Cellular Fatty Acid Composition as a Chemotaxonomic Marker for the Differentiation of Phenospecies and Hybridization Groups in the Genus *Aeromonas*

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Ninety genotypically characterized *Aeromonas* strains, including members of all 14 currently established genospecies, were studied by performing gas-liquid chromatographic analysis of their cellular fatty acid methyl esters (FAMES). A total of 44 fatty acids and two alcohols were found in members of the genus *Aeromonas*. All 90 strains contained 12:0, 13:0 iso, 14:0, 15:0 iso 3OH, 16:0, 16:1 ω 7c, 17:0 iso, iso 17:1 ω 9c, summed feature 3 (16:1 iso I and/or 14:0 3OH), and summed feature 7 (18:1 ω 7c, 18:1 ω 9t, and/or 18:1 ω 12t), whereas all but one strain (99%) also contained 15:0 iso. Although the FAME profiles were very similar, minor quantitative variations could be used to differentiate phenospecies and/or hybridization groups. A cluster analysis of the mean data revealed five FAME clusters, which were compared with phenotypic and genotypic groups identified in the genus *Aeromonas*. Hybridization groups that constituted the *Aeromonas hydrophila* complex, the *Aeromonas caviae* complex, and the *Aeromonas sobria* complex were basically grouped into distinct FAME clusters. The taxonomic positions of hybridization groups 7 and 11 in these clusters, however, remained unclear. All of our results were highly reproducible. A new database of *Aeromonas* FAME fingerprints was generated, and this database can be used for rapid identification of unknown aeromonads. Using a large set of well-characterized aeromonads, we demonstrated for the first time that gas-liquid chromatographic FAME analysis can be used to differentiate the majority of the phenospecies and/or hybridization groups in the genus *Aeromonas*.

Since Popoff and coworkers (26) placed 55 *Aeromonas* strains into DNA hybridization groups (HGs), the taxonomy of this genus has depended on a complex mixture of phenotypic and genotypic data. For this reason, biochemically distinct species are referred to as phenospecies, whereas distinct DNA HGs are usually called genospecies or genomic species. The four-species concept described in *Bergey's Manual of Systematic Bacteriology* (25), which was based on phenotypic characteristics, recognizes the following four groups or complexes in the genus *Aeromonas*: *Aeromonas hydrophila* (HGs 1, 2, and 3), *Aeromonas caviae* (HGs 4, 5A, 5B, and 6), *Aeromonas sobria* (HGs 7, 8, and 9), and *Aeromonas salmonicida* (HG 3). These groups are treated as if they are members of one (pheno)species, although each consists of one or more HGs. In contrast, strains belonging to the recently described taxa *Aeromonas veronii* biogroup *veronii* (HG 10), *Aeromonas schubertii* (HG 12), and *Aeromonas trota* (HG 13) seem to be genotypically homogeneous; in these cases each phenospecies corresponds to one HG. So far, the genus *Aeromonas* contains at least 11 phenospecies and at least 14 HGs (17, 37). Several of the genospecies are difficult to separate biochemically or have not been named yet because of a lack of phenotypic characteristics that correlate well with DNA-DNA homology data (4). Along with the ever-changing taxonomic situation of the genus *Aeromonas*, the question of whether aeromonads should be considered human opportunistic pathogens has been controversial. In fact, aeromonads have been associated with a broad range of infections in cold- and warm-blooded animals, but until now there has been little or no evidence that putative virulence

factors of aeromonads (e.g., hemolysin) can be unequivocally linked to the disease process itself (17).

For the past 10 years there has been a strong need for the development and application of new typing methods to address the current taxonomic and pathogenic problems in the genus *Aeromonas*. Until recently, it seemed that most chemotaxonomic typing methods could be used only for epidemiological purposes. Methods such as serotyping (13), sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins (32), and phage typing (2) are very useful for differentiating individual *Aeromonas* strains but are too sensitive to have any taxonomic value. Data obtained by biotyping (9, 19) and multilocus enzyme electrophoresis (3, 24, 33) correlated well with DNA-DNA hybridization data; however, these techniques are time consuming and require too many reagents to be of practical use in routine analyses.

Gas-liquid chromatographic analysis of fatty acid methyl esters (FAMES) does not have the disadvantages described above and has been used extensively for routine microbial identification and classification (18, 31, 34–36, 38). Only a few major studies of the cellular fatty acid compositions of *Aeromonas* species have been published previously. Originally, fatty acid analysis seemed to be useful only for separating clinical aeromonads as a group from nonclinical aeromonads (5, 13). Later, Canonica and Pisano (6) and Hansen and coworkers (12) described minor differences in the FAME profiles of clinical strains as well as nonclinical strains of *A. hydrophila*, *A. caviae*, and *A. sobria*. The cellular fatty acid compositions of the more recently described *Aeromonas* species (7, 8, 14, 15), however, have received very little attention (12). In addition, no previously described FAME study included *Aeromonas* strains belonging to particular HGs or paid attention to the striking taxonomic differences between the phenospecies and genospecies identified in the genus *Aeromonas*.

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To our knowledge, this paper is the first report that describes the fatty acid profiles of genotypically characterized *Aeromonas* strains which represent all currently established HGs in the genus *Aeromonas*. Two recently described species, *Aeromonas ichthiosmia* (29) and *Aeromonas enteropelogenes* (28), were not included in this study because on the basis of phylogenetic evidence it appears that these organisms are not new species but rather are identical to *A. veronii* biogroup *veronii* and *A. trota*, respectively (10). Strains of the nonmotile psychrophilic species *A. salmonicida* grow weakly when they are cultivated at 28°C and are therefore not discussed in this paper. Our aims were (i) to determine whether there are qualitative and/or quantitative differences in the mean FAME profiles of the different HGs, (ii) to correlate our findings with previously published phenotypic, genotypic, and FAME data, and (iii) to create a database of *Aeromonas* FAME fingerprints which can be used for rapid identification of unknown aeromonads.

MATERIALS AND METHODS

Strains. All of the bacterial cultures which we used were obtained from the Culture Collection of the Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium, the Culture Collection of the University of Göteborg, Göteborg, Sweden, and the Institute of Medical Microbiology, University of Zürich, Zürich, Switzerland. Details concerning the strains are shown in Table 1. The strains used for fatty acid analysis were cultured aerobically on Trypticase soy agar containing 3% (wt/vol) Trypticase soy broth (BBL) and 1.5% (wt/vol) Bacto Agar (Difco) at 28°C for 24 and/or 48 h.

FAME analysis. FAMES were prepared and extracted by using the standardized protocol of the Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Del.). The extracts were analyzed with a Hewlett-Packard model HP5890A gas chromatograph equipped with a flame ionization detector, an automatic sampler, an integrator, and a computer, as described previously (23). Identification of the FAME fingerprints, generation and validation of the database, and unweighted arithmetic average cluster analysis were performed by using the MIDI software package (version 3.7).

RESULTS AND DISCUSSION

Reproducibility. Bacterial fatty acid composition varies with the culture medium, the incubation temperature, and the physiological age of the cell population. However, several investigators have demonstrated that highly reproducible fatty acid profiles can be obtained if all of these experimental parameters are standardized (23). We recultivated five strains three or four times and analyzed their FAME extracts under the highly standardized conditions described in the Microbial Identification System protocol. In general, the data for the most common fatty acids varied less than 3%, thus indicating that the procedure had good reproducibility.

Influence of the physiological cell age. Five strains belonging to HGs 1, 2, and 3 (phenotypically *A. hydrophila*), HGs 4, 5A, and 5B (phenotypically *A. caviae* or *Aeromonas media*), or HG 8 (phenotypically *A. veronii* biogroup *sobria*) were incubated on Trypticase soy agar at 28°C for 24 and 48 h. More reproducible FAME patterns were obtained after incubation for 48 h. At the genospecies level, more characteristic differences that could be used for differentiation of HGs 4, 5A, and 5B were obtained after 48 h of incubation. Only slightly better separation of HGs 1, 2, and 3 was obtained with the 24-h data (data not shown). To delineate the groups at the phenospecies

level, the FAME profiles obtained after 48 h clearly had more differentiating characteristics than the FAME profiles obtained after 24 h. These results indicate that better taxonomic resolution would be obtained if all subsequent analyses are performed after 48 h of incubation. This is in contrast to the results of previous fatty acid analyses of aeromonads (6, 12, 13), in which cells were harvested after 24 h. It is interesting that, in close agreement with our results, more characteristic differences between taxa were obtained with the FAME profiles of *Xanthomonas* strains after 48 h of growth (30, 38).

Fatty acids in *Aeromonas* strains. A total of 44 fatty acids and two alcohols were detected in a gas-liquid chromatographic analysis of 90 genotypically characterized *Aeromonas* strains. Seventeen FAMES were present in less than 5% of the strains tested or were present at levels of less than 0.1%; these FAMES were not included in our analysis. The frequencies and mean percentages of the other 29 FAMES are shown in Table 2.

All 90 strains contained 12:0, 13:0 iso, 14:0, 15:0 iso 3OH, 16:0, 16:1 ω 7c, 17:0 iso, iso 17:1 ω 9c, summed feature 3 (16:1 iso I and/or 14:0 3OH), and summed feature 7 (18:1 ω 7c, 18:1 ω 9t, and/or 18:1 ω 12t), and all but one strain (LMG 13453, belonging to HG 3) contained 15:0 iso. Clearly, these 11 features should be considered the most characteristic fatty acids of the genus *Aeromonas*. Together with two other fatty acids (15:0 and 17:1 ω 8c), which appeared in 85 (94%) of the strains, the 11 features mentioned above accounted for almost 93% of the total cellular fatty acid composition of the genus *Aeromonas*. Consequently, differentiation among phenospecies and/or HGs depended almost entirely on quantitative differences among the FAME profiles.

Unsaturated fatty acid 16:1 ω 7c was the predominant fatty acid (mean level, 29.9%), whereas the saturated fatty acid 16:0 and the unsaturated fatty acid 18:1 (summed feature 7) were the second and third most common fatty acids (relative percentages, 14.4 and 9.9%, respectively). These three major components accounted for almost 55% of the total cellular fatty acids. Hansen and coworkers (12) reported that these components accounted for up to 60% of the total fatty acids of 39 *Aeromonas* strains which they studied, although the experimental conditions used by these authors (growth medium, incubation temperature, and incubation time) were totally different from the conditions which we used. This finding indicates that standardization of these parameters probably would have little or no influence on the relative amounts of the major fatty acids.

Lambert and coworkers (21) found that members of the *Vibrionaceae* (the genera *Vibrio*, *Aeromonas*, *Photobacterium*, and *Plesiomonas*) could be differentiated from *Escherichia coli* (*Enterobacteriaceae*) because the concentration of 16:1 ω 7c in the members of the *Vibrionaceae* was at least 1.5 times greater than the concentration of 16:0, while in *E. coli* this ratio was less than 1.0. Using the mean values for the fatty acid contents, we found a 16:1 ω 7c/16:0 ratio of 2.1 for the genus *Aeromonas*, which may indicate that there is a close relationship between the genus *Aeromonas* and the *Vibrionaceae*. In addition, all 90 *Aeromonas* strains lacked the cyclopropane fatty acids usually found in most genera of the *Enterobacteriaceae*. In the study of Lambert et al. (21), *A. hydrophila* and *A. salmonicida* were differentiated from all other members of the *Vibrionaceae* because they did not contain 13:1 iso and contained only trace amounts of 12:0 3OH. This may support the view that establishment of a separate family, the *Aeromonadaceae*, would be appropriate (11). The results of a FAME analysis of 12 clinical *Aeromonas* strains performed by Canonica and Pisano (5) demonstrated that the hydroxy fatty acid profiles obtained

TABLE 1. Strains used

Phenospecies	HG ^a	Cluster ^b	Strain ^{c,d}	Designation as received and/or other designation ^{c,d}	Source of isolation
<i>A. hydrophila</i>	1	E	LMG 2844 ^T	ATCC 7966 ^T , A 306 ^T , CCUG 14551 ^T	Milk with fishy odor
			LMG 13439	A 17	Unknown
			LMG 13440	A 19	Unknown
			LMG 13441	A 34	Feces
			LMG 13442	A 52	Feces
			LMG 13443	A 229	Feces
			LMG 13657	A 145	Feces
			LMG 13658	A 162	Feces
			LMG 13659	A 167	Feces
			LMG 13660	A 188	Contaminated Bactec vial
	2	E	LMG 13444 ^R	A 307 ^R	Diseased fish
			LMG 13445	A 2	Human
			LMG 13446	A 13	Environment
			LMG 13447	A 39	Water
			LMG 13448	A 169	Feces
			LMG 13662	A 295	Feces
			LMG 13449 ^R	A 99 ^R	Feces
	3	E	LMG 13450	A 63	Feces
			LMG 13451	A 308	Freshwater
			LMG 13452	A 7	Environment
			LMG 13453	A 8	Environment
			LMG 13674	A 14	Environment
			LMG 13675	A 15	Environment
<i>A. caviae</i>	4	B	LMG 3775 ^T	ATCC 15468 ^T , A 309 ^T , CCUG 25939 ^T	Epizootic, guinea pig
			LMG 13454	A 1	Human
			LMG 13455	A 30	Blood culture
			LMG 13456	A 41	Feces
			LMG 13457	A 102	Feces
			LMG 13658	A 234	Feces
			LMG 13676	A 12	Environment
			LMG 13677	A 20	Feces
			LMG 13678	A 26	Blood culture
			LMG 13680	A 61	Feces
	5A	C	LMG 13459 ^R	A 310 ^R	Fish
			LMG 13460	A 6	Environment
			LMG 13461	A 75	Feces
			LMG 13462	A 93	Feces
			LMG 13463	A 196	Feces
			LMG 13681	A 163	Feces
			LMG 13682	A 170	Feces
			LMG 13683	A 208	Feces
			LMG 13684	A 225	Feces
	5B	C	LMG 13464	A 904	Fish
			LMG 13465	A 117	Feces
			LMG 13466	A 213	Feces
			LMG 13467	A 242	Feces
			LMG 13468	A 284	Feces
<i>A. media</i>	5B	C	LMG 9073 ^T	ATCC 33907 ^T , A 912 ^T	Water
<i>A. eucrenophila</i>	6	C	LMG 3774 ^T	NCMB 74 ^T , A 311 ^T , CCUG 30340 ^T	Freshwater fish
			LMG 13057	CCUG 30341, A 914	Unknown
			LMG 13058	CCUG 30342, A 1650	Carp
			LMG 13060	CCUG 30344, A 1652	Water
			LMG 13061	CCUG 30345, A 1653	Water
			LMG 13687	CCUG 30343, A 1651	Water
			LMG 13691	CCUG 30347, A 1655	Groundwater
<i>A. sobria</i>	7	E	LMG 3783 ^T	CIP 74.33 ^T , A 312 ^T , CCUG 14830 ^T	Fish
			LMG 13469	A 915	Fish
<i>A. veronii</i> biogroup <i>sobria</i>	8	A	LMG 13067	CCUG 30356, A 10	Environment
			LMG 13068	CCUG 30357, A 64	Feces
			LMG 13069	CCUG 30358, A 66	Feces
			LMG 13070	CCUG 30359, A 68	Feces
			LMG 13071	CCUG 30360, A 132	Feces
			LMG 13072	CCUG 30361, A 151	Feces
			LMG 13073	CCUG 30362, A 155	Feces
			LMG 13074	CCUG 30363, A 916	Feces
			LMG 13693	A 11	Environment
			LMG 13694	A 27	Unknown

Continued on following page

TABLE 1—Continued

Phenospecies	HG ^a	Cluster ^b	Strain ^{c,d}	Designation as received and/or other designation ^{c,d}	Source of isolation
<i>A. jandaei</i>	9	A	LMG 13695	A 28	Feces
			LMG 13700	A 168	Feces
			LMG 12221 ^T	ATCC 49568 ^T , A 1642 ^T , CCUG 30353 ^T	Feces
			LMG 13064	CCUG 30348, A 179	Feces
			LMG 13065	CCUG 30350, A 919	Feces
			LMG 13066	CCUG 30351, A 950	Unknown
			LMG 13077	CCUG 30352, A 1641, ATCC 49570	Wound below left eye
<i>A. veronii</i> biogroup <i>veronii</i>	10	D	LMG 13078	CCUG 30354, A 1643, ATCC 49572	Prawn, Hawaii
			LMG 13079	CCUG 30355, A 1644, ATCC 49571	Leg wound, diver
			LMG 9075 ^T	ATCC 35624 ^T , A 901 ^T	Near-drowning victim
			LMG 13470	A 925	Kidney of tarbot
			LMG 13471	A 923	Kidney of brook trout
			LMG 13472	A 924	Kidney of rainbow trout
			LMG 13075	CCUG 30364, A 902, ATCC 35941	Ankle fracture
<i>A. veronii</i>	11	C	LMG 13076	CCUG 30365, A 926	Water, Mohawk River
<i>A. schubertii</i>	12	A	LMG 9074 ^T	ATCC 43700 ^T , A 903 ^T , CCUG 27820 ^T	Abscess on forehead
			LMG 12655	CCUG 25582	Human leg wound
			LMG 12668	CCUG 25583	Human leg wound
<i>A. trota</i>	13	A	LMG 13473	A 922, ATCC 43701	Human skin
			LMG 12223 ^T	ATCC 49657 ^T , A 1646 ^T , CCUG 30367 ^T	Stool, India
			LMG 13080	CCUG 30366, A 1645, ATCC 49659	Appendix
			LMG 13081	CCUG 30368, A 1647, ATCC 49660	Stool, Thailand
			LMG 13082	CCUG 30369, A 1648, ATCC 49658	Stool, Bangladesh

^a HG as determined in the DNA homology study of Altwegg and coworkers (4).

^b FAME cluster comprising one or more HGs as determined by cluster analysis of the mean fatty acid profiles (Fig. 1).

^c T = type strain. R = hybridization group reference strain.

^d A, Institute of Medical Microbiology, Zürich, Switzerland; ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; LMG, Laboratorium voor Microbiologie Culture Collection, Ghent, Belgium; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland.

from clinical strains are, in part, qualitatively different from the profiles of isolates that have nonclinical origins; e.g., 12:0 3OH was found only in clinical aeromonads. In our study, the fatty acid profiles of 90 strains revealed that not a single strain produces 13:1 iso and that 34 strains (38%) contain 12:0 3OH in trace amounts (up to 0.6%); 28 of these 34 strains are clinical isolates, and 6 are nonclinical isolates. In general, these findings are in agreement with the results of both previously described FAME studies (5, 13), although clear differentiation between strains that have clinical and nonclinical origins remains problematic.

Cluster analysis. Mean profiles were determined for all strains belonging to a particular HG. Unweighted arithmetic average clustering of the resulting 14 HG-specific profiles revealed five FAME clusters when the taxa were delineated at an Euclidian distance of 7 (Fig. 1). In general, the same clusters (clusters A through E) were found when the individual fatty acid profiles of all of the strains were used. However, since the clustering method which we used was based mainly on an overall comparison of FAME profiles, no clear differentiation of genospecies was obtained within the major FAME clusters (clusters A through E). Therefore, Fig. 1 is a simplified dendrogram in which the mean fatty acid profiles of the HGs are grouped together. Table 3 shows the mean fatty acid contents and standard deviations for the 14 HGs. The significant differences in the FAME profiles at the phenospecies level as well as at the genospecies level were mainly quantitative and are discussed below.

FAME cluster A contained 27 strains belonging to HGs 8, 9, 12, and 13 and was very well separated from the other four clusters at a Euclidian distance of 12.5. Together with the members of FAME cluster B, the members of this cluster contained the lowest relative amounts (22.0 to 25.8%) of 16:1

ω 7c found in the genus *Aeromonas*. Except for *A. trota* (HG 13) all members of cluster A contained significant amounts of the saturated fatty acid 13:0 (0.6 to 1.3%).

FAME cluster B was delineated at a Euclidian distance of 10.5 and was defined by the mean profile of 10 *A. caviae* HG 4 strains. Together with the members of HG 12, the members of this FAME group contained the highest relative amount of the branched fatty acid 13:0 iso (3.1%).

FAME cluster C comprised HGs 5A, 5B, 6, and 11, and its members were linked at a Euclidian distance of 7.7. This cluster was the only cluster whose members produced significantly fewer fatty acids (they produced 13 to 15 fatty acids) and lacked both 16:0 N alcohol and 16:1 ω 7c alcohol. Together with the members of HG 4, the members of all of these HGs lacked the branched fatty acid 17:0 10 Me. In addition, they produced only trace amounts (up to 0.6%) of 15:0, 16:0 iso, 17:0, and 17:1 ω 8c.

FAME cluster D was defined by the mean profile of four *A. veronii* biogroup *veronii* (HG 10) strains and was delineated at a Euclidean distance of 7. The dendrogram in Fig. 1 clearly indicates that this FAME group is closely related to FAME clusters C and E.

FAME cluster E was defined by the mean FAME data for 25 strains belonging to *A. sobria* (HG 7) and *A. hydrophila* (HGs 1, 2, and 3) and was closely related to cluster D, as discussed above. The members of HGs 1, 2, and 3 are characterized by the highest relative amounts (17.3 to 18.2%) of the saturated fatty acid 16:0 found in the genus *Aeromonas*.

Generation of an *Aeromonas* database. In a previous paper (16), we described the first results which indicated the resolving power of a new database, AER48. This database contains the mean FAME data for 70 genotypically characterized aeromonads, including members of all currently established

TABLE 2. Major fatty acids of 90 genotypically identified strains belonging to the genus *Aeromonas*^a

Compound	Frequency (%)	% in <i>Aeromonas</i> strains	
		Mean ^b	Range
Saturated fatty acids			
12:0	100.0	7.1	3.1–14.6
13:0	64.4	0.4	0.0–1.6
14:0	100.0	2.9	1.1–5.6
15:0	94.4	1.2	0.0–4.5
16:0	100.0	14.4	7.4–23.2
17:0	81.1	0.9	0.0–2.8
18:0	66.7	0.3	0.0–1.2
Unsaturated fatty acids^c			
16:1 ω 7 ^c	100.0	29.9	17.9–40.1
17:1 ω 8 ^c	94.4	1.7	0.0–5.4
17:1 ω 6 ^c	46.7	0.3	0.0–1.4
Branched fatty acids			
13:0 iso	100.0	1.9	0.4–4.4
14:0 iso	18.9	0.1	0.0–1.5
15:1 iso F ^d	23.3	0.1	0.0–1.2
15:0 iso	98.9	3.0	0.0–7.2
16:0 iso	83.3	1.1	0.0–3.6
iso 17:1 ω 9 ^c	100.0	3.9	0.6–13.5
17:0 iso	100.0	4.6	0.5–14.7
17:0 anteiso	26.7	0.1	0.0–0.7
17:0 10 methyl	40.0	0.6	0.0–4.4
Hydroxy fatty acid (12:0 3OH)	37.8	0.1	0.0–0.6
Branched hydroxy fatty acids			
14:0 iso 3OH	50.0	0.3	0.0–1.6
15:0 iso 3OH	100.0	4.3	0.8–10.4
Unknowns^e			
Unknown 13.961	37.8	0.3	0.0–1.0
Unknown 14.503	58.9	0.4	0.0–1.2
Summed features^f			
Summed feature 2	32.2	0.4	0.0–1.8
Summed feature 3	100.0	8.0	3.5–17.1
Summed feature 7	100.0	9.9	5.7–22.3
Alcohols			
16:0 N	34.4	0.4	0.0–3.0
16:1 ω 7 ^c	48.9	1.4	0.0–10.4

^a Fatty acids 11:0 iso, 11:0, unknown 11.541, 12:0 iso, unknown 11.798, 14:1 iso E, 15:0 anteiso, 15:1 ω 8^c, 15:1 ω 6^c, 15:0 2OH, 15:0 3OH, 18:0 iso, 19:0 anteiso, 19:1 ω 12^c, 20:1 ω 9^t, summed feature 5, and summed feature 6 were present in less than 5% of the strains tested or were present at levels of less than 0.1% (mean value). When the MIDI system was used, gas-liquid chromatography also revealed the presence of two C₁₆ alcohols.

^b Mean values were calculated by using FAME data for the 90 strains listed in Table 1, regardless of the fact that every fatty acid was not detected in all of the strains.

^c The position of the double bond can be located by counting from the methyl (ω) end of the carbon chain. A *cis* isomer is indicated by the suffix *c*.

^d The double bond position indicated by a capital letter is unknown.

^e The unknown fatty acids have no name listed in the Peak Library File of the MIDI system and therefore can be identified only by their equivalent chain lengths.

^f Summed features represent groups of two or three fatty acids which could not be separated by gas-liquid chromatography with the MIDI system. Summed feature 2 contained one or more of the following fatty acids: 13:0 3OH, 15:1 iso I, and/or 15:1 iso H. Summed feature 3 contained 16:1 iso I and/or 14:0 3OH. Summed feature 7 contained one or more of the following isomers: 18:1 ω 7^c, 18:1 ω 9^t, and/or 18:1 ω 12^t (*cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively).

Aeromonas genospecies. *Aeromonas allosaccharophila* (22) and enteric group 501 (14) were not investigated because they have not been assigned to an HG yet. Using AER48, we classified 228 (92%) of 249 presumptive aeromonads isolated from various drinking water supplies in the genus *Aeromonas*, whereas 186 (82%) and 125 (55%) strains were assigned to

FIG. 1. Dendrogram showing the FAME clusters revealed by a numerical analysis of the mean fatty acid profiles of all 14 HGs in the genus *Aeromonas*. The numbers of strains tested are indicated in parentheses.

phenospecies and HGs, respectively. These values suggest that this database has shortcomings and/or indicate that there are additional, currently undescribed phenospecies and/or HGs. However, certain HGs (HGs 7 and 11) are represented by only a small number of strains, and this may give rise to statistical variations in the cluster analysis results and in identification of unknown aeromonads. To address these problems, we recently added 20 well-characterized aeromonads to the database, which now contains the fatty acid profiles of all 90 strains mentioned in this paper (data not shown).

Comparison of FAME data with phenotypic and genotypic data. The FAME data were compared mainly with data from previous taxonomic studies which included basically all of the strains used in this investigation. Kämpfer and Altwegg (19) used 329 biochemical characteristics to differentiate 176 *Aeromonas* strains representing all currently established genospecies and identified 16 phenotypes which contained two or more strains. Altwegg (1) and Altwegg et al. (4) determined the levels of DNA relatedness for 116 aeromonads by the hydroxy-apatite method and included strains in the same genospecies or HG if the relative binding ratios were more than 70% at 60°C and/or more than 60% at 75°C. Data from these two studies correlated well with each other, and moreover, our FAME clusters are basically in agreement with the majority of the phenotypic (19) and genotypic (1, 4) groups identified previously.

FAME cluster A contains the genospecies *A. veronii* biogroup *sobria* (HG 8), *Aeromonas jandaei* (HG 9), *A. schubertii* (HG 12), and *A. trota* (HG 13), all of which contain strains isolated from clinical sources (7, 8, 14, 20). According to the four-species concept (25), strains belonging to genospecies 7, 8, and 9 (*A. sobria* complex) are phenotypically highly related. The tight grouping in this complex has also been confirmed by DNA-DNA hybridizations data; levels of DNA homology

TABLE 3. Major fatty acid compositions of the *Aeromonas* FAME clusters

Compound ^a	% in:													
	FAME cluster A ^b				FAME cluster B				FAME cluster C				FAME cluster D	
	HG 8 (n = 12) ^c	HG 13 (n = 4)	HG 9 (n = 7)	HG 12 (n = 4)	HG 10 (n = 10)	HG 5A (n = 9)	HG 11 (n = 2)	HG 5B (n = 6)	HG 6 (n = 7)	HG 1 (n = 10)	HG 7 (n = 2)	HG 2 (n = 6)	HG 3 (n = 7)	
Saturated fatty acids														
12:0	6.1 (1.4) ^d	4.8 (1.0)	3.8 (0.6)	6.6 (1.5)	10.6 (2.0)	9.5 (0.9)	5.8 (2.7)	8.1 (1.6)	7.0 (0.7)	6.3 (0.8)	6.1 (0.2)	6.8 (1.1)	7.5 (0.7)	
13:0	0.6 (0.3)	tr ^e	0.8 (0.2)	1.3 (0.3)	tr	tr	ND ^f	tr	tr	0.6 (0.2)	tr	0.6 (0.1)	tr	
14:0	2.6 (0.8)	2.9 (0.7)	4.0 (0.7)	3.1 (0.7)	1.9 (0.3)	2.5 (0.9)	2.9 (0.0)	2.2 (0.7)	2.6 (0.3)	3.9 (1.0)	3.9 (0.9)	3.6 (0.7)	1.6 (0.6)	
15:0	1.2 (0.5)	1.0 (0.4)	2.1 (0.5)	2.5 (0.4)	1.3 (1.6)	0.5 (0.2)	0.4 (0.0)	tr	tr	2.0 (1.0)	1.4 (1.1)	1.7 (0.3)	1.0 (0.4)	
16:0	10.6 (1.2)	13.7 (1.4)	11.1 (1.2)	9.6 (2.2)	14.2 (2.5)	15.4 (1.6)	16.6 (2.2)	15.5 (2.9)	14.4 (3.2)	17.3 (2.2)	16.3 (6.7)	17.4 (2.2)	18.2 (2.9)	
17:0	0.9 (0.5)	0.9 (0.3)	1.7 (0.6)	1.2 (0.2)	0.8 (0.9)	tr	tr	tr	tr	1.5 (0.6)	1.1 (0.9)	1.1 (0.2)	1.2 (0.5)	
18:0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.5 (0.0)	tr	0.5 (0.1)	
Unsaturated fatty acids														
16:1 ω7c	25.8 (4.2)	25.1 (3.2)	25.2 (1.2)	22.0 (3.2)	26.6 (1.7)	35.4 (1.0)	37.6 (3.5)	34.2 (2.8)	33.3 (3.8)	29.7 (2.4)	30.2 (2.3)	32.6 (1.8)	34.7 (3.7)	
17:1 ω8c	2.3 (1.3)	1.5 (0.6)	3.9 (1.2)	2.6 (1.2)	1.6 (1.6)	0.6 (0.2)	0.5 (0.1)	0.4 (0.4)	0.5 (0.3)	1.8 (0.7)	1.5 (0.6)	1.5 (0.4)	1.6 (0.5)	
17:1 ω6c	tr	tr	0.9 (0.3)	0.9 (0.4)	tr	ND	ND	tr	ND	tr	tr	tr	tr	
Branched fatty acids														
13:0 iso	2.2 (0.7)	2.1 (0.1)	1.6 (0.6)	3.0 (0.6)	3.1 (0.6)	1.6 (0.3)	1.2 (0.7)	1.9 (0.4)	2.1 (0.6)	1.2 (0.5)	1.7 (1.1)	0.9 (0.3)	0.8 (0.2)	
14:0 iso	tr	tr	tr	0.5 (0.3)	ND	ND	ND	ND	tr	tr	tr	ND	ND	
15:1 iso F	tr	tr	tr	0.6 (0.4)	tr	ND	tr	ND	tr	tr	ND	ND	ND	
15:0 iso	4.3 (1.8)	4.5 (1.3)	4.0 (2.3)	4.3 (1.6)	2.9 (1.3)	2.2 (0.7)	2.5 (0.4)	2.2 (0.3)	5.1 (2.4)	2.1 (1.3)	3.0 (2.4)	1.6 (0.9)	0.6 (0.3)	
16:0 iso	1.7 (1.0)	1.9 (1.2)	2.1 (1.2)	1.7 (0.8)	0.8 (0.5)	tr	tr	tr	tr	1.2 (0.6)	1.5 (0.3)	1.2 (0.6)	1.0 (0.3)	
iso 17:1 ω9c	6.6 (3.2)	8.4 (2.1)	4.7 (2.5)	5.4 (1.9)	3.2 (2.0)	1.4 (0.5)	4.1 (0.0)	4.4 (2.6)	5.1 (2.1)	2.1 (1.5)	2.7 (2.3)	2.1 (1.5)	1.7 (0.5)	
17:0 iso	7.0 (3.6)	7.6 (2.3)	5.7 (3.1)	4.4 (1.4)	3.7 (2.2)	4.0 (1.0)	5.2 (0.9)	5.2 (2.4)	5.3 (1.2)	2.3 (1.6)	4.4 (4.4)	2.7 (1.8)	3.6 (1.1)	
17:0 10 methyl	tr	tr	1.2 (0.8)	2.1 (1.7)	ND	ND	ND	ND	ND	1.1 (1.1)	tr	1.3 (1.1)	0.8 (0.9)	
Branched hydroxy fatty acids														
14:0 iso 3OH	0.7 (0.6)	0.5 (0.5)	0.6 (0.4)	0.8 (0.1)	tr	tr	ND	ND	ND	tr	0.7 (0.5)	tr	tr	
15:0 iso 3OH	6.0 (1.8)	4.8 (0.5)	3.3 (1.3)	5.2 (1.1)	5.9 (1.8)	4.2 (0.8)	3.6 (2.4)	4.6 (0.8)	5.6 (1.3)	2.1 (1.0)	3.3 (2.8)	2.2 (1.1)	2.6 (0.8)	
Unknowns														
Unknown 13.961	tr	ND	tr	ND	0.8 (0.1)	0.7 (0.3)	ND	tr	tr	tr	ND	tr	0.5 (0.2)	
Unknown 14.503	tr	tr	ND	ND	0.8 (0.4)	0.8 (0.1)	0.5 (0.2)	0.5 (0.1)	0.5 (0.1)	tr	0.8 (0.2)	tr	0.8 (0.1)	
Summed features														
Summed feature 2	0.5 (0.5)	ND	0.5 (0.3)	0.7 (0.2)	tr	ND	ND	ND	ND	tr	ND	tr	ND	
Summed feature 3	7.3 (2.8)	5.5 (1.2)	4.7 (1.2)	6.1 (1.5)	11.9 (3.2)	10.6 (1.3)	7.5 (3.1)	8.5 (2.8)	7.6 (1.1)	7.6 (1.6)	7.1 (0.5)	7.3 (1.4)	7.8 (1.4)	
Summed feature 7	9.5 (2.2)	10.8 (1.7)	12.1 (5.0)	9.4 (1.6)	8.3 (1.5)	9.0 (1.1)	10.8 (0.7)	10.6 (3.0)	8.3 (1.4)	12.0 (2.6)	11.3 (3.0)	8.9 (2.3)	10.2 (2.1)	
Alcohols														
16:0 N	tr	tr	0.9 (0.5)	1.2 (1.1)	ND	ND	ND	ND	ND	0.9 (0.8)	0.5 (0.7)	1.1 (1.2)	0.5 (0.5)	
16:1 ω7c	1.2 (1.6)	1.0 (1.0)	3.0 (1.6)	3.2 (2.4)	ND	ND	ND	ND	ND	2.3 (2.0)	1.3 (1.8)	3.8 (3.7)	1.9 (2.0)	

^a Fatty acid nomenclature is explained in the footnotes to Table 2.^b FAME clusters were delineated by unweighted arithmetic average clustering at a Euclidian distance of 7.^c n is the number of strains in the FAME cluster.^d Mean (standard deviation).^e tr, trace (<0.4%).^f ND, not detected.

ranging from 36 to 76% at 60°C have been found (1). HGs 12 and 13 are phenotypically quite similar to HGs 7, 8, and 9. In fact, Kämpfer and Altwegg (19) placed these five genospecies in a distinct phenon which is, except for the presence of HG 7, basically similar to FAME cluster A. The absence of HG 7 in the latter group and the lack of FAME relatedness between the phenotypically very similar genospecies *A. sobria* (HG 7) and *A. veronii* biogroup *sobria* (HG 8) are probably due to the fact that only two strains of HG 7 were included, which, on the basis of their FAME profiles, are quite heterogeneous. This suggestion was readily confirmed by the results of a cluster analysis of the type and reference strains of all *Aeromonas* genospecies; in this analysis tight clustering was found between LMG 3783 and LMG 13074, the type strain and the reference strain of HGs 7 and 8, respectively (data not shown).

FAME cluster B comprises *A. caviae* HG 4, a member of the *A. caviae* complex which also contains HGs 5A (*A. caviae*) and 5B (*A. caviae* and *A. media*), and the aerogenic species *Aeromonas eucrenophila* (HG 6) (27). The levels of DNA-DNA relatedness within this complex range from 38 to 69% at 60°C (1). The phenotypic and genotypic diversity in genospecies 5 indicates that this genospecies should be subdivided into two subgroups. These subgroups correspond to HGs 5A and 5B and were well differentiated in our FAME study. Kämpfer and Altwegg (19) assigned 10 strains of HG 4 to phenon 15, which is in fact quite similar to FAME cluster B. The clinical genospecies HG 4 is more easily separated from other members of the *A. caviae* complex on the basis of FAME data than on the basis of phenotypic data (19).

Together with FAME cluster B, FAME cluster C represents the entire *A. caviae* complex, as mentioned above. In addition, it also contains the mean fatty acid profile of two strains belonging to the genospecies *A. veronii* HG 11. On the basis of phenotypic data (19), HGs 5A, 5B, and 6 constitute a distinct phenogroup which clusters tightly with HG 4 and exhibits very little similarity to HG 11. So far, no correct name has been proposed for DNA homology group 11; moreover, only two strains (LMG 13075 and LMG 13076) belonging to this genospecies have been described. Strain LMG 13075 was formerly assigned to enteric group 77 (now *A. veronii* biogroup *veronii* HG 10), but differed from this taxon genetically (it exhibited less than 70% DNA-DNA relatedness with type strain LMG 9075 of HG 10 [15]). Kämpfer and Altwegg (19) demonstrated that HG 11 is phenotypically similar to the *A. hydrophila* complex, whereas the results of a numerical analysis of FAME data indicated that this taxon exhibits a high level of similarity with the *A. caviae* complex (Fig. 1). In a recent phenotypic study Carnahan and Joseph (9) used numerical taxonomy to systematically assess 167 aeromonads, including one of the strains belonging to HG 11 (LMG 13075). In contrast to the results of Kämpfer and Altwegg (19), the simple matching dendrogram of Carnahan and Joseph (9) strongly supports the data obtained for FAME cluster C by placing LMG 13075 in the *A. caviae* complex. The lack of agreement between the results of the two phenotypic studies (9, 19) may have been caused by the different markers used for numerical analysis. Kämpfer and Altwegg (19) used 30 conventional tests, including carbon source utilization, sugar fermentation, and enzyme production tests, whereas Carnahan and Joseph (9) used a much broader spectrum of characteristics, including 50 biochemical tests and antimicrobial resistance and virulence-associated markers.

FAME cluster D contains only *A. veronii* biogroup *veronii* (HG 10), which in fact belongs to a HG (HG 8/10) that also contains *A. veronii* biogroup *sobria* (HG 8) and cannot be distinguished from the latter by genotypic data (4). Because of

the physiological diversity in this DNA group, it seemed reasonable to establish two biogroups in *A. veronii* which correspond to HGs 8 and 10. Kämpfer and Altwegg (19) confirmed this suggestion by separating the two groups into distinct phena.

FAME cluster E consists of all of the members of the *A. hydrophila* complex (HGs 1, 2, and 3) and *A. sobria* (HG 7). As mentioned above, HG 7 is assigned to the *A. sobria* complex but is probably misplaced because of the heterogeneity of the two strains used. Strains belonging to the *A. hydrophila* complex are usually difficult to separate phenotypically; on the other hand, they cannot be clearly classified into one phenon (19). However, Carnahan and Joseph (9) systematically placed 51 geographically and clinically diverse *A. hydrophila* strains in one group of phenon by using 50 physiological tests. Moreover, DNA-DNA hybridization experiments (1) revealed a wide homology range (42 to 60% at 60°C) between members of the *A. hydrophila* complex. Our FAME data confirmed this grouping and, in addition, permitted separation of the clinically relevant taxon HG 1 from HGs 2 and 3 on the basis of minor quantitative variations in their fatty acid patterns.

Comparison of FAME data with data from other fatty acid studies. Only two major studies (6, 12) describing the fatty acid compositions of *Aeromonas* species have been published previously. In general, our results confirm the data in these studies. Differences in FAME data can be explained by the different culture conditions and/or different sets of strains used.

Canonica and Pisano (6) observed no qualitative differences in the fatty acid compositions of 12 *Aeromonas* strains that included *A. caviae*, *A. hydrophila*, and *A. sobria* strains but found some variations in the relative amounts of certain fatty acids. The ratio of 16:0 to 17:0 clearly separated the 12 aeromonads into three groups, which corresponded to the species designations. Our data revealed that the ratio of 16:0 to 17:0 in HGs 5A, 5B, and 6 is approximately two times greater than the ratios in all of the other genospecies. However, this feature alone does not differentiate all of the taxa found in this study. Previously, Hansen and coworkers (12) made a similar statement in their study of the FAME compositions of 39 *Aeromonas* strains belonging to the species *A. caviae*, *A. hydrophila*, *A. sobria*, *A. veronii*, *A. media*, and *A. schubertii*. These authors found that all of their fatty acid profiles were very similar both qualitatively and quantitatively and concluded that FAME analysis is not suitable for species differentiation in the taxonomically homogeneous genus *Aeromonas*.

In conclusion, this is the first report of the successful use of the FAME technique for differentiating the majority of a large number of genotypically characterized *Aeromonas* strains. Our results were achieved mainly by using highly standardized cultivation and extraction procedures and specialized software. The differences between taxa were mainly quantitative, which suggests that differentiation of genotypically distinct HGs in the genus *Aeromonas* by FAME analysis is possible only when a high-resolution identification system like the MIDI system is used. We have recently begun additional fatty acid analyses of other groups of well-characterized aeromonads to determine the precise chemotaxonomic value of the FAME technique with regard to the genus *Aeromonas*.

ACKNOWLEDGMENTS

This research was carried out in the framework of contract G. O. A. 91/96-2 of the Ministerie van de Vlaamse Gemeenschap, Bestuur Wetenschappelijk Onderzoek (Belgium), and contract BIOT-CT91-0294 of the Commission of European Communities.

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