Cellular Fatty Acids of *Flavobacterium meningosepticum* and *Flavobacterium* Species Group IIb

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The cellular fatty acid profiles of *Flavobacterium meningosepticum* and *Flavobacterium* species group IIb were markedly different from those of related bacteria. The profiles were characterized by the presence of 13-methyl-tetradecanoate and three uncommon acids: 2-hydroxy-13-methyl-tetradecanoate, 15-methyl-hexadecanoate, and 3-hydroxy-15-methyl-hexadecanoate.

In searching for new methodology for more rapid and specific identification of nonfermentative, gram-negative bacilli, we have used gas-liquid chromatography (GLC) to study the chemical composition and metabolic products from these organisms. We found that short-chain acid products and long-chain cellular fatty acid components provided additional valuable information for identification and classification of pseudomonads from clinical materials (7-9). Similar studies have been made with related organisms such as *Alcaligenes* (7), *Achromobacter* (3), *Acinetobacter* (5), and *Moraxella* (4). In the genus *Flavobacterium* two clinically significant groups have been recognized, *Flavobacterium meningosepticum* and *Flavobacterium* species group IIb (1, 14). The cellular fatty acid composition of representative strains of these organisms is described in this report.

Four strains of *F. meningosepticum* and three strains of *Flavobacterium* species group IIb were obtained from the Special Bacteriology Section, Center for Disease Control, where they were identified with conventional cultural and biochemical tests (6, 14). Cells for fatty acid analysis were grown for 24 h on a plate of Trypticase soy agar (Baltimore Biological Laboratory) and processed for cellular fatty acids as described previously (3, 7). The fatty acid methyl ester samples were analyzed with GLC on a 3% OV-1 column, using a flame ionization detector. The conditions for GLC analysis, quantitation of peak areas, and tentative identification of peaks were described earlier (3). All peaks were positively identified with results obtained from hydrogenation (3), acetylation (3), GLC-mass spectrometry (2, 12), and retention time data.

The fatty acid profile of *F. meningosepticum* 4433 shown in Fig. 1 is characterized by the presence of two major and three minor peaks. Essentially identical profiles were obtained from

the other six cultures, as shown by the quantitative data in Table 1. The first peak in the chromatogram, which constituted approximately 35% of the total acids, was identified by retention time data as a saturated, 15-carbon, branched-chain acid. The mass spectrum of the methyl ester of this compound showed that the M-31 ($m/e = 225$) ion was greater than the M-29 ($m/e = 227$) ion, indicating an iso-branched fatty acid (2, 12). These data firmly established the identity of this peak as 13-methyl-tetradecanoic acid.

The second large peak in the chromatogram gave a molecular ion of 272, which indicated a 15-carbon, saturated hydroxy acid. Also, the spectrum showed a peak at m/e 90 and a large M-59 ($m/e = 213$) peak, which is characteristic of the methyl esters of 2-hydroxy acids (12). The possibility that this compound also contained a branched methyl group could not be confirmed from the mass spectrum, and no reference standard was available for retention time comparisons by GLC. Thus, branching was confirmed by plotting relative retention times against carbon numbers, using reference standards and 2-hydroxy-9-methyl-decanoic acid from *Pseudomonas maltophilia* (8) and 2-hydroxy-15-methyl-hexadecanoic acid from a myxococcus (11). A plot of the latter two acids formed a straight line; the unknown fit precisely on this line at the 15-carbon intercept, thus confirming that this peak represented 2-hydroxy-13-methyl-tetradecanoic acid (i-2-OH 15:0).

The third peak in the chromatogram (i-17:1) was identified with mass spectrometry both before and after hydrogenation. The unsaturated ester showed characteristic fragment ions for an iso-branched monoenoic acid at M-55 ($m/e = 217$), M-87 ($m/e = 195$), and M-105 ($m/e = 177$), in contrast to M-69, M-101, and M-119 ions, which are characteristic for anteiso-branched

FIG. 1. Gas chromatogram of esterified fatty acids from saponified whole cells of *F. meningosepticum*. Analysis was made on a 3% OV-1 column.

TABLE 1. Cellular fatty acid composition of *F. meningosepticum* and *Flavobacterium* species group IIB

Species	CDC ^a no.	Fatty acid													
		i-14:0 ^b	14:0	i-15:0	i-16:0	16:1	i-2-OH 15:0	16:0	i-3-OH 15:0	i-17:1	2-OH 16:0	3-OH 16:0	18:1	18:0	i-3-OH 17:0
<i>F. meningosepticum</i>	14 ^c	T ^d	T	36	T	T	32	T	6	10	T	4	T	T	12
	4571	T	T	30	T	T	39	T	8	5	T	5	T	T	13
	4904	T	T	36	T	T	35	T	9	10	T	T	T	T	10
	4433	T	T	35	T	T	28	T	12	10	T	5	T	T	10
<i>Flavobacterium</i> spe- cies group IIB	7133	T	T	39	T	T	17	T	10	17	T	T	T	T	17
	B8190	T	T	34	T	T	22	4	6	26	T	T	T	T	8
	B6829	T	T	36	T	T	20	2	9	22	T	T	T	T	11

^a CDC, Center for Disease Control.

^b Number to left of the colon refers to number of carbon atoms; number to the right refers to number of double bonds; i- indicates a methyl branch at the iso carbon atom; 2- and 3-OH refer to a hydroxyl group at the 2- or 3-carbon atom, respectively.

^c ATCC 13252.

^d Numbers refer to percentage of total acids; T = <2%.

monoenoic methyl esters (2). Moreover, when hydrogenated (8), this compound had retention times and mass spectra identical to those of a saturated, iso-branched, 17-carbon reference standard. The identity of peak 4 (3-OH 16:0) was confirmed by comparing retention time data and mass spectra of the methyl ester and the acetylated methyl ester (8) from the bacteria with those of a reference standard. The last peak (i-

3-OH 17:0) was identified with mass spectrometry and with a plot of relative retention times, which indicated that this compound was 3-hydroxy-15-methyl-hexadecanoic acid.

The above data show that no distinction can be made between *F. meningosepticum* and *Flavobacterium* species group IIB on the basis of cellular fatty acids. However, as a group these organisms have a fatty acid profile which is

markedly different from those of closely related organisms (3-5, 7-10). The most striking feature of the group is the presence of relatively large amounts of i-2-OH 15:0 acid, which to our knowledge has only been found in *Bdellovibrio bacteriovorus* (13). Two other uncommon acids (i-17:1 and i-3-OH 17:0) are additional markers which comprise this apparently unique fatty acid profile. Thus, determination of cellular fatty acid composition provides a rapid and relatively simple procedure for identifying these organisms.

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