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Cellular Fatty Acid Composition of Group IVe, a Nonsaccharolytic Organism from Clinical Sources

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The cellular fatty acid composition of a group of gram-negative nonfermentative organisms designated group IVe was studied by gas-liquid chromatography. Strains of this group are isolated most frequently from urine and most closely resemble the *Alcaligenes* in conventional biochemical tests. On the basis of cellular fatty acids, however, we found these organisms to be strikingly different from *Alcaligenes* and other gram-negative species with similar phenotypic characteristics. The gas-liquid chromatography procedure offers an additional diagnostic test for rapid identification of unclassified bacteria like group IVe.

Over the last 30 years, King and her successors received over 35,000 cultures isolated from human specimens (10). Some of these isolates could not be classified under established genera and species and consequently were placed into groups with letter and number designations. Many groups most closely resembled species of either *Pseudomonas*, *Achromobacter*, or *Alcaligenes*, and a few of these have been further characterized and are now classified as new species under the above genera (5, 9, 13). Recently, our laboratory has studied the cellular fatty acid composition of a number of these groups, using gas-liquid chromatography, and found that the fatty acid profiles of some groups are similar to those of authentic species of *Pseudomonas* and *Flavobacterium* (3).

In the present report, we describe the cellular fatty acid composition of group IVe, which is composed of human isolates obtained mainly from urine. Their cellular fatty acid composition is compared to that of *Bordetella bronchiseptica*, *Brucella suis*, *Brucella melitensis*, *Brucella abortus*, and *Alcaligenes denitrificans*, presently classified as *Alcaligenes faecalis* (4). All of the latter species have phenotypic characteristics similar to those of group IVe (12). Our data show that isolates of group IVe are markedly different from *Alcaligenes*, *B. bronchiseptica*, and *Brucella*. The usefulness of cellular fatty acids as an additional test for identifying these bacteria is discussed.

MATERIALS AND METHODS

The eight reference strains of group IVe tested were D2312, C9573, D3123, E4723, E4236, E2690, E4326, and E3484. Four strains of *A. denitrificans*, five of *B. bronchiseptica*, and two each of *B. suis*, *B. melitensis*, and *B. abortus*, all of which were obtained from a variety of clinical sources, were also analyzed for cellular fatty acids. The morphological and cultural characteristics of each strain were determined as previously described (10, 12). For fatty acid analysis, strains were cultured on plates of heart infusion agar containing 5% rabbit blood for 24 h at 35°C. Because of the light growth of group IVe organisms within 24 h, two plates of blood agar were inoculated for each of these strains. After incubation, cells were removed from both plates and combined and then processed for fatty acid methyl esters according to published procedures (1). The fatty acid methyl esters were analyzed by gas-liquid chromatography on a 3% OV-101 column, using a flame ionization detector (6). Bacterial fatty acids were identified by comparison of their gas-liquid chromatography retention times to those of authentic standards (Applied Science, State College, Pa.; Analabs, North Haven, Conn.). Hydroxy and unsaturated fatty acids were further identified by acetylation and hydrogenation procedures, respectively (1). In addition, the identities of all fatty acids were confirmed by gas-liquid chromatography-mass spectrometry (8).

RESULTS AND DISCUSSION

The cellular fatty acids of eight strains of group IVe were essentially identical and were significantly different from those of other gram-negative, nonfermenting species we have tested (1-3, 6, 7). Octadecenoic acid (18:1) was the major acid identified in strain D3123, a representative isolate of group IVe, as shown in the top chromatogram in Fig. 1. In addition to 18:1, relatively large amounts of 14:0, 16:0, and 3-hydroxy-tetradecanoic (3-OH-14:0) acids and small to trace amounts of 16:1, 3-OH-16:0, 18:0, and 19:0 cyclopropane (19:0Δ) acids characterized group IVe strains. Relative percentages of these acids are given in Table 1. The values shown are averages obtained from processing each strain through the entire procedure two to

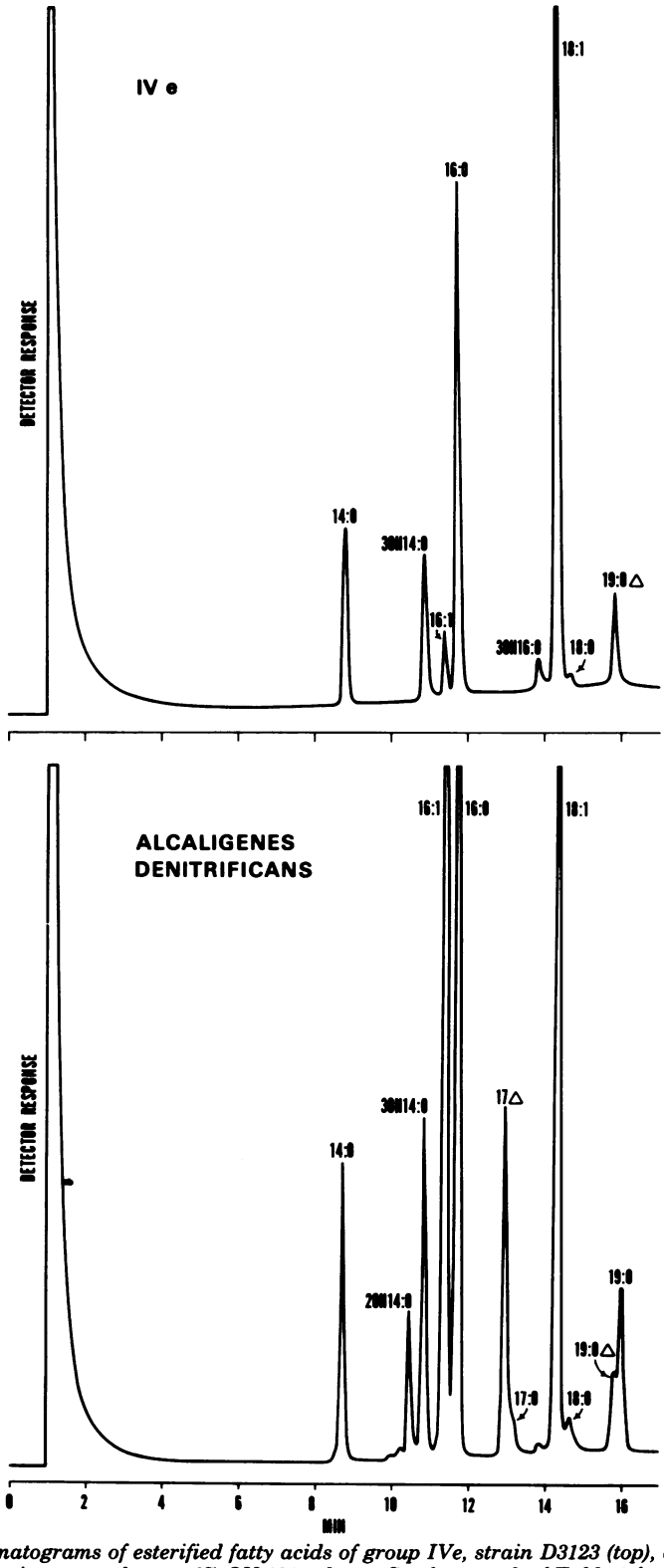

FIG. 1. *Gas chromatograms of esterified fatty acids of group IVe, strain D3123 (top), and A. denitrificans E370 (bottom). Analysis was made on a 3% OV-101 column. See footnote b of Table 1 for peak identification.*

three times. As indicated in Table 1, 18:1 comprised approximately 45% of the total cellular acids in group IVe strains.

The fatty acid profile of *A. denitrificans* E370 is shown in the bottom chromatogram in Fig. 1. The profile of this organism is representative of *A. denitrificans* and two other *Alcaligenes* species, *A. faecalis* and *A. odorans*, which are frequently isolated from clinical specimens. Previous studies have shown that these three species cannot be distinguished by cellular fatty acids (1). In contrast to group IVe organisms, which contained one major acid (18:1), *Alcaligenes* contained three major acids (16:1, 16:0, 18:1), which together constituted approximately 65% of the total acids (Table 1). In addition, *A. denitrificans* contained small amounts of 2-OH-14:0 and a 17-carbon cyclopropane acid (17:0Δ) which were not detected in group IVe strains. Three of four strains of *A. denitrificans* had fatty acid profiles that were essentially identical to that shown in Fig. 1; however, the fourth strain differed by the presence of relatively large amounts of 16:0 and 17:0Δ and the presence of 12:0, 2-OH-12:0, and 3-OH-12:0 acids. These results are consistent with our previous findings, which indicated that strains of *A. denitrificans* exhibit two different fatty acid profiles depending upon the strain tested (1). However, both fatty acid profiles of *A. denitrificans* are sufficiently distinct from group IVe so that they can be distinguished with ease. In addition, five strains of *B. bronchiseptica* were tested and were found to have a fatty acid composition essentially identical to that of strains of *Alcaligenes* sp., which contain 16:0 and 17:0Δ as major fatty acids (1). As shown in Table 1, these organisms also contain relatively small amounts of 2-OH-12:0, 14:0, 3-OH-14:0, 16:1, 18:1, and 18:0. Although these organisms are very similar to group IVe in conventional microbiological tests, *B. bronchiseptica* can be readily distinguished from this group on the basis of cellular fatty acids (Table 2).

TABLE 1. *Cellular fatty acid composition of group IVe, A. denitrificans, B. bronchiseptica, and Brucella spp.*

Organism	Fatty acids (% of total)[a]														
	12:0[b]	2-OH-12:0	3-OH-12:0	14:0	2-OH-14:0	3-OH-14:0	16:1	16:0	17:0Δ	17:0	3-OH-16:0	18:1	18:0	19:0Δ	19:0
Group IVe (8)[c]	—	—	—	10	—	9	3	26	—	—	2	45	T	5	—
A. denitrificans (3)	—	—	—	6	3	7	28	21	8	2	T	16	3	2	4
B. bronchiseptica (5)	T	5	T	8	—	6	14	29	25	T	T	7	6	T	T
B. suis (2)	—	—	—	—	—	—	2	17	T	2	—	22	7	50	T
B. melitensis (2)	—	—	—	—	—	—	3	19	T	4	—	24	8	42	T
B. abortus (2)	—	—	—	—	—	—	T	13	T	2	—	36	13	36	T

[a] T, Less than 2%; —, not detected.
[b] Number to left of colon refers to number of carbon atoms; number to right refers to number of double bonds; OH, hydroxy acid; Δ, cyclopropane acid.
[c] Numbers in parentheses refer to number of strains tested.

TABLE 2. *Identification of clinical isolates of group IVe and similar organisms by selected conventional tests and by cellular fatty acids*

Organism	Flagella	Conventional tests[a]					Major cellular fatty acids[b]
		Oxidase	Hydrolysis of urea	Nitrite reduction, gas	Acid in OF[c] medium		
					Glucose	Xylose	
Group IVe	Peritrichous[d]	+	+	v	−	−	18:1[e]
A. denitrificans	Peritrichous	+	v	+	−	−	16:1, 16:0, 18:1 or 16:0, 17:0Δ[f]
B. bronchiseptica	Peritrichous	+	+	−	−	−	16:0, 17:0Δ
Brucella spp.	−	+	+	v	v	+	19:0Δ

[a] +, 90% or more positive in 1 to 2 days; −, no reaction (90% or more); v, 11 to 89% positive.
[b] See Fig. 1 and 2 and Table 1.
[c] OF, Oxidative-fermentative.
[d] Weakly motile, one to two polar and long lateral flagella (group IVe only).
[e] Number to the left of the colon refers to number of carbon atoms; number to the right refers to number of double bonds; Δ, cyclopropane acid.
[f] Major fatty acids of four strains are 16:1, 16:0, and 18:1, but two strains have 16:0 and 17:0Δ as major acids.

The cellular fatty acid profile of *B. suis* B6537 is shown in Fig. 2. This profile is representative of both *B. suis* and *B. melitensis* and is included because other *Brucella* species resemble group IVe in the early stages of identification. Their similarities in morphology on blood agar and their rapid hydrolysis of urea may lead initially to a misidentification, especially if xylose is not included in the screening procedure. However, the fatty acid profile of *B. suis* (Fig. 2) is distinct and can be readily used to distinguish these organisms from group IVe. The major difference in *Brucella* is the presence of relatively large amounts of a 19-carbon cyclopropane acid (19:0Δ), which comprises 40 to 50% of the total cellular acids (Table 1); a second difference is the complete absence of hydroxy acids. Although 18:1 is the major acid in group IVe strains, it is the second largest peak (22 to 24%) in *Brucella*. The only other acid present in relatively large amounts (17 to 19%) was 16:0. An additional strain of *B. suis* and two strains each of *B. melitensis* and *B. abortus* gave profiles essentially identical to that shown in Fig. 2. However, the ratio of 19:0Δ to 18:1 determined for strains of *B. abortus* was 1:1, whereas that ratio in strains of *B. suis* and *B. melitensis* was approximately 2:1. Our data are in agreement with previous investigators who found that 19:0Δ was the most abundant fatty acid detected in the "free lipid" extracts of *B. melitensis* and *B. abortus* (11).

A selected number of conventional biochemical tests that are most useful in distinguishing group IVe from similar organisms is given in Table 2. The most outstanding feature of group IVe is a unique flagellar arrangement. These organisms also rapidly hydrolyze urea. As indicated previously, *Brucella* spp. are similar to group IVe in morphology and ability to hydrolyze urea. In addition, both organisms are oxidase positive and positive for reduction of nitrate to nitrite, but are variable in their ability to reduce inorganic nitrite to gas. Although *Brucella* spp. may be distinguished from group IVe by the absence of flagella and the oxidation of xylose, strains of group IVe may be mistakenly identified as *Brucella*. The most likely reason for this is that xylose has been omitted from the screening procedure, and demonstrating flagella and motility is difficult with weakly motile strains of IVe. Concurrently, *B. bronchiseptica* and urease-positive strains of *A. denitrificans* may be mistakenly identified as group IVe. As shown in Table 2, the species in question are unequivocally distinguished by cellular fatty acids. Their clear distinction indicates the value of these data as additional parameters for the identification of group IVe. The gas-liquid chromatography test for cellular fatty acids is reproducible and relatively simple to perform. When used with a selected number of conventional tests, the gas-liquid chromatography procedure provides a rapid and reliable diagnostic aid for identifying unclassified bacteria like group IVe.

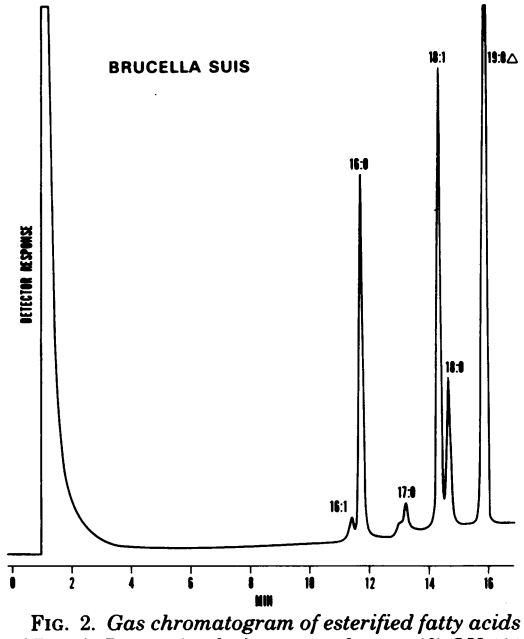

FIG. 2. *Gas chromatogram of esterified fatty acids of B. suis B6537. Analysis was made on a 3% OV-101 column. See footnote b of Table 1 for peak identification.*

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