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Cellular Fatty Acid Composition of *Pseudomonas paucimobilis* and Groups IIk-2, Ve-1, and Ve-2

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The cellular fatty acid composition of *Pseudomonas paucimobilis* (IIk-1) and groups IIk-2, Ve-1, and Ve-2 was determined by gas-liquid chromatography. The unnamed groups were readily distinguished from *P. paucimobilis* by cellular fatty acids. The data strongly suggest that these bacteria may be additional species of *Pseudomonas* and *Flavobacterium*.

Among the nonfermenting, gram-negative bacteria routinely isolated in clinical laboratories are a number of unnamed groups which have been recognized as phenotypically distinct on the basis of conventional biochemical criteria (3-6, 12, 13). Some groups which have characteristics similar to those of *Pseudomonas* were designated by King as IIk-1, IIk-2, Ve-1, and Ve-2 (12). With the exception of group IIk-1 which has been recently studied by Holmes et al. and given the name *Pseudomonas paucimobilis* (6), legitimate taxons have not been proposed for these groups.

In our laboratory we have used gas-liquid chromatography (GLC) to demonstrate differences in the cellular fatty acid composition of medically important species of *Pseudomonas* and related bacteria (1, 2, 9, 10). On the basis of these differences, we assigned 15 species of *Pseudomonas* to eight distinct GLC groups (9). Since the bacteria in groups IIk-2, Ve-1, and Ve-2 are similar to *Pseudomonas* by conventional tests, it seemed appropriate to investigate the cellular fatty acids of representative strains of these groups. In this report, we compare the fatty acid compositions of the above-mentioned groups and *P. paucimobilis* (IIk-1). Our data show that strains of each group were homogeneous and that the unclassified groups have fatty acid compositions similar to those of well-established species.

MATERIALS AND METHODS

Twenty reference strains of groups IIk and Ve isolated from a variety of human sources were obtained from the Special Bacteriology Section, Center for Disease Control. Included in the IIk group was the type strain of *P. paucimobilis*, NCTC 11030 (6). The pertinent biochemical features of these organisms have been described in previous publications (6, 12). For cellular fatty acid analysis, the cultures were grown on plates of Trypticase soy agar for 24 h at 35°C, and the cells were removed and processed by published procedures (1). The resulting fatty acid methyl esters were analyzed by GLC on a 3% OV-101 column with a flame ionization detector. The conditions for GLC analysis of methyl esters have been discussed (9). Bacterial fatty acids were identified by comparison of retention times to those of standards (Applied Science, State College, Pa.; Analabs, North Haven, Conn.) and by additional techniques of GLC-mass spectrometry, acetylation, and hydrogenation (1, 8).

RESULTS AND DISCUSSION

The chromatograms in Fig. 1A and B show the cellular fatty acids of the *P. paucimobilis* type strain and B3159, group IIk-2, respectively. Comparison of the profiles shows that these two organisms are strikingly different. Most of the fatty acids (75%) in *P. paucimobilis* (Fig. 1A) were unsaturated, whereas the II-2 strain was characterized by large amounts of branched-chain acids. The major peak in the top chromatogram at 14.7 min was identified as octadecenoic (18:1) acid, which comprises approximately 50% of the total fatty acids in *P. paucimobilis* (Table 1). Three additional peaks present in moderate amounts were hexadecenoic (16:1), hexadecanoic (16:0), and heptadecenoic (17:1) acids. Unsaturation was confirmed by hydrogenating the sample and observing the shift in retention time to the corresponding saturated isomer. In hydrogenated samples, the small shoulder on the 16:1 peak (12.0 min, Fig. 1A) was not present, indicating that this peak was an unsaturated acid. Small to trace amounts of other fatty acids (14:0, 15:0, 17:0, 18:0, 19:0) were also detected. Peaks at 11.1 and 15.7 min (Fig. 1A) were hydroxy acids, which were identified by acetylation and mass spectrometry (8) as 2-hydroxytetradecanoate (2-OH 14:0) and 3-hydroxyheptadecanoate (3-OH 17:0), respectively. No branched-chain acids were detected. The fatty acid profiles of all strains of biotype 1,

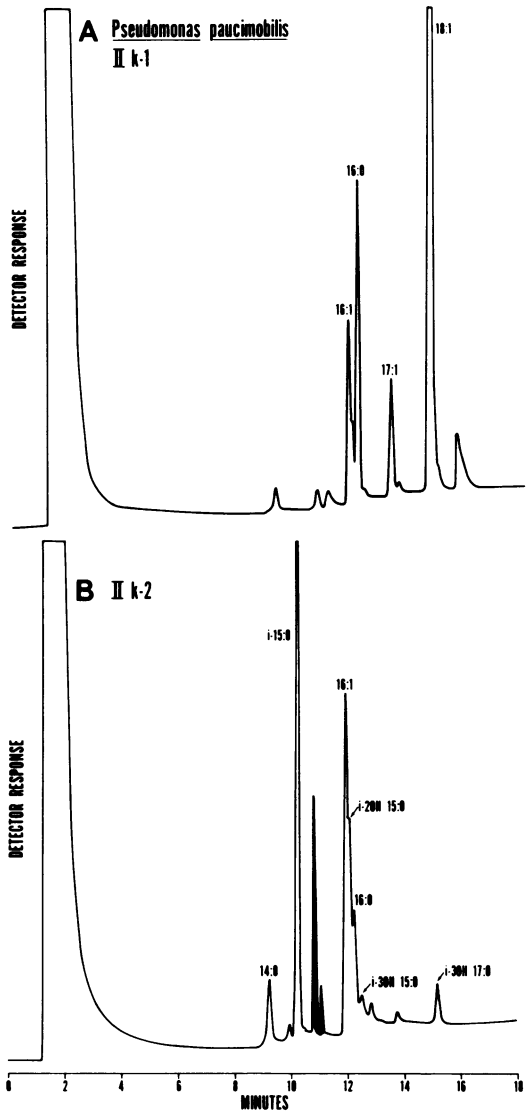

FIG. 1. *Gas chromatograms of the esterified fatty acids of P. paucimobilis, NCTC 11030, type strain (top), and group IIk-2, strain B3159 (bottom). Analysis was made on a 3% OV-101 column.*

group IIk, were essentially identical to that of the type strain of *P. paucimobilis*.

Approximately 60% of the total fatty acids of the IIk-2 strain were branched-chain acids (Fig. 1B). The most abundant acid, which eluted from the column at 10.2 min, was 13-methyl-tetradecanoate (i-15:0). The organism was also characterized by several relatively uncommon fatty acids. For example, the third largest peak at 12.0 min was identified as 2-hydroxy-13-methyl-tetradecanoate (2-OH-i-15:0). Relative amounts of this and the β-substituted acid (3-OH-i-15:0) were determined from acetylated samples. The trifluoroacyl derivatives of the methyl esters which eluted at 12.0 and 12.5 min are represented in Fig. 1 by the shaded peaks in the chromatogram at retention times of 10.5 and 11.0 min, respectively. In addition to these acids, trace amounts of 13-methyl-tetradecanoate (i-15:1, 9.9 min), 15-methyl-hexadecenoate (i-17:1, 12.8 min), and 3-hydroxy-15-methyl-hexadecanoate (3-OH-i-17:0, 15.2 min) were detected. Other fatty acids which characterized the IIk-2 strain were 14:0, 16:1, and 16:0. Essentially identical chromatograms were obtained with five other IIk-2 strains.

Relative amounts of the fatty acids of groups IIk and Ve are shown in Table 1. The numbers represent an average percentage of the acids determined for all strains. The relative amounts of fatty acids were essentially the same for all strains of *P. paucimobilis* (IIk-1). Since their fatty acid profiles were markedly different from those of other pseudomonads which have been examined (9), the newly named species has been placed into a separate GLC group, which we have designated group 9.

Group IIk-2, which is generally differentiated from IIk-1 strains by a small number of biochemical tests, was clearly distinguished by cellular fatty acids (Table 1). Although organisms of group IIk-2 have not been assigned to a genus by existing criteria, fatty acid data indicate that they are a distinct group and are not similar to species of *Pseudomonas* (9), *Alcaligenes* (1), and *Achromobacter* (2). However, data in Table 1 show that group IIk-2 closely resembles *Flavobacterium* species which have been recently investigated (10). The most abundant fatty acids identified in isolates of *F. meningosepticum* and *Flavobacterium* sp., group IIb, were i-15:0 and 2-OH-i-15:0 which are also major fatty acids in group IIk-2 (Table 1). Although differences in relative amounts of 16:1, 16:0, and i-17:1 show that group IIk-2 and the *Flavobacterium* species tested are not identical, our data indicate that IIk-2 organisms are more closely related to *Flavobacterium* than to other species that we have studied (1, 2, 9). Further information such as that provided by deoxyribonucleic acid homology studies on a representative number of isolates would be highly desirable to determine whether this group represents another species of *Flavobacterium*. Our data support the results of other investigators who show that group IIk-2 is more similar to *F. meningosepticum* and *Flavobacterium* group IIb than to *Pseudomonas* on the basis of guanine-plus-cytosine composition of their deoxyribonucleic acids (6).

TABLE 1. *Cellular fatty acids of P. paucimobilis (IIk-1) and groups IIk-2, Ve-1, and Ve-2*

Organism	Straight-chain acids									Branched-chain acids				Hydroxy acids						Branched hydroxy acids			Cyclo-propane acids	
	12:0[a]	14:0	15:0	16:1	16:0	17:1	17:0	18:1	18:0	i-13:0	i-15:1	i-15:0	i-17:1	3-OH 10:0	2-OH 12:0	3-OH 12:0	2-OH 14:0	3-OH 16:0	3-OH 17:0	2-OH i-15:0	3-OH i-15:0	3-OH i-17:0	17:0	19:0
P. paucimobilis (5)[b] (Group IIk-1)	—[c]	T	T	13	26	9	T	52	T	—	—	—	—	—	—	—	T	—	T	—	—	—	T	T
Group IIk-2 (6)	—	3	T	27	10	—	T	T	T	T	T	37	T	—	—	—	—	—	—	18	3	2	T	—
Flavobacterium sp.[d]	—	T	T	T	T	—	T	T	T	—	—	35	14	—	—	—	—	2	—	28	9	12	T	—
Group Ve-1 (5)	5	T	T	22	23	—	T	29	T	—	—	—	—	5	4	4	—	—	—	—	—	—	2	6
Group Ve-2 (4)	6	T	T	19	26	—	T	33	4	—	—	—	—	5	5	2	—	—	—	—	—	—	—	—
GLC Group 1[e] *P. aeruginosa* *P. putida* *P. fluorescens*	5	T	T	18	22	—	T	25	T	—	—	—	—	5	9	2	—	—	—	—	—	—	5	9

[a] Number to the 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- and 3-carbon atom, respectively.

[b] Number in parentheses refers to number of strains tested.

[c] Numbers refer to percentage of total acids; T, less than 2%; —, not detected.

[d] See reference 10.

[e] See reference 9.

Group Ve (biotypes 1 and 2) was markedly different from biotypes of group IIk (Table 1). Strains of both biotypes (group Ve) were characterized by relatively large amounts of 16:1, 16:0, and 18:1 and the presence of hydroxy acids, 3-OH 10:0, 2- and 3-OH 12:0. Other fatty acids detected were 12:0, 14:0, 15:0, 17:0, and 18:0. Only Ve-1 strains contained cyclopropane acids (17:0 Δ, 19:0 Δ). When compared with other bacteria that we have tested (7), these organisms were most similar to species of *Pseudomonas* (9). No distinction could be made between the fatty acid composition of biotype 1 (Ve) and *Pseudomonas* species of GLC group 1 listed in Table 1. These species, *P. aeruginosa*, *P. putida*, and *P. fluorescens*, constitute a GLC group established in a previous investigation of *Pseudomonas* (1, 8, 9). In addition to small qualitative differences shown in the table, biotype 1 may be distinguished from biotype 2 by the ability to hydrolize esculin and arginine, by the production of nitrite from nitrate, and by the number of flagella (5, 11, 12).

Results in this report show that the fatty acid compositions of *P. paucimobilis* (IIk-1) and group IIk-2 are distinct. Fatty acid profiles of groups IIk-2 and Ve-1 strongly resemble those of well-documented species of *Flavobacterium* and *Pseudomonas*, respectively. The fatty acids of group Ve-2 were found to be more like those of GLC group 1 than other pseudomonads. Studies of additional strains will be carried out to establish whether differences in the fatty acid composition of groups Ve-1 and Ve-2 are consistent.

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