JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1988, p. 1592–1594
0095-1137/88/081592-03$02.00/0

Vol. 26, No. 8

Cellular Fatty Acid Composition of *Kingella* Species, *Cardiobacterium hominis*, and *Eikenella corrodens*

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We determined the cellular fatty acid composition of reference strains and clinical isolates of each of the three *Kingella* species, *Cardiobacterium hominis*, and *Eikenella corrodens* by using capillary gas chromatography. *Kingella denitrificans* and *Kingella kingae* contained myristic (14:0) and palmitic (16:0) acids as major acids, whereas *cis*-vaccenic (18:1 ω7c) and palmitic acids were the major acids in *Kingella indologenes*, *C. hominis*, and *E. corrodens*. *C. hominis* differed from the other four species by the absence of 3-hydroxylauric (3-OH-12:0) acid, from *K. indologenes* by the presence of 3-hydroxypalmitic (3-OH-16:0) acid, and from *E. corrodens* by the presence of 3-hydroxymyristic (3-OH-14:0) acid. *E. corrodens* contained a small amount (2%) of myristic acid, while the other four species contained moderate to large amounts (11 to 31%) of this acid.

In recent years, we have used gas-liquid chromatography, mass spectrometry, and associated analytical techniques to study the chemical composition and metabolic activity of microorganisms as a basis for their identification and classification. Chemical data from cellular fatty acids, isoprenoid quinones, sphingolipids, and cell wall amino acids have provided valuable information for the recognition of genus, species, and unclassified groups of various bacteria (3–7). Over the years, we have determined the fatty acid composition of all organisms currently included in the family *Neisseriaceae* by Bøvre (1) except *Kingella* species (6, 7). In this report, we describe the fatty acid compositions of the three *Kingella* species and compare these data with those from two phenotypically similar species, *Cardiobacterium hominis* and *Eikenella corrodens*.

Strains. The type, reference, and clinical strains used in this study were obtained from the culture collection of the Special Bacterial Reference Laboratory, Centers for Disease Control, Atlanta, and were identified by conventional cultural and biochemical tests (2). The following strains were tested: *Kingella denitrificans*, the type strain (ATCC 33394 [KC1435, B4363, NCTC 10995]) and nine clinical isolates (E6676, E7177, E7905, E8090, F7162, F7601, F7605, F7906, and F8841); *Kingella kingae*, one reference strain (ATCC 23332 [KC1746]) and 12 clinical isolates (F578, F659, F1260, F1655, F1812, F2122, F5666, F5857, F7517, F7580, F7724, and F8695); *Kingella indologenes*, the type strain (ATCC 25869 [KC1142, NCTC 10717]) and four clinical isolates (F4654, F652, F760, and G46); *C. hominis*, the type strain (ATCC 15826 [KC1776]), four reference strains (ATCC 29308 [KC1408], ATCC 29309 [KC1409], ATCC 29310 [KC1410], and ATCC 29311 [KC1411]) and seven clinical isolates (6573, 6815, 8202, C7913, D3490, E4940, and F6453); and *E. corrodens*, the type strain (ATCC 23834 [KC566, 333/54-55]) and eight clinical isolates (E960, E3669, E3824, E3825, E9816, E9825, F184, and G385).

Preparation and gas-liquid chromatography analysis of fatty acids. For fatty acid analysis, all strains were grown for 24 to 48 h at 35°C on heart infusion agar enriched with 5% defibrinated rabbit blood. After incubation, approximately 1.0 ml of sterile distilled water was added to the surface of one plate, the cells were harvested by gentle scraping, and the cell suspension was transferred to a tube (13 by 100 mm) fitted with a Teflon-lined cap and was processed for cellular fatty acids as described previously (7). The cellular fatty acids (as methyl esters) were analyzed by using the HP5898A Microbial Identification System (Hewlett-Packard Inc., Avondale, Pa.) which includes a 5890A gas chromatograph equipped with a hydrogen flame ionization detector, an automatic injector, a sample controller and sample tray, and an electronic integrator controlled by a minicomputer. The gas chromatograph was equipped with a fused silica capillary column (25 m by 0.2 mm [inner diameter]) containing methylphenyl silicone (SE54) as the stationary phase. The computer-controlled operating parameters of the instrument were as follows: injector temperature, 250°C; detector temperature, 300°C; oven temperature, programmed from 170°C to 300°C at 5°C per min and held at 300°C for 1 min before recycling to the initial temperature. The cellular fatty acid methyl esters were identified by comparing retention times with known standards and by computer calculation of equivalent chain lengths. Combined gas-liquid chromatography-mass spectrometry was used to confirm the identification of the fatty acids.

The fatty acid data for the three *Kingella* species, *C. hominis*, and *E. corrodens* are shown in Table 1. The cellular fatty acid compositions of both *K. denitrificans* and *K. kingae* were characterized by large amounts (24 and 31%, respectively) of myristic acid (14:0), which is consistent with results from two strains of *K. kingae* reported earlier by Jantzen et al. (4). In addition to 14:0, both species contained moderate to large amounts (10 to 31%) of palmitic (16:0) acid; small to moderate amounts of lauric (12:0), palmitoleic (16:1 ω7c), linoleic (18:2), oleic (18:1 ω9c), 3-hydroxylauric (3-OH-12:0), and 3-hydroxymyristic (3-OH-14:0) acids; and trace to small amounts of *cis*-vaccenic (18:1 ω7c) acid. Although the mean values for the 14:1, 14:0, 16:1 ω7c, 16:0, and 18:2 acids are different for *K. denitrificans* and *K. kingella*, these values were not useful for their differentiation because of the overlap of one or two strains of each species. However, both species were easily distinguished from *K. indologenes*, which contained large amounts (27%) of 18:1 ω7c compared with trace amounts in *K. denitrificans* and only small amounts (3%) in *K. kingae*.

C. hominis and *E. corrodens* also contained large amounts of 18:1 ω7c, which readily distinguished these species from

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K. denitrificans and K. kingae. The overall fatty acid profiles of C. hominis and K. indologenes were most similar, but these species were distinguished by the presence of 3-OH-14:0 and 3-OH-16:0 in C. hominis, the presence of 3-OH-12:0 in K. indologenes, and higher amounts (41% versus 27%) of 18:1 ω7c and lower amounts (3% versus 19%) of 16:1 ω7c in C. hominis (Table 1). C. hominis differed from each of the other four species by the absence of 3-OH-12:0.

The fatty acid composition of E. corrodens most closely resembled that of K. indologenes, with the major exception being the amount of myristic acid (14:0). E. corrodens consistently contained small amounts (2 to 3%) of 14:0, compared with moderate amounts (8 to 17%) in K. indologenes. Two other minor differences were the complete absence of 3-OH-14:0 and the presence of trace amounts of 3-OH-16:0 in E. corrodens. E. corrodens was easily differentiated from C. hominis by the smaller amount of myristic acid (14:0), the presence of 3-OH-12:0, and the complete absence of 3-OH-14:0.

The use of the fused silica capillary column permitted complete resolution and accurate quantitation of the two monounsaturated 18-carbon acids (18:1 ω9c, 18:1 ω7c) that were present in each of the 49 strains tested. The ability to resolve these 18-carbon isomers provides a clear means for differentiating K. denitrificans and K. kingae from K. indologenes, E. corrodens, and C. hominis. The presence of large amounts of 18:1 ω7c in K. indologenes but not in K. denitrificans or K. kingae suggests that this species is not closely related to the other two. Originally, K. indologenes was added to Kingella on the basis of biochemical similarities and percent DNA base composition (9). However, in a recent study with DNA-rRNA hybridization, Rossau et al. (8) showed that K. indologenes is not related on the generic level to the other Kingella species and is not a Kingella species.

On the basis of both biochemical characteristics and cellular fatty acid composition, we found K. indologenes to be most similar to C. hominis. In Bergey's Manual of Systematic Bacteriology (10), these two species are separated on the basis that C. hominis produces acid from fermentation of sorbitol and usually produces acid from fermentation of mannitol whereas K. indologenes does not produce acid from either sugar. However, the sorbitol reaction can be weak (10), and at least 5% of C. hominis strains received by the Centers for Disease Control have been mannitol negative and 43% have shown a delayed (3 to 7 days) positive reaction (2). Strains with these atypical reactions are differentiated with supplemental tests, such as Tween hydrolysis, casein hydrolysis, presence of alkaline phosphatase, and growth in 4% NaCl (9). However, the use of cellular fatty acids for identification readily distinguishes C. hominis from K. indologenes, thus eliminating the need for this additional testing.

The large amount of myristic acid (14:0) such as that observed in K. denitrificans and K. kingae is an unusual feature in most bacteria. To our knowledge, comparable concentrations are found only in Haemophilus species, Actinobacillus species, and some unnamed groups of organisms currently under study (C. W. Moss, unpublished observations). However, these organisms are readily distinguished from K. denitrificans and K. kingae by the absence of 3-OH-12:0 and by much smaller amounts of 12:0 (0% to 1%, compared with 6% to 13%). The fatty acid composition of E. corrodens, although distinct from that of the organisms included in this study, is essentially identical to that of Centers for Disease Control groups M-5 and M-6 and some

TABLE 1. Cellular fatty acid composition of *Kingella* species, *C. hominis*, and *E. corrodens*

Organism[a]	Amt of fatty acid[b]													
	12:0	12:1	13:0	3-OH 12:0	14:1	14:0	15:0	3-OH 14:0	16:1 ω7c	16:0	17:0	3-OH 16:0	18:2	18:1 ω9c	18:1 ω7c	18:0	20:4
K. denitrificans (10)	10 (3.0)	T (0.5)	–	3 (1.1)	T (0.8)	24 (13.1)	T (0.5)	2 (1.4)	5 (8.4)	28 (5.6)	T (0.5)	–	12 (9.1)	9 (5.3)	T (2.0)	4 (2.6)	1 (1.2)
K. kingae (13)	9 (2.5)	–	T (0.5)	5 (2.0)	4 (3.0)	31 (14.2)	1 (0.6)	2 (2.9)	15 (7.6)	14 (3.8)	T (0.4)	–	6 (3.2)	5 (3.2)	3 (2.0)	4 (2.2)	1 (0.4)
K. indologenes (5)	5 (3.7)	T (0.3)	–	2 (0.9)	T (0.3)	11 (5.8)	T (0.1)	T (0.4)	19 (7.7)	26 (7.3)	T (0.3)	–	3 (2.8)	2 (2.0)	27 (10.4)	2 (2.2)	T (0.3)
C. hominis (12)	9 (3.8)	–	–	–	T (0.2)	14 (6.0)	T (0.2)	3 (1.4)	3 (1.3)	26 (5.5)	–	1 (0.7)	1 (1.2)	T (0.6)	41 (8.5)	1 (0.9)	–
E. corrodens (9)	6 (0.9)	–	–	5 (1.6)	–	2 (1.2)	–	–	21 (5.6)	29 (4.4)	–	T (0.5)	3 (3.5)	2 (2.2)	28 (6.2)	3 (1.5)	T (0.7)

[a] Number in parentheses is the numbers of strains analyzed.

[b] Number before the colon is the number of carbon atoms, and the number after the colon is the number of double bonds. 3-OH indicates a hydroxyl group at the 3-carbon. Values are percentages of total fatty acids and are arithmetic means; T, trace (less than 0.7%); –, not detected. Values are 2 standard deviations from the mean.

Neisseria species (4–7). Thus, conventional cultural and biochemical tests are required for their differentiation. The combined use of fatty acid data and selected conventional tests provide a means for rapid and accurate identification of many bacteria encountered in the clinical laboratory.

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