Fatty Acids of *Fusobacterium* Species: Taxonomic Implications

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Fatty acids of *Fusobacterium* species were examined by gas–liquid chromatography. *Fusobacterium nucleatum*, *F. necrophorum*, *F. mortiferum*, *F. gonidiaformans* and *F. varium* showed similar patterns, characterized by the presence of 3-hydroxytetradecanoate, *n*-tetradecanoate, hexadecanoate, *n*-hexadecanoate, octadecanoate, *n*-octadecanoate and a component having the properties of octadecadienoate. *Fusobacterium nucleatum* contained 3-hydroxyhexadecanoate as a distinctive character. Simpler fatty acid patterns characterized by the absence of 3-hydroxytetradecanoate and other hydroxy fatty acids were observed in *F. plauti*, the single strain of *F. prausnitzii* and in the majority of strains classified as *F. russii* and *F. naviforme*. Neither methyl-branched nor cyclopropane fatty acids could be detected in any of the strains examined. In addition to fatty acid methyl esters, the chromatographic profiles of all species except *F. mortiferum*, *F. gonidiaformans* and *F. naviforme* contained substantial amounts of fatty aldehyde dimethyl acetals of chain lengths C₁₄ to C₁₈.

INTRODUCTION

The genus *Fusobacterium* (Knorr, 1923) includes species of obligately anaerobic, non-spore-forming, motile or non-motile Gram-negative rods. Their habitat is the mucous membranes of man and animals (Moore & Holdeman, 1974). Most species do not normally cause infection in healthy humans, but some, especially *F. nucleatum* and *F. necrophorum*, are not infrequently isolated from clinical material (Sutter *et al.*, 1974). Thus, interest in differentiating species of *Fusobacterium* has increased, and there have been considerable improvements in the nomenclature and classification of the genus (Holdeman *et al.*, 1977). However, the number of stable and specific tests available for differentiation is still limited. Additional taxonomic characters are required for a more precise definition of the genus as well as for a more accurate description of the species of the genus.

Gas–liquid chromatography (g.l.c.) of metabolic end-products plays an important role in the classification of anaerobic bacteria including *Fusobacterium* (Holdeman *et al.*, 1977). G.l.c. of cellular fatty acids has proved very useful in differentiating facultative as well as obligately anaerobic bacteria (Lambert & Armfield, 1979; Lechevalier, 1977; Shaw, 1974) but has only been applied to a limited extent in the study of *Fusobacterium* (Miyagawa *et al.*, 1979).

The present paper deals with the fatty acid composition of nine species of *Fusobacterium*. The aim was to establish new and stable chemotaxonomic characters that would be useful in the classification and identification of these anaerobic organisms.

METHODS

Strains and cultivation. The 31 test strains examined are listed in Table 1. The strains of *F. plauti* were originally isolated from cultures of *Entamoeba histolytica* (Moore & Holdeman, 1974). The majority of the other

Table 1. *Fusobacterium* strains studied

Species	Strain designation	Source*
<i>F. nucleatum</i>	Fev 1	1
<i>F. nucleatum</i>	F1	Own isolate
<i>F. nucleatum</i>	F8	Own isolate
<i>F. nucleatum</i>	F15	Own isolate
<i>F. nucleatum</i>	ATCC 10953	2
<i>F. necrophorum</i>	SPH 1	3
<i>F. necrophorum</i>	VPI 6161	4
<i>F. necrophorum</i>	N167	5
<i>F. mortiferum</i>	VPI 4249	4
<i>F. mortiferum</i>	VPI 5696 = Finegold B609	4
<i>F. mortiferum</i>	VPI 0473	4
<i>F. mortiferum</i>	VPI 4123A = ATCC 25557 = Prévot 350 A	4
<i>F. gonidiaformans</i>	VPI 4381 = Prévot 470 D	4
<i>F. gonidiaformans</i>	VPI 0482A = ATCC 25563 = Prévot 3554 A	4
<i>F. gonidiaformans</i>	VPI 4879	4
<i>F. gonidiaformans</i>	VPI 11360	4
<i>F. varium</i>	VPI 4234 = Prévot 587 A	4
<i>F. varium</i>	VPI 0499A = Prévot 1669 BA	4
<i>F. varium</i>	ATCC 8501	6
<i>F. varium</i>	Lille 109	6
<i>F. naviforme</i>	VPI 4877 = Prévot 1800	4
<i>F. naviforme</i>	VPI 11936C	4
<i>F. naviforme</i>	VPI 12188	4
<i>F. russii</i>	VPI 0307 = ATCC 25533 = Prévot 593 A	4
<i>F. russii</i>	VPI 10646	4
<i>F. russii</i>	VPI 11018	4
<i>F. plauti</i>	VPI 0310 = Prévot S 1	4
<i>F. plauti</i>	VPI 0311 = Prévot S 2	4
<i>F. plauti</i>	VPI 0312 = Prévot S 4	4
<i>F. plauti</i>	VPI 4145 = Prévot S 3	4
<i>F. prausnitzii</i>	VPI 4815	4

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strains are human isolates. Strains were cultivated on *Brucella* blood agar plates (7% human blood) at 37 °C for 48 h in a deoxygenated CO₂ atmosphere (BBL GasPak jars). Bacterial growth was then washed from the agar using a right-angled glass rod and sterile distilled water. Biomass was sedimented by centrifugation at 3000 g for 20 min, washed twice with distilled water, freeze-dried and stored under nitrogen in closed vials at -20 °C.

Chemicals. Solvents of analytical grade were distilled before use. The 2 M-HCl in methanol was obtained by bubbling dry HCl gas (Fluka, Buchs, Switzerland) into dried methanol until saturation and subsequent dilution. Fatty acid methyl ester standards, including linoleate (*cis,cis*-9,12-octadecadienoate) and linolealdate (*trans,trans*-9,12-octadecadienoate), were obtained from Applied Science Laboratories (State College, Pa., U.S.A.) and fatty aldehyde dimethyl acetal standards from Supelco (Bellefonte, Pa., U.S.A.).

Methanolysis and extraction. Freeze-dried bacteria (1 to 10 mg) were suspended in 2 M-HCl in methanol (3 ml) in Teflon-lined screw-capped vials at 85 °C for 18 h (Jantzen *et al.*, 1974*a, b*, 1978). After cooling, the methanolysates were concentrated to about 1.5 ml in a stream of nitrogen and then 3 ml of a 50% saturated NaCl solution was added; the mixture was extracted twice with 4.5 ml hexane and the hexane phases were collected using a syringe. The hexane was evaporated to near dryness using dry nitrogen at room temperature, and the volume was adjusted with hexane to 100 µl. The samples were stored in sealed glass tubes at -20 °C.

Separation of methyl esters and methyl acetals. Where substantial amounts of dimethyl acetals were present (see below), these were separated from the fatty acid methyl esters by the procedure of Meyer & Meyer (1971). After methanolysis and extraction as described above, the methyl esters were saponified in 0.5 M-NaOH in 90% methanol (1 ml) for 2 h at 85 °C. This treatment did not affect dimethyl acetals which could then be extracted with hexane (2 × 3 ml). The remaining fatty acids (converted to sodium salts) were remethylated by adjusting the pH to

2 with 6 M-HCl, subsequent treatment with 14% (w/v) BCl_3 in methanol (Merck; 0.5 ml, 100 °C, 5 min) and extraction with hexane (2×3 ml).

Thin-layer chromatography (t.l.c.). The presence of hydroxy fatty acids and dimethyl acetals in bacterial methanolysates was examined by t.l.c. Analytical plates (0.2 mm silica gel F₂₅₄; Merck) were developed in hexane/diethyl ether (85:15, v/v). The spots were visualized either by charring of plates sprayed with 10% sulphuric acid in ethanol or by an acidified alcoholic solution of 2,4-dinitrophenylhydrazine.

Gas-liquid chromatography. G.l.c. of fatty acid methyl esters and dimethyl acetals was carried out on a Hewlett-Packard 5710 chromatograph equipped with a flame ionization detector. Two standard glass columns (200 $\times$ 0.2 cm) were used routinely: non-polar – 10% SE-30 (methyl silicone) on Gas Chrom Q, 100 to 120 mesh; polar – 10% Silar-10C (cyanopropyl silicone) on Gas Chrom Q, 100 to 120 mesh (Applied Science Laboratories). Additional analyses were performed on a 25 m WCOT SE-30 glass capillary column (Chrompack, Middelburg, The Netherlands). The two standard columns were operated from 120 to 250 °C increasing at 2 °C min⁻¹ with a carrier gas (nitrogen) flow rate of 20 ml min⁻¹, injection port temperature of 250 °C and flame ionization detector temperature of 300 °C. The glass capillary column was operated in splitless mode using a Hewlett-Packard 18740B capillary column control and with a carrier gas (helium) flow rate of 1.5 ml min⁻¹; the column oven temperature was programmed from 170 to 250 °C at a rate of 4 °C min⁻¹.

Peak areas were recorded by a Hewlett-Packard 3380A digital integrator as a percentage of the total peak area. These values were then adjusted by the response factor of each fatty acid (K. Bryn & E. Jantzen, unpublished results). The responses of underivatized hydroxy fatty acids were generally poor and variable in our chromatographic system. These constituents were therefore quantified after trifluoroacetylation (see below), 'on column' injection and chromatography on the standard SE-30 column.

Identification of fatty acids. Fatty acid methyl esters were primarily identified by comparing their retention times on both the non-polar and polar columns with those of the standards. The identities of hydroxy fatty acids were confirmed by determination of their retention times before and after conversion to trifluoroacetyl derivatives (Jantzen *et al.*, 1974a, 1978). The presence of unsaturation and/or cyclopropane substitution in the alkyl chain was examined by the reduction method of Brian & Gardner (1968). Since methanolic HCl largely degrades cyclopropane fatty acids, a comparison of fatty acid profiles obtained after alkaline and acidic methanolysis, respectively, provides another check for the presence of cyclopropane fatty acids.

Trifluoroacetyl derivatives were formed by heating to boiling for 5 min with equal volumes of trifluoroacetic anhydride (Merck) and acetonitrile (Merck).

Generally, the fatty acid identities were confirmed by mass spectrometry (m.s.) using a Hewlett-Packard 5992A gas chromatographic/mass spectrometer computer system equipped with a glass capillary column (25 m $\times$ 0.5 mm, CP Sil 5, methyl silicone; Chrompack). The quadrupole mass spectrometer was automatically calibrated (AUTOTUNE program) with a perfluorotetrabutylamine reference compound to a fixed set of mass spectral conditions; the intensity ratio of the peaks at m/e 69, 219 and 502 was adjusted to 100:35:2. The ionization conditions used were 70 eV at 170 °C.

RESULTS

The fatty acid composition of the 31 test strains is given in Table 2, and four examples of fatty acid profiles are shown in Fig. 1. In all strains the main constituents were straight-chain saturated and monoenoic acids of chain lengths from C₁₂ to C₁₈. In addition, most chromatograms contained peaks which changed position after trifluoroacetylation, thus indicating a compound with a free hydroxyl group in the chain. By g.l.c./m.s. these peaks showed the abundant fragment of m/e 103 characteristic of 3-hydroxy fatty acids (Ryhage & Stenhagen, 1960), and comparison with reference compounds established their identity to 3-hydroxydodecanoate (3-OH-12:0), 3-hydroxytetradecanoate (3-OH-14:0) and 3-hydroxyhexadecanoate (3-OH-16:0), respectively.

The monoenoic fatty acids hexadecenoate (16:1) and octadecenoate (18:1) were found in all strains examined. In addition, all of the strains contained an additional constituent eluting just in front of 18:1 on the methyl silicone column (Fig. 1). This constituent chromatographed together with the 18:2 standard *cis,cis*-9,12 octadecadienoate (linoleate) on both the polar and non-polar columns. Furthermore, g.l.c./m.s. analysis of this peak revealed a molecular ion of m/e 294 and a fragmentation pattern identical to that of linoleate. Thus, both the mass spectrum and the g.l.c. retention time data are consistent with the presence of octadecadienoate (18:2). Neither cyclopropane nor methyl-branched fatty acids, which frequently occur in bacterial lipids, could be detected.

Table 2. *Fatty acid composition of Fusobacterium strains*

Taxon	Strain	Fatty acid composition* (% w/w. of total in strain)											
		12:0	3 OH 12:0	14:0	3 OH 14:0	15:0	16:1	16:0	3 OH 16:0	17:0	18:2	18:1	18:0
<i>F. nucleatum</i>	Fev 1	1.7	—	24.5	15.2	0.4	14.6	26.1	4.6	—	1.4	9.9	1.3
<i>F. nucleatum</i>	F1	2.5	—	28.3	14.9	1.5	18.7	22.6	4.3	—	1.5	5.0	1.2
<i>F. nucleatum</i>	F8	1.8	—	26.5	16.2	tr	13.3	25.8	5.9	tr	1.9	6.6	1.9
<i>F. nucleatum</i>	F15	2.2	—	24.5	14.1	1.0	16.5	24.6	6.2	—	1.4	7.8	1.7
<i>F. nucleatum</i>	10953	2.3	—	27.8	15.4	0.7	19.3	21.1	5.5	—	1.2	4.7	1.8
<i>F. necrophorum</i>	SPH 1	2.5	—	15.9	24.8	1.4	5.8	18.2	—	tr	6.7	19.9	4.6
<i>F. necrophorum</i>	6161	3.1	—	14.4	24.7	0.6	8.6	20.9	—	0.1	7.4	15.2	5.0
<i>F. necrophorum</i>	N167	3.3	—	13.0	28.2	1.1	6.8	21.6	—	tr	6.1	15.5	4.3
<i>F. mortiferum</i>	4249	1.2	—	19.5	13.9	3.8	13.0	23.8	—	—	5.4	9.6	9.7
<i>F. mortiferum</i>	5696	0.5	—	12.1	10.5	1.2	13.2	26.1	—	tr	6.6	9.8	20.0
<i>F. mortiferum</i>	0473	0.9	—	15.6	16.9	2.4	23.4	28.7	—	—	2.6	7.7	2.0
<i>F. mortiferum</i>	4123A	tr	—	13.7	20.4	0.9	21.8	28.5	—	0.1	2.1	8.5	4.1
<i>F. gonidiaformans</i>	4381	3.6	—	15.8	12.0	—	7.1	16.5	—	0.2	8.1	12.4	24.5
<i>F. gonidiaformans</i>	0482A	4.7	—	9.9	18.5	tr	3.6	30.3	—	tr	6.1	10.8	16.0
<i>F. gonidiaformans</i>	4879	0.7	—	4.6	7.9	—	2.9	24.1	—	—	10.7	11.8	36.3
<i>F. gonidiaformans</i>	11360	2.6	—	7.9	19.4	0.1	2.9	28.7	—	—	8.8	12.0	17.4
<i>F. varium</i>	4234	0.8	—	19.4	22.1	0.4	19.6	22.1	—	0.1	3.7	5.2	6.5
<i>F. varium</i>	0499A	0.9	—	20.6	29.8	tr	20.4	21.5	—	—	1.6	3.7	1.5
<i>F. varium</i>	8501	0.7	—	14.0	31.9	0.1	23.0	19.3	—	—	1.4	5.2	4.3
<i>F. varium</i>	109	1.1	—	20.1	28.3	tr	20.9	20.3	—	—	2.6	4.8	1.9
<i>F. naviforme</i>	4877	6.1	8.5	8.7	8.1	tr	1.7	26.3	—	0.2	11.6	16.9	11.8
<i>F. naviforme</i>	11936C	8.6	—	31.1	—	2.9	7.5	30.0	—	0.4	3.2	9.1	7.2
<i>F. naviforme</i>	12188	5.8	—	11.3	—	0.2	6.1	40.3	—	tr	11.7	12.0	12.7
<i>F. russii</i>	0307	4.6	—	12.1	16.1	tr	14.4	34.5	—	tr	1.3	11.9	4.1
<i>F. russii</i>	10646	11.0	—	37.7	—	0.1	2.8	37.8	—	—	2.6	4.0	4.2
<i>F. russii</i>	11018	6.7	—	21.4	—	0.2	3.3	40.2	—	—	10.0	12.9	5.5
<i>F. plauti</i>	0310	tr	—	33.1	—	4.4	4.1	35.2	—	3.4	4.6	10.1	5.0
<i>F. plauti</i>	0311	0.1	—	26.8	—	0.9	3.8	36.0	—	1.0	6.9	16.8	7.7
<i>F. plauti</i>	0312	tr	—	33.4	—	tr	3.1	40.7	—	tr	4.5	12.9	5.3
<i>F. plauti</i>	4145	0.2	—	26.7	—	—	5.0	31.7	—	0.9	13.3	16.5	5.4
<i>F. prausnitzii</i>	4815	2.7	—	25.6	—	tr	tr	56.5	—	4.1	2.3	4.8	3.9

tr, less than 0.1%; —, not detected.

* The figure before the colon indicates the number of carbon atoms in the chain, that after the colon denotes the number of double bonds (the position of the double bonds has not been determined); 3-OH indicates the presence of a 3-hydroxy group.

Fig. 1. Typical g.l.c. fatty acid profiles of *Fusobacterium* strains. Bacteria were digested with 2 M-HCl in methanol. Fatty aldehyde dimethyl acetals were removed by saponification and extraction. Hydroxy fatty acids were trifluoroacetylated before injection on the 25 m SE-30 glass capillary column.

The majority of the *Fusobacterium* strains showed g.l.c. profiles containing several peaks of non-fatty acid nature. These constituents chromatographed in a non-reproducible manner and changed position or disappeared completely after trifluoroacetylation. This non-reproducible g.l.c. behaviour is compatible with fatty aldehyde dimethyl acetals, well-known reaction products of alk-1'-enyl glycerol ethers (plasmalogens) subjected to methanolysis (Farquhar, 1962; Gray, 1967).

Fatty aldehyde dimethyl acetals are easily separated from fatty acid methyl esters by extraction after first converting the methyl esters to solvent-insoluble sodium salts by a mild saponification procedure. By applying the method of Meyer & Meyer (1971), fatty acid profiles without interfering substances were obtained (Fig. 1). In most cases, the

Table 3. *Differentiating fatty acid and aldehyde features of the Fusobacterium species examined*

Species*	Non-hydroxy-fatty acid characteristics†	Hydroxy-fatty acids present	Chain length of the fatty aldehydes present
<i>F. nucleatum</i> (5)	18:0 (1.2–1.9%), 18:2 (1.2–1.9%)	3-OH-14:0, 3-OH-16:0	C _{16:0}
<i>F. necrophorum</i> (3)	16:1 (5.8–8.6%), 18:1 (15.2–19.9%)	3-OH-14:0	C _{14:0} , C _{16:1} , C _{16:0} , C _{18:0}
<i>F. mortiferum</i> (4)	12:0 (tr–1.2%), 15:0 (0.9–3.8%)	3-OH-14:0	ND
<i>F. gonidiaformans</i> (4)	16:1 (2.9–7.1%), 18:0 (16.0–36.3%)	3-OH-14:0	ND
<i>F. varium</i> (4)	16:1 (19.6–23.0%), 18:1 (3.7–5.2%)	3-OH-14:0	C _{14:0} , C _{16:1} , C _{16:0}
<i>F. naviforme</i> 4877‡	16:1 (1.7%)	3-OH-12:0, 3-OH-14:0	ND
(2)	12:0 (5.8–8.6%), 16:0 (30.0–40.3%)	ND	ND
<i>F. russii</i> 0307‡	16:0 (34.5%)	3-OH-14:0	ND
(2)	12:0 (6.7–11.0%), 14:0 (21.4–37.7%)	ND	C _{16:0} , C _{18:0}
<i>F. plauti</i> (4)	12:0 (tr–0.2%), 14:0 (26.7–33.4%), 16:0 (31.7–40.7%)	ND	C _{14:0} , C _{16:0}
<i>F. prausnitzii</i> (1)	16:0 (56.5%), 17:0 (4.1%)	ND	ND

ND, Not detected.

* Numbers in parentheses indicate the number of strains examined: see Table 1 for descriptions.

† Fatty acids are designated as in Table 2. Numbers in parentheses indicate the range among the strains (data from Table 2).

‡ Deviating strain.

corresponding g.l.c. and g.l.c./m.s. profiles of the non-fatty acid material showed a few abundant and several minor peaks. The main peaks in a majority of chromatograms showed identical retention times to those of the *n*-tetradecanal dimethyl acetal (14:0 DMA) and/or *n*-hexadecanal dimethyl acetal (16:0 DMA) standards. In addition, some chromatograms showed peaks whose retention times indicated the presence of hexadecenal dimethyl acetal (16:1 DMA) and/or *n*-octadecanal dimethyl acetal (18:0 DMA) (Table 3). Several of the less abundant peaks were probably decomposition products which were formed in the injection port of the gas chromatograph (Mahadevan *et al.*, 1967).

Mass spectrometry of the non-fatty acid constituents provided further structural evidence. Thus, the mass spectra of the main g.l.c. peaks were all characterized by the diagnostic fragments $\text{CH}(\text{OCH}_3)_2^+$ (m/e 75), $(\text{M} - \text{CH}_3\text{OH})^+$ and, in some cases, M^+ (McFadden *et al.*, 1964). Most of the minor g.l.c. peaks yielded an abundant fragment of m/e 71 which may be $(\text{CH}_2\text{CH}=\text{CHOCH}_3)^+$, a fragment one would expect to be formed from methyl vinyl ethers, which are reported to be a heat-induced degradation product of fatty aldehyde dimethyl acetals (Mahadevan *et al.*, 1967).

The presence of fatty acid dimethyl acetals was also confirmed by t.l.c. analysis. In these experiments methanolysates of selected strains were applied to analytical thin-layer plates. In addition to the spots of hydroxylated fatty acid methyl esters and unsubstituted fatty acid

methyl esters, several strains (Table 3) showed a distinct spot of R_F value and colour corresponding to that of the dimethyl acetal standard (14:0 DMA + 16:0 DMA).

Strains classified as *F. nucleatum*, *F. necrophorum*, *F. mortiferum*, *F. gonidiaformans* and *F. varium* contained the common lipopolysaccharide constituent 3-OH-14:0. Within this cluster of hydroxy fatty acid-containing species, the *F. nucleatum* strains formed a homogeneous sub-group with 3-OH-16:0 as a distinct character (Fig. 1, Table 2). The remaining species had a grossly similar fatty acid pattern and could only be distinguished on the basis of minor quantitative differences. Thus, the three species of *F. necrophorum* contained relatively small amounts of 16:1 and large amounts of 18:1 compared with the others within this group, whereas *F. gonidiaformans* could be distinguished by its high content of 18:0, and *F. varium* by high contents of 3-OH-14:0 and 16:1. The fatty acid pattern of *F. mortiferum* was less distinct, although the content of 15:0 appeared to be somewhat higher and that of 12:0 somewhat lower than for other species within this group. The hydroxy fatty acid-containing strains of *F. russii* (VPI 0307) and of *F. naviforme* (VPI 4877), the only strain containing 3-OH-12:0, were both easily distinguishable from the other strains with these constituents (Table 2).

All four strains of *F. plauti*, the strain of *F. prausnitzii*, *F. russii* strains VPI 10646 and VPI 11018, and *F. naviforme* strains VPI 11936C and VPI 12188 formed another cluster. This cluster showed a fatty acid pattern characterized by the absence of hydroxy acids and the presence of major amounts of 14:0 and 16:0.

DISCUSSION

On the basis of their fatty acid patterns, representatives of the *Fusobacterium* species examined fell into two distinct groups. One group was characterized by the presence of 3-OH-14:0 and contained all of the strains of *F. nucleatum*, *F. necrophorum*, *F. gonidiaformans*, *F. mortiferum* and *F. varium* together with *F. russii* VPI 0307 and *F. naviforme* VPI 4877. The second group, characterized by the absence of 3-OH-14:0, comprised all strains of *F. plauti*, the single strain of *F. prausnitzii* and the remaining strains of *F. russii* and *F. naviforme*. Within both groups several interspecific and intraspecific differences (Table 3) were detected.

Hydroxylated fatty acids are most frequently reported to occur in Gram-negative bacteria (Lechevalier, 1977) where they are constituents of endotoxic lipopolysaccharides (LPS) (Lüderitz *et al.*, 1974). Hase *et al.* (1977) showed that 3-OH-14:0 and 3-OH-16:0 were present as an integral part of LPS isolated from three strains of *F. nucleatum*. This finding has recently been confirmed by a study of nine additional strains of this species (Hofstad & Skaug, 1980). The same authors found that LPS preparations from *F. necrophorum*, *F. mortiferum*, *F. gonidiaformans* and *F. varium* contained 3-OH-14:0 as the only hydroxylated fatty acid. Accordingly, the hydroxy fatty acids detected in whole cells of *Fusobacterium* species are, at least in part, of LPS origin. The presence of 3-OH-16:0 solely in *F. nucleatum* strains suggests that this compound may be a useful taxonomic marker of this species.

Hydroxylated fatty acids have also been found in ornithine lipids of different bacteria (Thiele & Schwinn, 1974) and in sphingolipids of the genus *Bacteroides* (Miyagawa *et al.*, 1978; White *et al.*, 1969). The sphingolipids of *Bacteroides* species contain small amounts of 3-OH-16:0 in addition to 3-hydroxy-15-methylhexadecanoic acid, which is the main hydroxylated fatty acid. However, organisms belonging to this genus are easily distinguished from *Fusobacterium* species by their content of methyl-branched non-hydroxylated fatty acids (Fritsche, 1974; Miyagawa *et al.*, 1979).

The absence of hydroxy fatty acids in *F. plauti*, *F. prausnitzii*, *F. russii* (VPI 10646 and VPI 11018) and *F. naviforme* (VPI 11936C and VPI 12188) suggests that these strains may have cell walls with atypical LPS composition, or no LPS. Support for this suggestion is

provided by the failure to isolate LPS from these strains by phenol/water extraction (Hofstad & Skaug, 1980). These findings imply that *F. plauti* and the other organisms lacking hydroxylated fatty acids may constitute a cluster which is phylogenetically distant to the other fusobacteria.

All strains examined contained a component having the properties of octadecadienoate (18:2). With a few exceptions (Fulco, 1970; Uchida, 1975) most bacteria apparently lack the biosynthetic apparatus for making di-unsaturated acids (Goldfine, 1972). Whether the octadecadienoate of the *Fusobacterium* species examined is a result of cellular uptake from the medium or a biosynthetic product was not determined. Thus, the taxonomic significance of the quantitative differences among the strains with respect to this compound is presently not known.

Fatty aldehyde dimethyl acetals were found in substantial amounts in many, but not in all, of the organisms studied (Table 3). Such lipid constituents are well-known reaction products formed by methanolysis of alk-1'-enyl glyceryl ethers (plasmalogens). Ether lipids of this kind occur in a number of anaerobic organisms (Hagen & Goldfine, 1967; Kamio *et al.*, 1969) including *Fusobacterium* (*Sphaerophorus ridiculosus*) (Hagen, 1974) but not in aerobic bacteria (Lechevalier, 1977). At present the influence of environmental factors on the biosynthesis of plasmalogens is not known. The taxonomic value of this group of lipids or their aldehyde constituents is therefore uncertain.

Two main taxonomic implications of the results of the present study are evident. Firstly, the spindle-shaped organism *F. nucleatum* contains 3-OH-16:0 and is therefore distinct, on the basis of fatty acid content, from the pleomorphic organisms *F. necrophorum*, *F. mortiferum*, *F. gonidiaformans* and *F. varium*, species classified as *Sphaerophorus* by Prévot (1938) and Prévot & Fredette (1966). Secondly, the marked difference in fatty acid composition between the hydroxy fatty acid-containing species and *F. plauti*, the strain of *F. prausnitzii* and the non-hydroxy fatty acid-containing strains of *F. naviforme* and *F. russii* may indicate that the latter are representatives of a group of non-spore-forming organisms phylogenetically distant to both *Fusobacterium* and *Bacteroides*. It is of relevance in this connection that *F. prausnitzii* with a G + C content of DNA of 50 to 57 mol % (Cato *et al.*, 1974) can be readily distinguished from the hydroxy fatty acid-containing species of *Fusobacterium* examined, which fall into the range 26 to 34 mol % (Moore & Holdeman, 1974; Page & Krywolap, 1976).

As presently defined, the only stable character differentiating *Fusobacterium* species from other anaerobic Gram-negative non-spore-forming rods is the production of butyric acid as a major product from the fermentation of glucose or peptone. The results of the present study suggest that other criteria, including lipid constituents, should be included in the generic description of *Fusobacterium*.

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