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Characterization of CDC Group DF-3 by Cellular Fatty Acid Analysis

P. LYNN WALLACE,[1]* DANNIE G. HOLLIS,[2] ROBERT E. WEAVER,[2] AND C. WAYNE MOSS[1]

Analytical Chemistry Laboratory[1] and Special Bacteriology Reference Laboratory,[2] Meningitis and Special Pathogens Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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Fourteen strains of Centers for Disease Control group DF-3 bacteria were examined for cellular fatty acid composition to evaluate their chemical relatedness to known bacterial species and groups. The fatty acids were liberated from whole cells by base hydrolysis, methylated, and analyzed by capillary gas-liquid chromatography. All group DF-3 strains possessed a distinct fatty acid profile which was characterized by large amounts (24%) of 12-methyltetradecanoate (a-$C_{15:0}$), moderate amounts of saturated iso-branched-chain acids (i-$C_{14:0}$ and i-$C_{15:0}$), and small to moderate amounts of both branched- and straight-chain hydroxy acids (3-OH $C_{15:0}$, i-3-OH $C_{16:0}$, 3-OH $C_{16:0}$, and i-3-OH $C_{17:0}$). This fatty acid profile was unique as compared with the profiles of other bacteria we have previously tested but was most similar to the profiles of *Capnocytophaga* species.

Over the past 20 years, the Special Bacteriology Reference Laboratory of the Centers for Disease Control (CDC) has received for identification many strains of fastidious, oxidase-negative, catalase-negative, nonmotile, gram-negative rods which are presently designated CDC group DF-3 (DF = dysgonic fermenter). Group DF-3 strains also are facultatively anaerobic; ferment glucose, xylose, lactose, sucrose, and maltose; usually produce indole weakly; hydrolyze esculin; do not grow on MacConkey agar; do not reduce nitrate; and produce acid on the slant and in the butt of triple sugar iron agar. These strains have been isolated from several clinical sources, such as blood, wounds, urine, peritoneal fluid, umbilicus, and stools.

In previous studies, we have found that cellular fatty acid analysis of bacterial cells provides useful information for their identification and classification (7, 10–12). In this report, we describe the cellular fatty acid composition of CDC group DF-3 strains and their biochemical and chemical relationships to other bacteria which contain similar branched-chain fatty acids. These bacteria include four *Capnocytophaga* species (previously included in DF-1 and DF-2), three *Flavobacterium* species (*Flavobacterium breve*, *F. meningosepticum*, and *F. odoratum*), and *Cytophaga johnsonae*.

MATERIALS AND METHODS

Strains. Fourteen clinical isolates of CDC group DF-3 strains were obtained from the stock collection of the Special Bacteriology Reference Laboratory, CDC, and were identified by conventional cultural and biochemical tests (3). The strains analyzed were as follows: D7608 (77036067), F2019 (82023922), F2098 (82029609), F2562 (82045692), F3644 (82087033), F3760 (83004053), F4311 (83037597), F7421 (86001100), F7620 (86014354), F9043 (87007805), F9047 (87007836), F9489 (87019984), G294 (87033906), and G919A (88016330).

Preparation and gas-liquid chromatographic analysis of fatty acids. For fatty acid analysis, bacterial cells were inoculated onto plates of heart infusion agar supplemented with 5% rabbit blood and incubated for 24 to 48 h at 35°C in a candle extinction jar. After incubation, the cells were harvested by gentle scraping into approximately 1.0 ml of sterile distilled water, and the cell suspension was transferred to a hexane-rinsed tube (13 by 100 mm) fitted with a Teflon-lined screw-cap. The cells were hydrolyzed, and the liberated fatty acids were derivatized to their corresponding methyl esters by a previously described method (11). The cellular fatty acids (as methyl esters) were analyzed by using the HP5898A Microbial Identification System (Hewlett-Packard Co., Avondale, Pa.) and gas-liquid chromatographic parameters previously described (12). The fatty acid methyl esters were identified by retention time comparison with known standards, calculated equivalent carbon-chain lengths, ancillary techniques (hydrogenation and acetylation), and combined gas-liquid chromatography–mass spectrometry.

Gliding motility. Cultures were inoculated with a loop onto the surface of heart infusion agar containing 5% rabbit serum and incubated for 18 to 24 h at 35°C in a candle extinction jar. After incubation, a cover slip was placed over a marginal section of growth, and the cells were observed at room temperature by bright-field microscopy with a 100× oil-immersion lens (magnification, approximately 1,000×). An observation of individual cell movement of two or more cell lengths was considered positive for gliding motility with this method (2).

RESULTS AND DISCUSSION

The cellular fatty acid composition of the 14 CDC group DF-3 strains was distinct from that observed in all bacteria we have previously tested. Each strain contained large amounts of a-$C_{15:0}$, moderate amounts of saturated iso-branched-chain acids (i-$C_{14:0}$ and i-$C_{15:0}$), and moderate amounts of branched- and straight-chain hydroxy acids (Table 1). Although the relative percentages of these acids varied widely between strains, the overall qualitative pattern was sufficient for differentiating group DF-3 strains from other bacteria. All of the acids listed in Table 1 were

* Corresponding author.

TABLE 1. Characteristic cellular fatty acid compositions of CDC group DF-3, four *Capnocytophaga* species, three *Flavobacterium* species, and *C. johnsonae*[a]

Organism	Fatty acid[b] composition[c]															
	i-$C_{13:0}$	i-$C_{14:0}$	$C_{14:0}$	i-$C_{15:0}$	a-$C_{15:0}$	$C_{15:0}$	$C_{16:1}\omega7c$	$C_{16:0}$	3-OH $C_{15:0}$	i-3-OH $C_{15:0}$	i-3-OH $C_{16:0}$	3-OH $C_{16:0}$	$C_{18:2}$	$C_{18:1}\omega9c$	$C_{18:0}$	i-3-OH $C_{17:0}$
Group DF-3	tr (0.4)	12 (6.7)	2 (0.6)	5 (1.8)	24 (8.1)	5 (1.9)	2 (1.1)	10 (3.7)	2 (0.8)	—(0)	11 (6.6)	5 (1.7)	9 (3.1)	4 (1.3)	1 (1.3)	.5 (2.9)
C. gingivalis	4	—	tr	70	1	1	—	3	—	3	—	3	2	2	1	10
C. ochracea	1	—	2	65	tr	—	tr	6	—	3	—	4	4	5	2	7
C. sputigena	tr	—	tr	78	tr	tr	tr	4	—	3	—	4	2	tr	2	7
C. canimorsus[d]	1	—	tr	77	1	—	—	2	—	4	—	1	1	1	tr	8
F. breve[e]	—	—	2	27	—	2	15	7	—	4	tr	4	1	tr	—	7
F. meningosepticum[f]	2	tr	tr	45	3	—	5	2	—	4	tr	3	tr	tr	tr	10
F. odoratum[g]	3	—	tr	57	1	2	1	1	—	6	tr	4	1	tr	—	8
C. johnsonae[h]	tr	—	2	25	2	10	21	10	1	4	tr	4	tr	—	tr	4

[a] Data were previously published (4–6, 9, 13), with the exception of data for group DF-3.

[b] The number before the colon is the number of carbon atoms; the number after the colon is the number of double bonds; 3-OH indicates a hydroxyl group at C-3; i indicates a branched-chain acid with the branched methyl group at the iso position; a indicates a branched-chain acid with the branched methyl group at the anteiso position.

[c] Values are percentages of total fatty acids and are arithmetic means; tr, <0.7%; —, not detected. Values in parentheses are 2 standard deviations from the means.

[d] In a recent report, CDC group DF-2 was placed in the genus *Capnocytophaga* as *C. canimorsus* on the basis of overall phenotypic similarity to this genus (2).

[e] *F. breve* also contained $C_{15:1}$ (1%), 2-OH $C_{14:0}$ (1%), i-2-OH $C_{15:0}$ (5%), $C_{16:1}\omega5$ (8%), i-$C_{17:1}$ (2%), i-$C_{17:0}$ (1%), and a second i-$C_{17:1}$ isomer (2%).

[f] *F. meningosepticum* also contained i-2-OH $C_{15:0}$ (13%), i-$C_{17:1}$ (5%), and i-$C_{17:0}$ (1%).

[g] *F. odoratum* also contained i-2-OH $C_{15:0}$ (4%) and i-$C_{17:1}$ (7%).

[h] *C. johnsonae* also contained 3-OH $C_{14:0}$ (1%), $C_{15:1}$ (2%), i-$C_{17:1}$ (2%), $C_{17:1}$ (1%), and a second $C_{17:1}$ isomer (2%).

identified by mass spectrometry. The mass spectra of i-3-OH $C_{16:0}$ (and other 3-hydroxy esters) showed prominent ions at *m/e* 103 and at M − 50, characteristic of 3-hydroxy methyl esters, while the mass spectra of 2-hydroxy methyl esters showed typical M − 59 and *m/e* 90 ions (8). The presence of M − 31 and M − 29 ions at about equal concentrations firmly established the branched methyl group at the anteiso position for a-$C_{15:0}$ and other anteiso acids (1). The chemical ionization spectra showed large M + 1 ions at the expected mass value for each acid listed in Table 1.

On the basis of cellular fatty acid composition, group DF-3 strains are most closely related to the bacterial species listed in Table 1. Each species (as well as group DF-3) is characterized by the presence of large amounts of saturated branched-chain acids and moderate amounts of branched- and straight-chain hydroxy acids (4–6, 9, 13). The presence and concentrations of a-$C_{15:0}$ and i-$C_{15:0}$ are particularly useful as markers to differentiate DF-3 strains from *Flavobacterium*, *Capnocytophaga*, and *Cytophaga* species. DF-3 strains contain a-$C_{15:0}$ as the major acid, while i-$C_{15:0}$ is the major acid in all the other species. The overall fatty acid composition of group DF-3 strains was most similar to that of the four *Capnocytophaga* species. However, group DF-3 strains contained i-$C_{14:0}$, i-3-OH $C_{16:0}$, and 3-OH $C_{15:0}$ (12 of 14 strains), which were absent from *Capnocytophaga* species. In addition, *C. johnsonae* and the three *Flavobacterium* species contained small amounts of several acids which were absent from both group DF-3 strains and *Capnocytophaga* species (Table 1, footnotes *e* to *h*).

Shown in Table 2 are some of the key biochemical tests used to distinguish group DF-3 strains from the eight species listed in Table 1. Three *Capnocytophaga* species (*Capnocytophaga gingivalis*, *C. ochracea*, and *C. sputigena*), which were formerly designated DF-1, were grouped together because they cannot be separated on the basis of routine biochemical characteristics. Group DF-3 strains are biochemically most similar to the four *Capnocytophaga* species, as both are gram-negative, fastidious fermenters which do not grow on MacConkey agar. Group DF-3 strains are separated from *Capnocytophaga* species (DF-1) and *C. canimorsus* by the production of acid from xylose. In addition, *C. canimorsus* is positive for catalase, while the three *Capnocytophaga* species (DF-1) and the group DF-3 strains are negative; in addition, *C. canimorsus* does not produce acid from sucrose (3). The three *Flavobacterium* species and *C. johnsonae* are all nonfermenters, and each species has at least three or more key biochemical differences from group DF-3 (3).

On the basis of both biochemical and cellular fatty acid data, CDC group DF-3 strains most closely resemble *Capnocytophaga* species. These findings suggest that group DF-3 strains may represent an additional species of *Capnocytophaga* or a separate genus that is closely related to *Capnocytophaga*. We feel that the latter is probably the case, since the fatty acid compositions of the four *Capnocytophaga* species are essentially identical (4, 6), whereas that of the DF-3 strains is distinctly different. In addition, all *Capnocytophaga* species exhibit gliding motility, which was not observed for three group DF-3 strains (D7608, F2019, and F2098) with the method previously described for *C. canimorsus* (2). This study shows that cellular fatty acid analysis is a rapid and reliable method for the identification of group DF-3; however, DNA-DNA hybridization studies will be required to establish the taxonomic status of this group.

TABLE 2. Review of some key biochemical tests of CDC group DF-3, four *Capnocytophaga* species, *Flavobacterium* species, and *C. johnsonae*[a]

Biochemical test	Test result[b] for:						
	CDC group DF-3 (n = 21)	*Capnocytophaga* species[c] (DF-1) (n = 155)	*C. canimorsus* (DF-2) (n = 27)	*F. breve* (n = 3)	*F. meningosepticum* (n = 148)	*F. odoratum* (n = 74)	*C. johnsonae* (n = 1)
Motility, flagella	nm	v[d]	nm	nm	nm[e]	nm	nm
Carbohydrate base	F	F	F[f]	OF	OF	OF	OF
Acid production from:							
Glucose	+ or (+) (86, 14)	+ (90, 10)	v (67, 18)	+ (100)	+ (94, 4)	− (0)	+
Xylose	+ or (+) (86, 14)	− (0)	− (0)	− (0)	− (2, 1)	− (0)	+
Mannitol	− (0)	− (0)	− (0)	− (0)	+(91, 8)	− (0)	−[g]
Lactose	+ or (+) (52, 43)	v (75, 11)	+ or (+) (81, 19)	− (0)	v (42, 15)	− (0)	−
Sucrose	+ or (+) (62, 33)	+ (90, 9)	− (0)	− (0)	− (0)	− (0)	−
Maltose	+ or (+) (81, 19)	+ or (+) (86, 14)	+ or (+) (81, 19)	+ (100)	+ (93, 7)	− (0)	+
Catalase	− (0)	− (7)	+(100)	+ (100)	+ (100)	+ (100)	w^+
Oxidase	− (0)	− (7)	+ or w^+ (96)	+ (100)	+ (99)	+ (99)	+
Growth on MacConkey medium	− (0)	− (0)	− (0)	+ (100)	+ or (+) (89, 3)	+ (91, 5)	+ or (+)
Urea (Christensen)	− (0, 4)	− (0)	− (0)	− (0)	− (3, 5)	+ (100)	+ or (+)
Nitrate reduction	− (0)	v (63)	− (0)	− (0)	− (0)	− (0)	−
Indole	v (71, 14)	− (0)	− (0)	+ (100)	+ (100)	− (0)	−
Gelatin hydrolysis	− (0)	− (0)	− (0)	+ (100)	+ (91)	+ (96)	−
Esculin hydrolysis	+(100)	v (91, 2)	v (77)	− (0)	+ (99)	− (0)	+

[a] Data were previously published (3), with the exception of data for *C. johnsonae*.

[b] nm, nonmotile; F, fermentation; OF, oxidation-fermentation; −, <10% positive at 7 days; +, 90% or more positive at 48 h; v, 11 to 89% positive at 48 h; + or (+), positive and late positive together totaling 90% or more; w^+, weakly positive; number in parentheses, percent positive; number before comma, percent positive at 48 h; number after comma, percent positive at 3 to 7 days.

[c] *Capnocytophaga* species formerly included in CDC group DF-1. This group includes *C. gingivalis*, *C. sputigena*, and *C. ochracea*.

[d] Delayed spreading in motility medium and an occasional single polar or lateral "flagellum" have been observed (3).

[e] Polar and lateral flagella have been demonstrated on some strains.

[f] One to two drops of rabbit serum per 3 ml of medium may be required; reactions may be obtained within 4 h by using the rapid sugar test (3).

[g] Positive at 8 to 21 days.

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