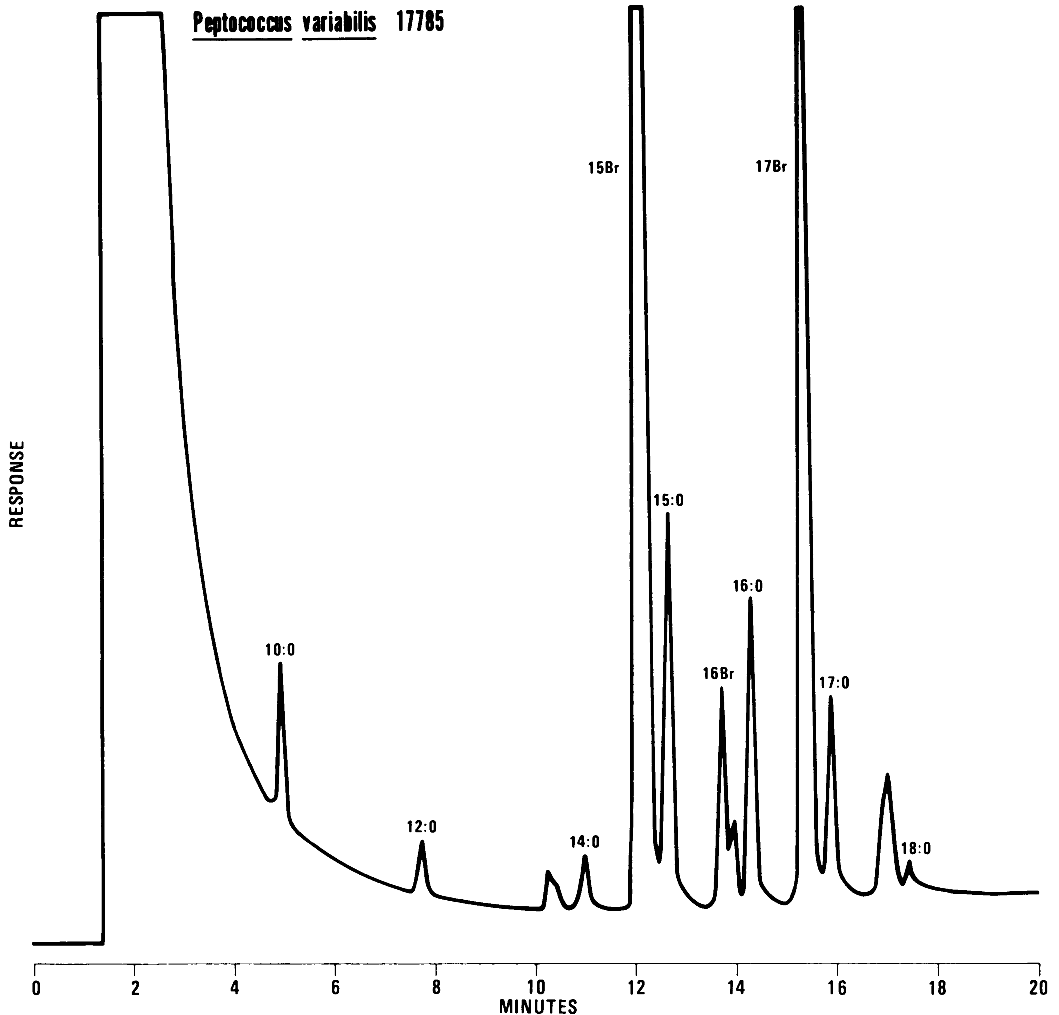

FIG. 3. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column.*

was the only metabolic product found in extracts of the media from *P. micros* (Table 2), whereas several acidic and basic metabolites were found in those from *S. intermedius, S. faecalis,* and *S. durans.* The retention time of the unidentified acidic compound found in these three species (Fig. 7) was very close to that of caproic acid; however, mass spectrometry showed that the two were not identical. Studies are presently underway to identify this compound.

Figure 8 is a representative chromatogram of the basic ethyl acetate extract from 5 ml of spent growth medium from one of the *S. intermedius* cultures. The two peaks were identified as β-phenethylamine and tyramine by GLC standards and by mass spectrometry. The size of the tyramine peak was approximately one-third that seen in Fig. 8 when broth samples were extracted with $CHCl_3$. When ethyl acetate, a more polar solvent, was used for extraction, the recovery of tyramine was increased as evidenced by a marked increase in the size of the peak. The recovery of β-phenethylamine was approximately the same in the two solvents.

Streptococcus mutans was readily differentiated by GLC from *P. micros* and the other three species of streptococci because the former contained large amounts of $C_{20:1}$ and did not contain lactobacillic acid. The metabolic profile was also different because no amines were detected in the medium.

DISCUSSION

GLC analysis of cellular fatty acids and metabolic products was useful in differentiating the

species of anaerobic and aerotolerant cocci examined in this study. The best results were obtained for those whose conventional identification is the least controversial: namely, *P. saccharolyticus, P. asaccharolyticus, P. anaerobius, P. micros*, and *S. intermedius*. Less consistent results were found for the species whose taxonomic position or biochemical reactions are in doubt: these included *P. prevotii, P. variabilis*, and *P. magnus*.

Most of the chromatograms of the fatty acids which we obtained resembled those shown by Wells and Field (25). Even though they did not identify all of the peaks, and the ratios of some acids differed from the ratios which we found, the overall fatty acid profiles were similar. These similarities and the fact that major differences resulting from media effects were not observed indicate that GLC analysis can be used with confidence by other laboratories.

When the fatty acid compositions of closely related species are not strikingly different, comparison of both the fatty acid and metabolic profiles may be useful for identification purposes. *P. asaccharolyticus, P. prevotii, P. magnus*, and *P. variabilis* were grouped together by Wells and Field (25) because of similarities in their fatty acid contents. Our GLC results also showed that these species are closely related. However, differences in the relative percentages of acids with less than 14 carbon atoms and the branched-chain acids, as well as differences in the metabolic products, were observed which indicated that these species were not exactly alike.

The production of indole is used by some

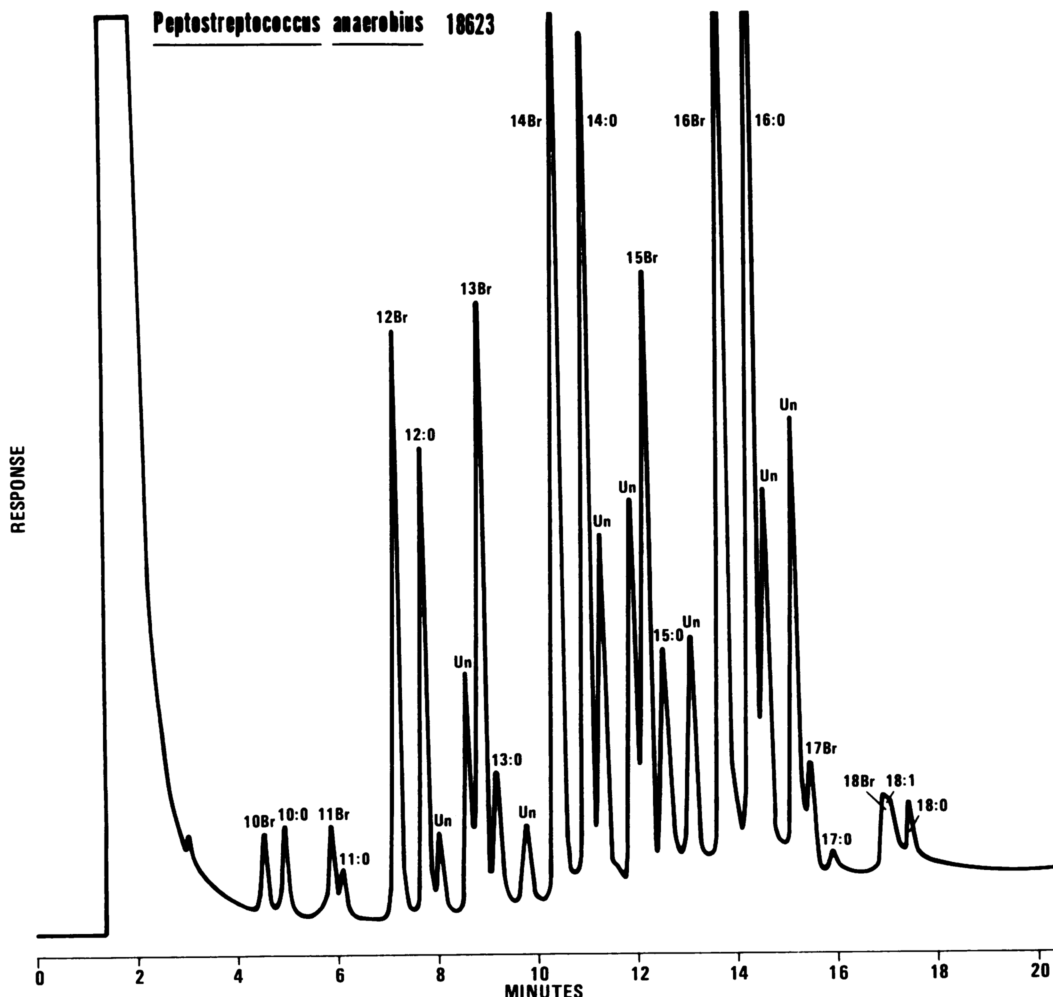

FIG. 4. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column. Un, Unidentified.*

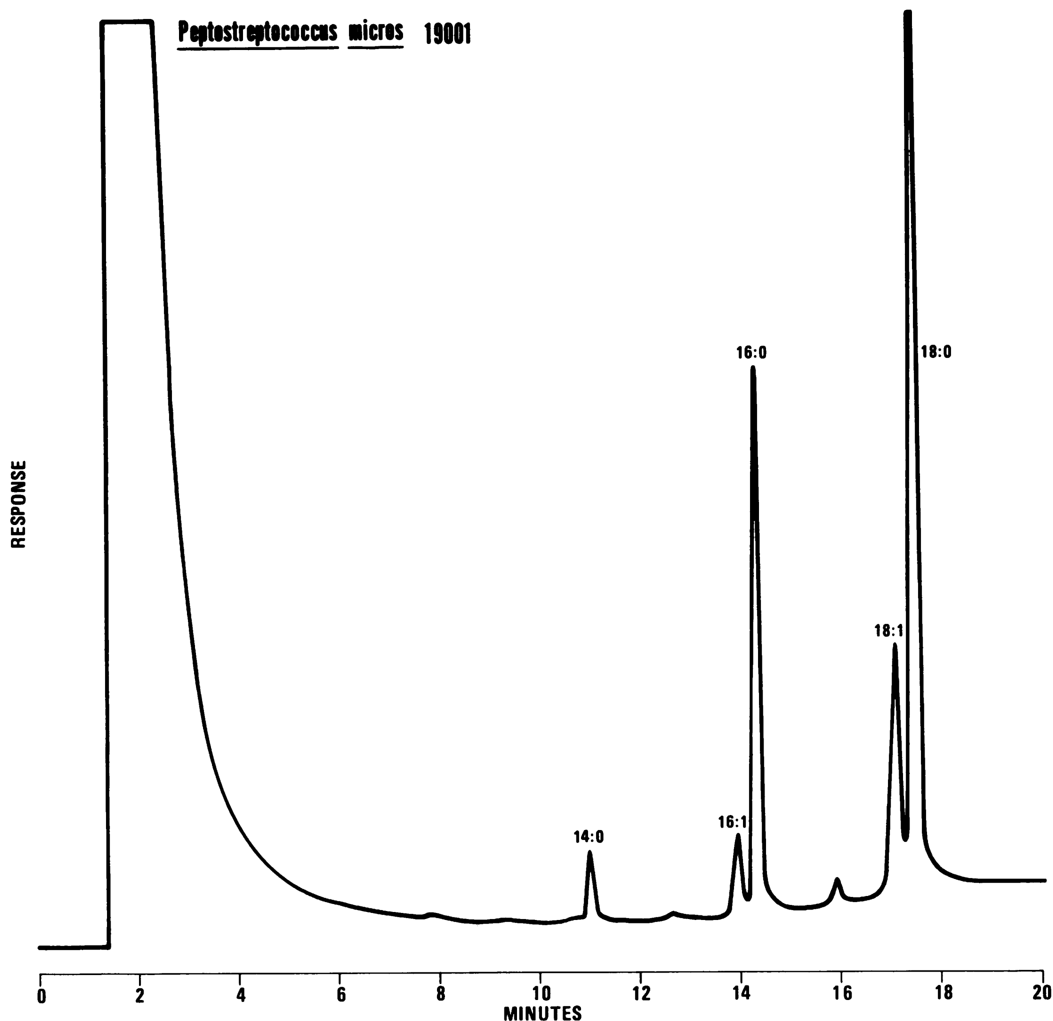

FIG. 5. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column.*

laboratories (5, 18, 24) to differentiate *P. asaccharolyticus* from *P. prevotii*; however, these two species are considered to be identical by Rogosa (23). The GLC profiles indicate that *P. prevotii* and *P. asaccharolyticus* are related. Since one of the strains (19115) appeared to be more like *P. asaccharolyticus* than the type strain (15684), additional cultures need to be examined before definite conclusions about these two species can be reached.

There is also controversy as to whether *P. magnus* and *P. variabilis* are the same or different organisms. Although it has been reported that *P. magnus* liquefies gelatin (18, 24), the Center for Disease Control Anaerobe Section has found that this species usually gives a negative gelatin reaction (5; G. L. Lombard, personal communication). In the present study, the only culture which did liquefy gelatin was *P. variabilis* 16284-78; this culture had GLC profiles which were almost identical to those of *P. magnus*. These results would support the opinion of some that *P. magnus* and *P. variabilis* are the same organism (18, 23, 24). However, our results suggest that there are two distinct species because of the differences found in the GLC profiles of *P. variabilis* 17785, the co-type strain of this species. Additional strains of *P. variabilis* and *P. magnus* should be examined by conventional tests, GLC, and genetic techniques to determine their taxonomic and genetic relatedness. There is certainly a need for agreement on

the nomenclature and the conventional criteria for separating these two species because the cotype strain of *P. variabilis* is currently listed by the Virginia Polytechnic Institute and State University as a reference strain of *P. magnus* (9).

The single strain of *P. micros* studied had a cellular fatty acid profile which was almost identical to that reported by Wells and Field (25) for eight strains of this species. Although some similarities in the fatty acid compositions of *P. micros* and *P. magnus* were noted, the data in Table 2 show that there are major differences in the relative percentages of the saturated C_{14}, C_{16}, and C_{18} acids, the branched-chain acids, and acids with less than 14 carbon atoms. These two species are very difficult to identify on the basis of conventional characteristics described by the Center for Disease Control and others because both species are nonreactive biochemically and have similar metabolic profiles (5, 9, 12). Because of this, *P. micros* and *P. magnus* are often differentiated by microscopic examination to determine relative differences in their cell sizes (9, 11; G. L. Lombard, personal communication). However, if differences in the cellular fatty acid profiles prove to be consistent when additional strains are tested, GLC analysis will provide a much more reliable method for differentiating *P. magnus* and *P. micros*.

We were able to distinguish *S. intermedius* from *P. micros* by GLC because the former contained lactobacillic acid. However, Wells and Field (25) found that the fatty acids of these two species were identical and did not report the presence of lactobacillic acid in any of the strains they examined. These workers used boron-trifluoride methanol (BF_3-CH_3OH) to esterify the

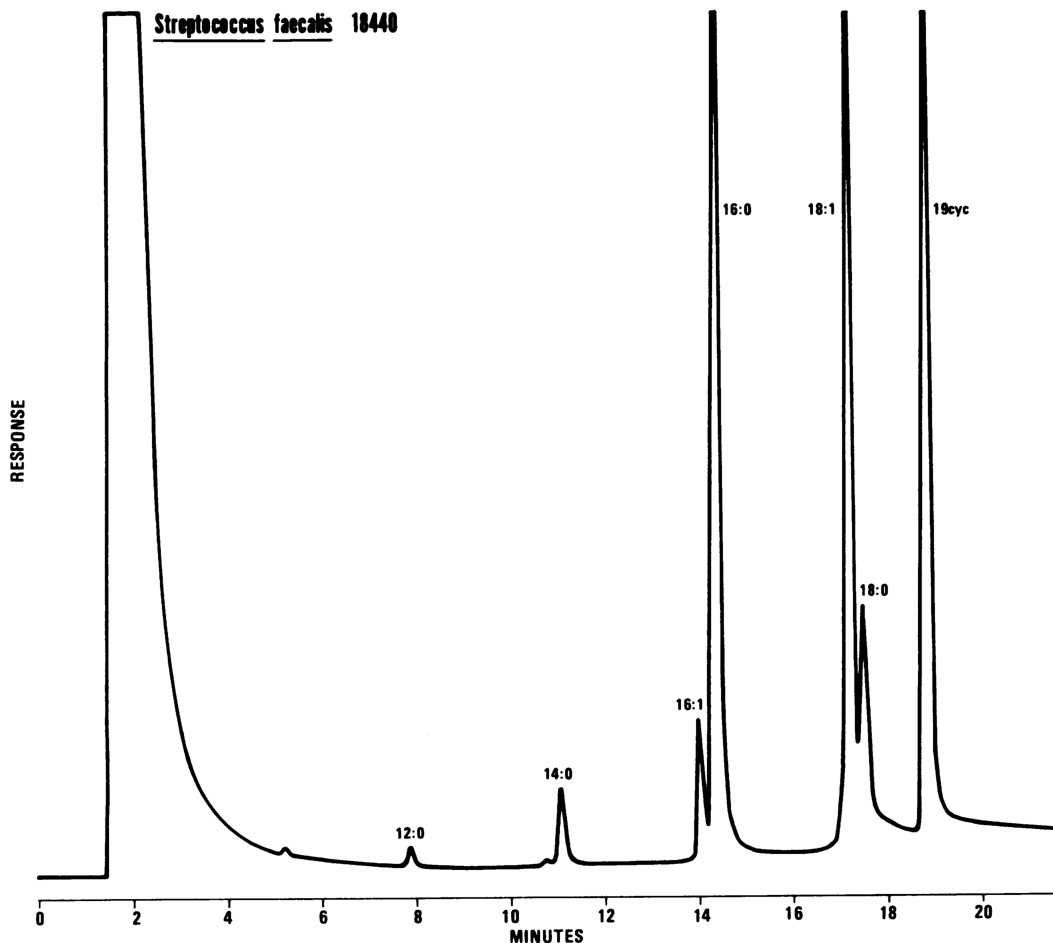

FIG. 6. *Gas-liquid chromatogram of methylated fatty acids from saponified cells. Analysis was on a 3% OV-101 column.*

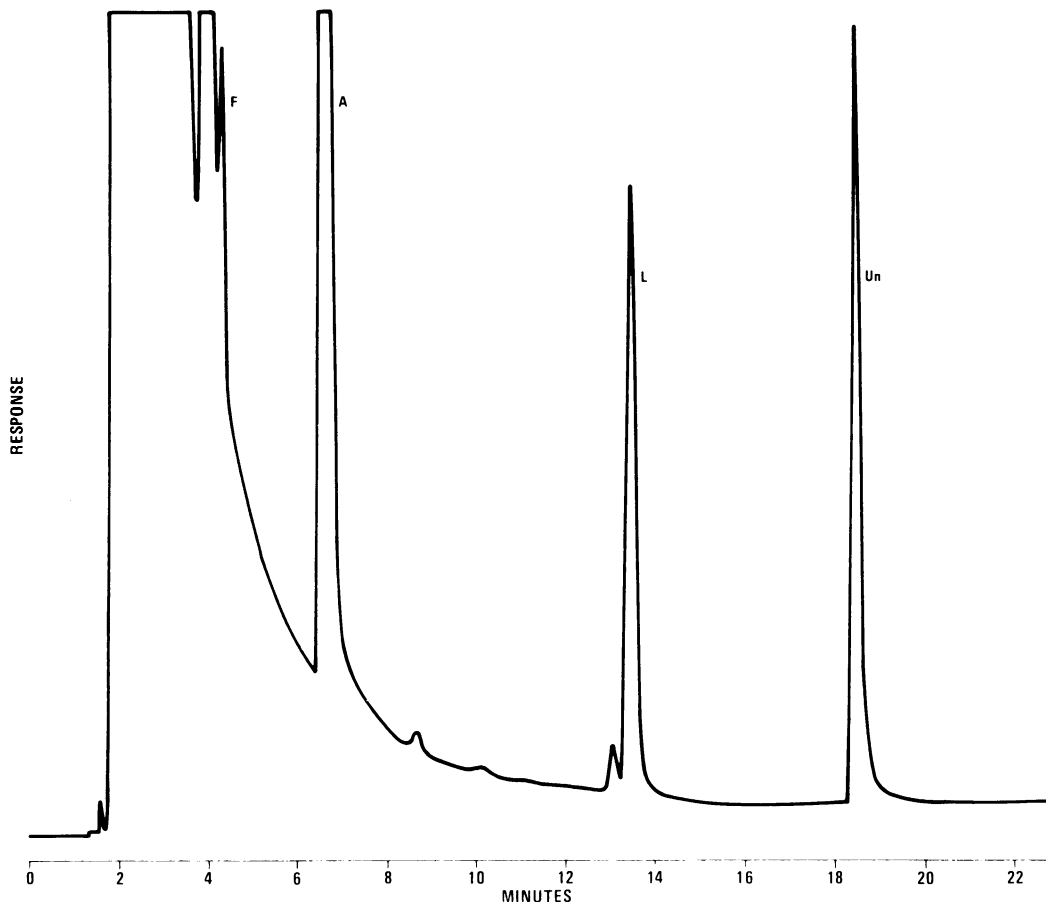

FIG. 7. *Gas-liquid chromatogram of esterified short-chain acids from the spent culture medium of S. intermedius 18849. F, Formic acid; A, acetic acid; L, lactic acid; and Un, unidentified component. Analysis was on a 15% Dexsil 300 column.*

bacterial fatty acids. Our laboratory (16) and others (3) have shown that this reagent destroys cyclopropane acids, and its use by Wells and Field may account for the absence of lactobacillic acid in the cultures which they tested. The GLC profiles which we found for *S. intermedius* and for the facultative enterococci (*S. faecalis* and *S. durans*) support the recent recommendation to transfer three species of the aerotolerant peptostreptococci to the genus *Streptococcus* (10). Reference strains of *Streptococcus constellatus* and *Streptococcus morbillorum* should also be examined by GLC to determine their fatty acid and metabolic profiles.

The presence of lactobacillic acid (1, 15) and the production of lactic acid (4, 23) have been reported previously as biochemical characteristics of several species of streptococci. Although the production of β-phenethylamine and tyramine by group D streptococci has also been reported (4), the presence of these amino acid decarboxylation end products is not currently used as a differential characteristic of streptococci (7). Now that relatively simple GLC techniques are available, the end products of amino acid metabolism can be rapidly determined, and the information can be used to differentiate closely related bacterial strains (4, 14).

The cellular fatty acid composition of *S. mutans* was identical to that found previously for 16 strains of this species (15) even though different cultural conditions were used. The presence of large amounts of $C_{20:1}$ acid is quite characteristic of *S. mutans* and can be used to distinguish it from other closely related species (15).

The cellular fatty acid profile of *P. anaerobius* (18623 and 18662) was somewhat different from that found in an earlier study for another strain of this species, 17642 (20). Although the relative amounts of most of the branched-chain and sat-

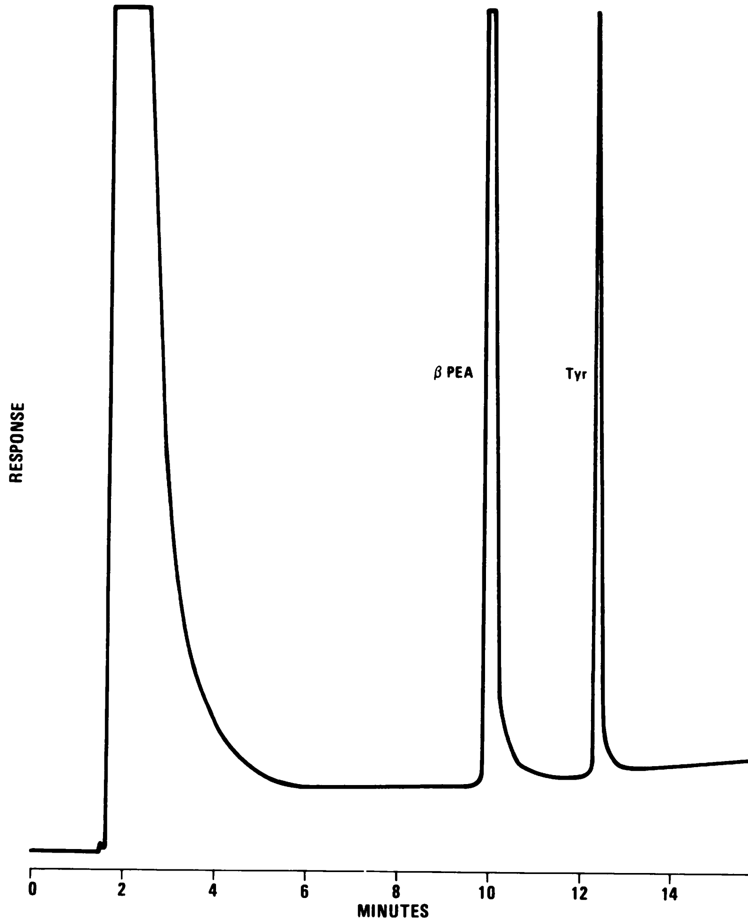

FIG. 8. *Gas-liquid chromatogram of acetylated β-phenethylamine (β-PEA) and tyramine (Tyr) from the spent culture medium of S. intermedius 18449. The derivatives were analyzed on a 3% OV-101 column.*

urated fatty acids were essentially the same in all three strains, the percentage of the iso-branched C_{10} acid (C_{10} Br) was much lower in *P. anaerobius* 18623 and 18662 than in 17642. In addition, these two strains contained small amounts of acids with greater than 16 carbon atoms and several unidentified compounds which were not found in *P. anaerobius* 17642. The strains of *P. anaerobius* which were examined by Wells and Field were characterized by an unidentified peak which eluted between the C_8 and C_{10} acids and whose average percentage composition ranged from 2.4 to 29% (25). This peak was probably the C_{10} Br acid which we found in widely varying amounts in the three cultures of *P. anaerobius*.

Results from this study and from others (5, 9, 20, 23-25) show that GLC analyses of cells and spent growth media are reliable for differentiating species of *Peptococcus, Peptostreptococcus,* and *Streptococcus* and can be easily adapted for routine use. These analyses can also be used to aid in clarifying the taxonomy of the members of these genera. However, for interlaboratory results to be reproducible and meaningful, the nomenclature of the gram-positive cocci should be standardized and some agreement on the major conventional criteria useful in identifying these bacteria should be reached. Further studies of the chemical, taxonomic, genetic, and serological relationships of these cultures are needed so that existing species can be better characterized and consolidated when appropriate.

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LITERATURE CITED

1. **Amstein, C. F., and P. A. Hartman.** 1973. Differentiation of some enterococci by gas chromatography. J. Bacteriol. **113**:38-41.

2. **Balows, A., R. M. De Hann, V. R. Dowell, Jr., and L. B. Guze (ed.).** 1974. Anaerobic bacteria: role in disease. Charles C Thomas, Springfield, Ill.
3. **Brian, B. L., R. W. Gracy, and V. E. Scholes.** 1972. Gas chromatography of cyclopropane fatty acid methyl esters prepared with methanolic BCl_3 and BF_3. J. Chromatogr. **66**:138-140.
4. **Deibel, R. H.** 1964. The group D streptococci. Bacteriol. Rev. **28**:330-366.
5. **Dowell, V. R., Jr., and T. M. Hawkins.** 1977. Laboratory methods in anaerobic bacteriology. CDC Laboratory Manual. (CDC)77-8272. U. S. Government Printing Office, Washington, D. C.
6. **Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson, and A. Y. Armfield.** 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. Center for Disease Control, Atlanta, Ga.
7. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23**:1131-1139.
8. **Graves, M. H., J. A. Morello, and F. E. Kocka.** 1974. Sodium polyanethol sulfonate sensitivity of anaerobic cocci. Appl. Microbiol. **27**:1131-1133.
9. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
10. **Holdeman, L. V., and W. E. C. Moore.** 1974. New genus, *Coprococcus*, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int. J. Syst. Bacteriol. **24**:260-277.
11. **Holland, J. W., S. M. Gagnet, S. A. Lewis, and L. Stauffer.** 1977. Clinical evaluation of a simple, rapid procedure for the presumptive identification of anaerobic bacteria. J. Clin. Microbiol. **5**:416-426.
12. **Holland, J. W., E. O. Hill, and W. A. Altemeier.** 1977. Numbers and types of anaerobic bacteria isolated from clinical specimens since 1960. J. Clin. Microbiol. **5**:20-25.
13. **Lambert, M. A., and C. W. Moss.** 1972. Gas-liquid chromatography of short-chain fatty acids on Dexsil 300GC. J. Chromatogr. **74**:335-338.
14. **Lambert, M. A., and C. W. Moss.** 1973. Use of gas chromatography for detecting ornithine and lysine decarboxylase activity in bacteria. Appl. Microbiol. **26**: 517-520.
15. **Lambert, M. A., and C. W. Moss.** 1976. Cellular fatty acid composition of *Streptococcus mutans* and related streptococci. J. Dent. Res. (Special Issue) **A55**:A96-A102.
16. **Lambert, M. S., and C. W. Moss.** 1977. Degradation of bacterial cyclopropane acids with boron trihalide reagents. Microbios **18**:51-58.
17. **Markowitz, A., and A. M. Lerner.** 1977. Differentiation of several isolates of *Peptococcus magnus* by counterimmunoelectrophoresis. Infect. Immun. **16**:152-154.
18. **Martin, W. J.** 1974. Anaerobic cocci, p. 381-387. *In* E. H. Lennette, E. P. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D. C.
19. **Moss, C. W., and S. B. Dees.** 1975. Identification of microorganisms by gas chromatographic-mass spectrometric analysis of cellular fatty acids. J. Chromatogr. **112**:595-604.
20. **Moss, C. W., M. A. Lambert, and G. L. Lombard.** 1977. Cellular fatty acids of *Peptococcus variabilis* and *Peptostreptococcus anaerobius*. J. Clin. Microbiol. **5**:665-667.
21. **Porschen, R. K., and E. H. Spaulding.** 1974. Fluorescent antibody study of the gram-positive anaerobic cocci. Appl. Microbiol. **28**:851-855.
22. **Prevot, A.-R., and V. Fredette.** 1966. Manual for the classification and determination of the anaerobic bacteria, p. 73-74. Lea and Febinger, Philadelphia.
23. **Rogosa, M.** 1971. *Peptococcaceae*, a new family to include the gram-positive, anaerobic cocci of the genera *Peptococcus, Peptostreptococcus*, and *Ruminococcus*. Int. J. Syst. Bacteriol. **21**:234-237.
24. **Sutter, V. L., V. L. Vargo, and S. M. Finegold.** 1975. Wadsworth anaerobic bacteriology manual, 2nd ed., p. 33-34. Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, Los Angeles.
25. **Wells, C. L., and C. R. Field.** 1976. Long-chain fatty acids of peptococci and peptostreptococci. J. Clin. Microbiol. **4**:515-521.