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Chemical Characterization of *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium*-Like groups IIe, IIh, and IIf

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The cellular fatty acid, sphingolipid, and isoprenoid quinone compositions of *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium*-like groups IIe, IIh, and IIf were determined, using thin-layer, gas-liquid, and reverse-phase high-performance liquid chromatography. The fatty acid data showed that groups IIe, IIh, and IIf were similar to recognized *Flavobacterium* species by the presence of relatively large amounts of iso-branched hydroxy and nonhydroxy acids. Groups IIe and IIh were essentially identical in fatty acid composition but were distinguished from group IIf, *F. breve*, and *F. odoratum* on the basis of minor qualitative and quantitative differences. All strains tested contained menaquinone 6 as the major isoprenoid quinone, and all lacked sphingolipids. Overall, the chemical data suggest that groups IIe, IIh, and IIf are additional *Flavobacterium* species and are different from sphingobacteria, which contain sphingolipid and menaquinone 7 as the major quinone.

In recent years, our laboratory has reported (5, 15) the similarity in the cellular fatty acid compositions of *Flavobacterium meningosepticum* and some unnamed groups of organisms (IIb, IIk-2, and IIk-3). These bacteria, which were originally described by the late Elizabeth O. King, Centers for Disease Control (CDC) (21), have since been designated by DNA studies as new species of flavobacteria (9–11, 22). The later discovery of sphingolipids in certain flavobacteria has led to the reclassification of groups IIk-2 and IIk-3 in a new genus, *Sphingobacterium*, which is distinct from *Flavobacterium* primarily on the basis of the presence of sphinganin compounds in the cell membrane (22). During recent years, some of the originial CDC group IIb strains have been reclassified as *Flavobacterium indologenes* (22), *Flavobacterium gleum* (10), and *Flavobacterium balustinum* (10); some remaining IIb strains are still unidentified.

In this report, we have extended our investigation to the *Flavobacterium*-like groups IIe, IIh, and IIf, which have not been assigned to any taxon (17–20), and have compared these groups with *Flavobacterium breve* and *Flavobacterium odoratum*, two species which are closely associated with them in conventional tests. Our data from the analysis of cellular fatty acids, sphingolipids, and isoprenoid quinones indicate that the unnamed groups are readily distinguished from sphingobacteria and are probably additional species of flavobacteria.

MATERIALS AND METHODS

Cultures. Type and reference strains of the following bacteria were obtained from the culture collection of the Special Pathogens Reference Laboratory, Center for Infectious Diseases, CDC: *F. odoratum*, 11 strains, including the neotype strain NCTC 11036/CDC-KC1416 (12); *F. breve*, 11 strains, including the type strain NCTC 11099/CDC-KC1428 (8, 13); CDC group IIe, 9 strains; and CDC groups IIh and IIf, 10 strains each. Detailed descriptions of the morphologic and biochemical characteristics of these bacteria have been published previously (19, 20).

Culture conditions. For all chemical tests, strains were inoculated onto heart infusion agar plates supplemented with 5% rabbit blood. The plates were incubated at 35°C for 24 h for cellular fatty acid and sphingolipid analysis and for 48 h for quinone analysis. Sufficient cell mass for each strain was obtained by harvesting growth from five plates for quinones, six plates for sphingolipids, and one plate for cellular fatty acids.

Determination of cellular fatty acids. Cells were harvested from approximately one-fifth of one heart infusion agar-rabbit blood plate, saponified by adding 1.5 ml of 15% NaOH in 50% aqueous methanol, and heated at 100°C for 30 min. Fatty acids were esterified by adding 2.2 ml of 25% HCl in methanol to the cooled hydrolysate, and the contents were again heated to 100°C and held for 10 min. The fatty acid methyl esters were then extracted according to a previously published procedure, except that the amount of ether-hexane (1:1) was reduced to 1.5 ml (3). After mixing, the aqueous layer was removed, 1.0 ml of phosphate buffer (pH 11.0) was added, and the contents were mixed by shaking. The phases were allowed to separate by standing for 2 to 3 min, and the organic layer containing the methyl esters was removed to a test tube (13 by 100 mm) for subsequent analysis.

GLC of cellular fatty acids. Fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) with a model 3700 hydrogen flame ionization gas chromatograph (Varian Instruments, Palo Alto, Calif.) equipped with a fused-silica capillary column (50 m by 0.2-mm inner diameter) containing OV-1 as the stationary phase; data were recorded with a 3390A electronic integrator (Hewlett-Packard, Avondale, Pa.). Conditions for GLC analysis were described in an earlier report (16). Cellular fatty acids were identified by comparing retention times with those of authentic standards (Alltech Associates, Inc., Applied Science Div., State College, Pa.; Supelco, Bellefonte, Pa.) before and after sample acetylation and hydrogenation (4). GLC-mass spectrometry

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(14) and a mathematical plot of carbon number and retention time were also used to support the identification of both branched-chain and straight-chain hydroxy acids when standards were not commercially available.

Determination of sphingolipid long-chain bases. The methods used for the hydrolysis and extraction of sphingolipid bases from whole cells were those described by Yabuuchi et al. (23) and Yano et al. (24). Extracts of long-chain bases obtained from alkaline hydrolyzed cells were analyzed both by thin-layer chromatography and by GLC as the trimethylsilyl-ether derivative, using the trimethylsilyl derivatization procedure for long-chain bases described previously (2).

Thin-layer chromatography of sphingolipid bases. Ether extracts of the long-chain bases were developed on activated silica gel thin-layer plates with a solvent system of chloroform-methanol-water (65:25:4) as previously described (2). Ether extracts of the bases of *Flavobacterium* (*Sphingobacterium*) *multivorum* (CDC B5533, type strain) and *F. meningosepticum* (CDC 14, type strain) were used as controls on each plate. The long-chain bases were visualized as dark blue areas after the plates were sprayed with a ninhydrin solution (0.2% in acetone).

GLC of long-chain bases. Trimethylsilyl derivatives of sphingolipid bases (1 μl) were analyzed with a 3% OV-101-packed column (3.05 m by 6.71-mm inner diameter); peaks were recorded with a 3390A electronic integrator. Conditions for GLC analysis and identification of long-chain bases were as previously described (2).

Determination of isoprenoid quinones. Cells were hydrolyzed by adding 0.2 ml of 50% aqueous KOH and 3 ml of 1% pyrogallol in methanol and boiling (100°C) for 10 min. The cooled hydrolysates were then extracted with ether-hexane (1:1) as previously described (2). Quinones were finally dissolved in methanol and examined by reverse-phase high-performance liquid chromatography (HPLC).

HPLC of isoprenoid quinones. Authentic quinone standards and extracts of bacterial quinones were separated by HPLC as previously described (2). The HPLC system contained a model 1220 variable wavelength detector (LDC Div., Milton Roy Co., Riviera Beach, Fla.) and a μBondapak (300 by 3.9 mm, 10-μm particle size) C_{18} reverse-phase column (Waters Associates, Inc., Milford, Mass.). The gradient system was methanol-isopropanol-water (75:25:5) with a flow rate of 1 ml/min. Quinone standards and bacterial extracts were monitored at 248 and 275 nm and were collected from the HPLC column for mass-spectrometric analysis. Identities of quinones were confirmed by analysis on a DuPont mass spectrometer equipped for both electron impact and chemical ionization (2).

RESULTS AND DISCUSSION

There are currently two major chemical groups of clinically important bacteria which have been recognized in the genus *Flavobacterium*. One group is classified under the taxon *Flavobacterium* and has been shown to differ in chemical composition from the second group, *Sphingobacterium* (22). The major differences between these groups are in their sphingolipid and isoprenoid quinone contents and in the relative amounts of characteristic cellular fatty acids. Sphingobacteria were so named because they contain relatively large amounts of sphingophospholipids, which are relatively uncommon in bacteria (22). These lipids contain major amounts of sphinganin-type long-chain bases, the most abundant of which was identified as *N*-2-hydroxy-13-methyltetradecanoyl-5-methylhexadecasphinganine (24). The species of flavobacteria which were found to contain sphingolipids and are presently classified as *Sphingobacterium* are *F. multivorum* (11) and *F. spiritivorum* (9). Also included in the genus is a newly described organism, *Sphingobacterium mizutae* (22). In addition to these species, we have demonstrated the presence of sphingolipids in a recently named *Flavobacterium* species, *F. thalpophilum* (2, 7). In the same study, we found that these lipids could not be detected in *F. meningosepticum*, *F. indologenes*, *F. balustinum*, *F. breve*, or *F. odoratum* (2).

In addition to the presence of sphingolipids, sphingobacteria possess menaquinone 7 as the major respiratory quinone, whereas flavobacteria have menaquinone 6 as the primary quinone (2). The cellular fatty acid compositions of the two groups are quite similar, but some differences in the relative amounts of particular fatty acids provide a means for distinguishing between the groups. Members of *Sphingobacterium* contain a monounsaturated 16-carbon acid ($16:1^{\Delta 9}$) in amounts up to 22% of the total fatty acids, compared with 2% or less of this acid in *F. meningosepticum*, *F. gleum*, *F. indologenes*, and *Flavobacterium* species group IIb (10, 15, 20). In addition, species of flavobacteria contain a monounsaturated 17-carbon branched-chain acid (i-17:1) which may be present in amounts as great as 20%, compared with 2% or less of this acid in sphingobacteria and *F. thalpophilum*. An additional distinction between these groups is that the relative amounts of i-15:0 and i-2-OH-15:0 are approximately equivalent in sphingobacteria, whereas in flavobacteria i-15:0 is present in amounts two to three times that of the i-2-OH-15:0 acid.

Other gram-negative bacteria in which we have observed a fatty acid composition similar to that of sphingobacteria and flavobacteria are the *Capnocytophaga* spp. (3), *Cytophaga johnsonae* (22), and CDC groups IIj and DF-2 (6). The three documented *Capnocytophaga* species differed from the above-mentioned organisms in that i-15:0 comprised approximately 75% of the total fatty acids, with only trace to small amounts of $16:1^{\Delta 9}$, i-3-OH-15:0, and i-3-OH-17:0 present. The i-2-OH-15:0 and i-17:1 acids were completely absent from *Capnocytophage* spp. The cellular fatty acid composition of CDC group DF-2 was essentially identical to that of *Capnocytophaga* spp., whereas CDC group IIj was found to be more similar to flavobacteria (6). The major fatty acids identified in group IIj were i-15:0 and i-17:1, which comprised approximately 50 and 18% of the total acids, respectively. Relatively small amounts of i-2-OH-15:0, i-3-OH-15:0, i-3-OH-17:0, 16:0, linoleic (18:2), and oleic (18:1) acids were observed, and $16:1^{\Delta 9}$ was completely absent from group IIj (6). Conversely, *C. johnsonae* differed from all previously described species in that a relatively large amount of 16:0 (27 to 35%) was observed, and approximately 60% of the total fatty acids were composed of 16:0, i-15:0, and $16:1^{\Delta 9}$ (22). Trace to small amounts of i-16:0, i-2-OH-15:0, i-3-OH-15:0, i-17:1, i-17:0, i-3-OH-17:0 and two straight-chain hydroxy acids, 2-OH-14:0 and 3-OH-14:0, were also reported in *C. johnsonae* (22).

In the present study, we have examined the chemical characteristics of three *Flavobacterium*-like groups, IIe, IIh, and IIf, and compared them with the biochemically similar species *F. breve* and *F. odoratum*. The major conventional biochemical tests which distinguish *F. breve* and *F. odoratum* from these groups are shown in Table 1. *F. breve*, IIe, and IIh strains are oxidative and metabolize both glucose and maltose, whereas *F. odoratum* and IIf strains

TABLE 1. Distinguishing biochemical characteristics of *F. odoratum*, *F. breve*, and unclassified *Flavobacterium*-like groups[a]

Biochemical test	*F. odoratum*[b] (74[c])	Group IIf (87)	*F. breve* (3)	Group IIe (18)	Group IIh (21)
Pigment	+ (yellow) or −	−	+ (light yellow)	−	−
Indole	−	+	+	+	+
Growth on MacConkey agar	+	−	+	−	−
Urease[d]	+	−	−	−	−
Esculin hydrolysis	−	−	−	−	+
Gelatin hydrolysis	+	+	+	−	−
Acid production in OF[e] base					
Glucose	−	−	+	+	+ or (+)
Maltose	−	−	+	+	+

[a] +, Positive reaction within 48 h (for gelatin hydrolysis, + is positive reaction within 14 days); + or (+), positive or delayed positive (3 to 7 days); −, negative.
[b] A fruity odor is often detected.
[c] Total number of isolates in the culture collection of the Special Pathogens Reference Laboratory.
[d] Christensen urea agar.
[e] OF, Oxidative fermentative.

are nonsaccharolytic and metabolize neither of these carbohydrates. Group IIh differs from the other bacteria in its ability to hydrolyze esculin, while *F. odoratum* is distinguished by its ability to hydrolyze urea and by the absence of indole production. Yellow pigment is produced by some strains of *F. odoratum* and by *F. breve*, but none has been observed in strains of IIe, IIh, or IIf. In addition, *F. breve*, *F. odoratum*, and IIf hydrolyze gelatin, and only *F. breve* and *F. odoratum* grow on MacConkey agar. Although IIe, IIh, and IIf are distinguishable by at least two or more conventional biochemical tests from other recognized species of flavobacteria and from each other, it is difficult to determine on the basis of these tests alone whether these organisms represent additional species of flavobacteria or whether they belong to other taxa.

The data obtained from our investigations showed that some chemical components of groups IIe, IIh, and IIf were consistent with those of flavobacteria rather than sphingobacteria. The cellular fatty acid composition of these unnamed groups was similar to that of *F. odoratum* and *F. breve* (and other flavobacteria) in that the major fatty acids were isobranched and accounted for 50 to 90% of the total acids present (Table 2). Representative chromatograms of the cellular fatty acids (as methyl esters) of *F. odoratum* and group IIf are shown in Fig. 1, and those of *F. breve* and group IIe are shown in Fig. 2. Minor qualitative and quantitative differences were observed among the four fatty acid profiles, and these were found to be consistent when each group of organisms was recultured and processed again through the entire procedure.

The most abundant acid in each organism was an isobranched 15-carbon acid (i-15:0) which was present at a level of approximately 50% of the total acids in both *F. odoratum* and group IIf and at 30% or less in *F. breve* and groups IIe and IIh (Table 2 and Fig. 1 and 2). Each organism also contained iso-branched-chain hydroxy acids (i-2-OH-15:0, i-3-OH-15:0, and i-3-OH-17:0), which along with i-15:0 are characteristic acids of all flavobacteria and sphingobacteria tested to date (2, 5, 15, 22). The fatty acid compositions of groups IIe and IIh were essentially identical, and each contained moderate amounts (12%) of an anteiso-branched-chain 15-carbon acid (a-15:0) that is a useful marker for distinguishing these organisms from other flavobacteria and sphingobacteria, which contain only small (1%) to trace amounts of a-15:0 (2, 5, 15, 22). Groups IIe and IIh also contained small amounts (1%) of 2-OH-15:0, and 2-OH-17:0 acids, which were not present in any of the other organisms studied. Several other hydroxy fatty acids (2-OH-14:0, 3-OH-14:0, i-3-OH-16:0, 3-OH-16:0, and i-3-OH-17:1) were identified, but these were generally present in small amounts (Table 2, footnote *e*).

F. breve differed from the other organisms under study by the presence of two monounsaturated 16-carbon acids which were tentatively identified as $16{:}1^{\Delta 9}$ and $16{:}1^{\Delta 11}$ (Fig. 2 and Table 2). The length of the carbon chain and the degree of unsaturation for both acids were confirmed by hydrogenation and mass spectrometry; designation of the positions of

TABLE 2. Cellular fatty acid composition of *F. breve*, *F. odoratum*, and *Flavobacterium*-like bacteria isolated from clinical specimens[a]

Fatty acid[b]	*F. breve* (11)[c]	Group IIe (9)	Group IIh (10)	*F. odoratum* (11)	Group IIf (10)
i-13:0	—[d]	1.6	3.4	3.1	1.0
i-15:0	23.8	28.0	33.4	54.8	50.3
a-15:0	—	12.8	11.4	1.2	—
$16{:}1^{\Delta 9}$	14.3	1.1	1.4	1.2	1.6
i-2-OH-15:0	3.6	9.5	9.1	2.8	7.0
$16{:}1^{\Delta 11}$	7.1	0.6	0.7	—	—
16:0	8.1	1.3	1.8	1.4	3.9
i-3-OH-15:0	4.9	3.5	3.8	5.9	4.3
2-OH-15:0	—	1.2	0.9	—	—
i-17:1(1)	1.7	16.3	9.2	5.7	4.7
i-17:1(2)	4.8	—	—	0.4	8.3
a-17:1	—	0.7	0.8	—	—
i-17:1(3)	—	1.0	1.3	—	—
i-3-OH-16:0	1.4	2.0	1.1	0.5	0.1
3-OH-16:0	5.3	0.3	1.6	5.2	0.1
18:2	0.4	1.3	2.0	0.8	1.5
18:1	0.7	0.6	1.3	0.7	1.4
i-3-OH-17:0	9.1	13.1	12.2	8.3	6.4
2-OH-17:0	—	1.0	1.3	—	—
Other[e]	14.8	4.1	4.3	8.0	9.4
Total branched chain	53.1	89.1	85.6	83.7	87.5

[a] Values are arithmetic means.
[b] Number to the left of colon is the number of carbons; number to right is the number of double bonds; i, iso; a, anteiso; OH, hydroxy; (1), (2), (3), isomers of i-17:1 in order of elution; 18:2, linoleic acid; 18:1, oleic acid.
[c] Numbers in parentheses are the total of strains tested.
[d] —, Not detected.
[e] Other fatty acids occurring in trace to 2% amounts were: *F. breve*, 14:1, 14:0, i-15:1, 15:0, 2- and 3-OH-14:0, i-16:0, i-17:0, 18:0; *F. odoratum*, 13:0, i-14:0, 14:0, 15:1, 15:0, 3-OH-14:0, i-16:0, i-3-OH-17:1; IIf, 14:0, i-15:1, 15:0, 3-OH-14:0, i-16:0, i-17:0, 18:0; IIe and IIh, i-14:0, 14:0, i-15:1, 15:0, 3-OH-14:0, i-16:0, i-17:0, 18:0, i-3-OH-17:1.

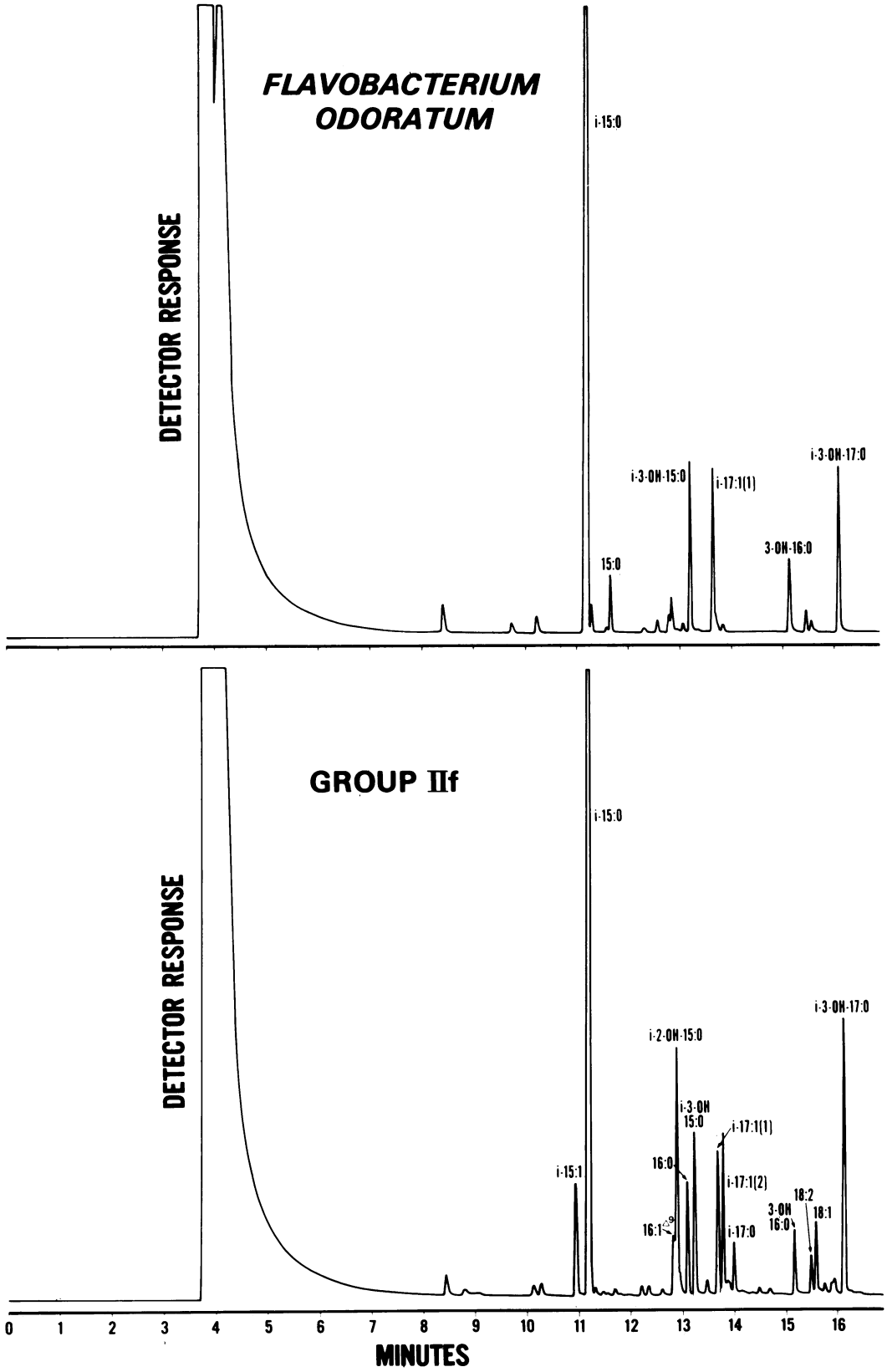

FIG. 1. Gas chromatograms of esterified fatty acids of *F. odoratum* type strain KC1416 and group IIf strain F721. Analysis was made on a fused-silica capillary column with OV-1 as the stationary phase. See Table 2, footnote *b*, for peak identification.

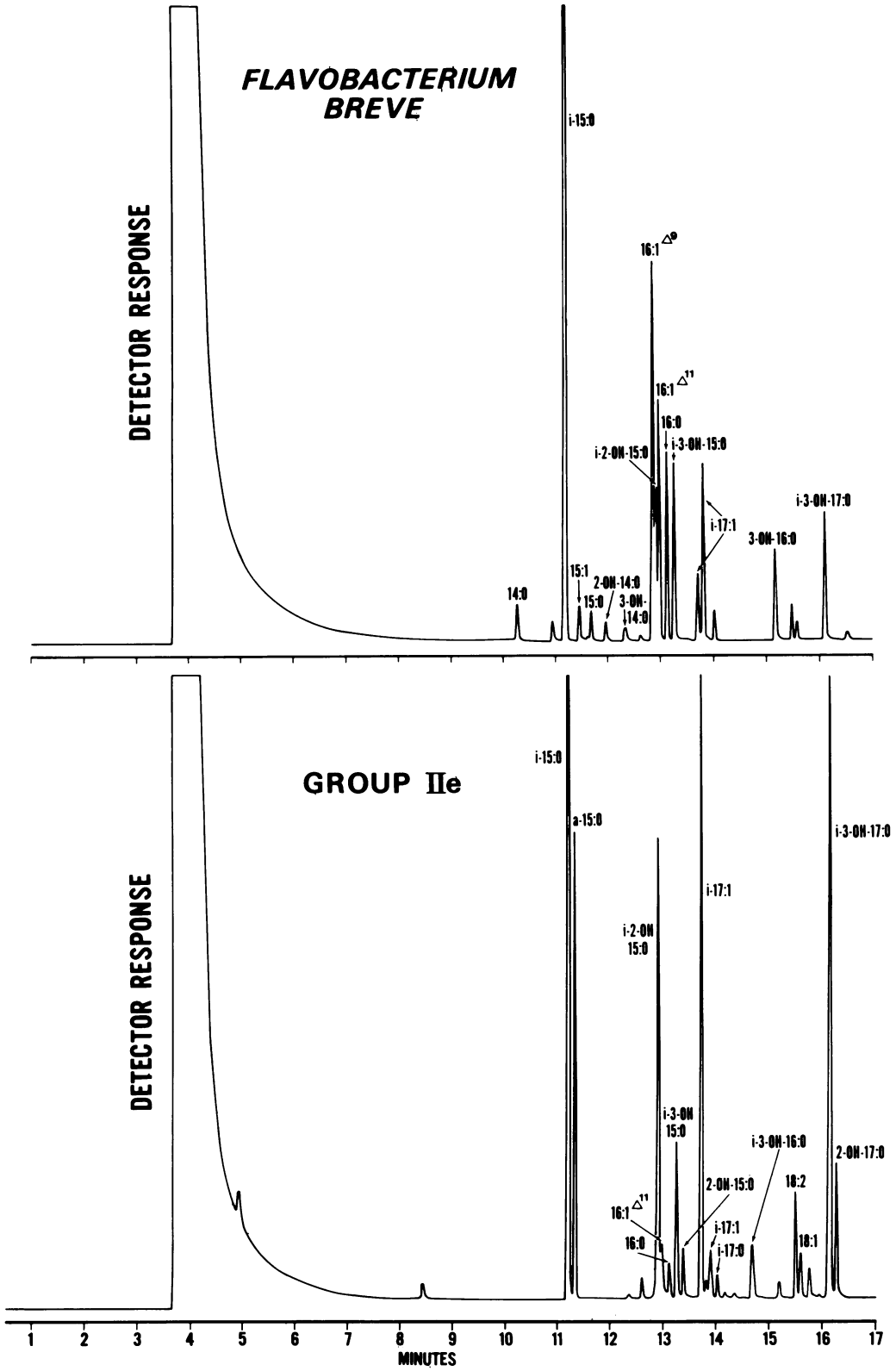

FIG. 2. Gas chromatograms of esterified fatty acids of *F. breve* type strain KC1428 and group IIe strain 8973. Analysis was made on a fused-silica capillary column with OV-1 as the stationary phase. See Table 2, footnote *b*, for peak identification.

the double bond was based on comparison of capillary GLC retention time with that of monounsaturated standards. The relatively large amount (14%) of 16:1$^{\Delta 9}$ present in *F. breve* was useful for distinguishing this organism from other flavobacteria, which contain 4% or less of this acid (15). In addition, the 16:1$^{\Delta 11}$ isomer was present in each of the 11 strains of this species (range, 4 to 11%) and was either absent or present in only trace amounts (<1%) in other flavobacteria (Table 2) (2, 15). The overall fatty acid compositions of *F. odoratum* and group IIf were most similar, with both containing large amounts (>50%) of i-15:0 (Fig. 1). However, *F. odoratum* could be distinguished from group IIf by the presence of small amounts (5%) of 3-OH-16:0, which was absent in group IIf, and also by the absence of an iso-branched-chain monounsaturated 17-carbon acid (i-17:1 [4]) which was present at approximately 8% in group IIf organisms (Table 2).

The results of thin-layer chromatography and GLC analyses showed that none of the bacteria contained sphingolipids. Data obtained from clinical strains of *F. breve* and *F. odoratum* were consistent with results presented in a previous report on the sphingolipid content of type strains of both species (2). The type strains of *F. multivorum* and *F. meningosepticum* were used as positive and negative controls, respectively. *F. multivorum* contained a branched-chain 17-carbon sphinganin (d-17:0 branched chain) as the major long-chain base and minor amounts of the d-16:0, d-18:1, d-18:0, and d-19:0 branched-chain bases, whereas these compounds were not detected in *F. meningosepticum*. The identities of d-16:0, d-17:0 branched chain, and d-18:0 detected in *F. multivorum* were confirmed by combined GLC and mass spectrometry of trimethylsilyl derivatives, which showed mass spectra essentially identical to those reported previously for these compounds (22). No ninhydrin-positive spots on thin-layer plates or GLC peaks were observed when the alkaline extracts of organisms other than the positive-control strain were examined. In contrast to the data presented in the present study, we have recently reported the presence of sphingolipids in *F. thalpophilum*, with a branched-chain 17-carbon sphinganin as a major component (2). These data are consistent with the sphingolipid composition reported for *Sphingobacterium* species by Yabuuchi et al. and Yano et al. (22, 24).

The major isoprenoid quinone found in extracts of these bacteria was menaquinone 6. The extracts were analyzed by HPLC, and the retention times of bacterial quinones were compared with those of authentic standards. Identities were confirmed by collection of the major quinone, menaquinone 6, from the HPLC column and subsequent analysis by mass spectrometry. Ubiquinones were not detected in these organisms. These data are consistent with those reported in a previous investigation in which we identified menaquinone 6 as the major quinone in the type strains of *F. breve*, *F. odoratum*, *F. balustinum*, *F. indologenes*, and *F. meningosepticum* (2). Our findings are also in agreement with previously published data of other investigators who found that clinically important species of flavobacteria contained menaquinones; however, in the previous study no specific menaquinone was identified. (1).

In summary, the chemical data presented in this report show that the unclassified groups IIe, IIh, and IIf resemble flavobacteria rather than sphingobacteria and may well be one or more additional species of the genus *Flavobacterium*. Although it is obvious that the true taxonomic status of these organisms cannot be determined until DNA hybridization studies are conducted, the chemical data in this report provide useful additional information for the identification and characterization of these unnamed bacterial groups.

LITERATURE CITED

1. **Callies, E., and W. Mannheim.** 1978. Classification of the *Flavobacterium-Cytophaga* complex on the basis of respiratory quinones and fumarate respiration. Int. J. Syst. Bacteriol. **28**:14–19.
2. **Dees, S. B., G. M. Carlone, D. Hollis, and C. W. Moss.** 1985. Chemical and phenotypic characteristics of *Flavobacterium thalpophilum* compared with those of other *Flavobacterium* and *Sphingobacterium* species. Int. J. Syst. Bacteriol. **35**:16–22.
3. **Dees, S. B., D. E. Karr, D. Hollis, and C. W. Moss.** 1982. Cellular fatty acids of *Capnocytophaga* species. J. Clin. Microbiol. **16**:779–783.
4. **Dees, S. B., and C. W. Moss.** 1975. Cellular fatty acids of *Alcaligenes* and *Pseudomonas* species isolated from clinical specimens. J. Clin. Microbiol. **1**:414–419.
5. **Dees, S. B., C. W. Moss, R. E. Weaver, and D. Hollis.** 1979. Cellular fatty acid composition of *Pseudomonas paucimobilis* and groups IIk-2, Ve-1, and Ve-2. J. Clin. Microbiol. **10**:206–209.
6. **Dees, S. B., J. Powell, C. W. Moss, D. G. Hollis, and R. E. Weaver.** 1981. Cellular fatty acid composition of organisms frequently associated with human infections resulting from dog bites: *Pasturella multocida* and groups EF-4, IIj, M-5, and DF-2. J. Clin. Microbiol. **14**:612–616.
7. **Holmes, B., D. G. Hollis, A. G. Steigerwalt, M. J. Pickett, and D. J. Brenner.** 1983. *Flavobacterium thalpophilum*, a new species recovered from human clinical material. Int. J. Syst. Bacteriol. **33**:677–682.
8. **Holmes, B., and R. J. Owen.** 1982. *Flavobacterium breve* sp. nov., nom. rev. Int. J. Syst. Bacteriol. **32**:233–234.
9. **Holmes, B., R. J. Owen, and D. G. Hollis.** 1982. *Flavobacterium spiritivorum*, a new species isolated from human clinical specimens. Int. J. Syst. Bacteriol. **32**:157–165.
10. **Holmes, B., R. J. Owen, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Flavobacterium gleum*, a new species found in human clinical specimens. Int. J. Syst. Bacteriol. **34**:21–25.
11. **Holmes, B., R. J. Owen, and R. E. Weaver.** 1981. *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as group IIk, biotype 2. Int. J. Syst. Bacteriol. **31**:21–34.
12. **Holmes, B., J. J. S. Snell, and S. P. Lapage.** 1977. Revised description, from clinical isolates, of *Flavobacterium odoratum* Stutzer and Kwaschnina 1929, and designation of the neotype strain. Int. J. Syst. Bacteriol. **27**:330–336.
13. **Holmes, B., J. J. S. Snell, and S. P. Lapage.** 1978. Revised description, from clinical strains, of *Flavobacterium breve* (Lustig) Bergey et al. 1923 and proposal of the neotype strain. Int. J. Syst. Bacteriol. **28**:201–208.
14. **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.
15. **Moss, C. W., and S. B. Dees.** 1978. Cellular fatty acids of *Flavobacterium meningosepticum* and *Flavobacterium* species group IIb. J. Clin. Microbiol. **8**:772–774.
16. **Moss, C. W., S. B. Dees, and G. O. Guerrant.** 1980. Gas-liquid chromatography of bacterial fatty acids with a fused-silica capillary column. J. Clin. Microbiol. **12**:127–130.
17. **Owen, R. J., and J. J. S. Snell.** 1973. Comparisons of group IIf with *Flavobacterium* and *Moraxella*. Antonie van Leeuwenhook J. Microbiol. Serol. **39**:473–480.
18. **Shewan, J. M., and T. A. McMeekin.** 1983. Taxonomy (and ecology) of *Flavobacterium* and related genera. Annu. Rev. Microbiol. **37**:233–252.
19. **Weaver, R. E., and D. G. Hollis.** 1983. Gram-negative organisms: an approach to identification (guide to presumptive identification). Centers for Disease Control, Atlanta, Ga.
20. **Weaver, R. E., D. G. Hollis, W. A. Clark, and P. Riley.** 1983. Revised tables for the identification of unusual pathogenic gram negative bacteria. Centers for Disease Control, Atlanta, Ga.

21. **Weaver, R. E., H. W. Tatum, and D. G. Hollis.** 1981. The identification of unusual pathogenic gram negative bacteria (Elizabeth O. King). Centers for Disease Control, Atlanta, Ga.
22. **Yabuuchi, E., T. Kaneko, I. Yano, C. W. Moss, and N. Miyoshi.** 1983. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting gram-negative rods in CDC groups IIK-2 and IIb. Int. J. Syst. Bacteriol. **33**:580–598.
23. **Yabuuchi, E., E. Tanimura, A. Ohyama, I. Yano, and A. Yamamoto.** 1979. *Flavobacterium devorans* ATCC 10829: a strain of *Pseudomonas paucimobilis*. J. Gen. Appl. Microbiol. **25**:95–107.
24. **Yano, I., I. Tomiyasu, and E. Yabuuchi.** 1982. Long chain base composition of strains of three species of *Sphingobacterium* gen. nov. FEMS Microbiol. Lett. **15**:303–307.