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Cellular Fatty Acids and Metabolic Products of *Pseudomonas* Species Obtained from Clinical Specimens

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The cellular fatty acid composition of 112 reference strains and clinical isolates of *Pseudomonas* species was determined by gas-liquid chromatography (GLC). The presence and relative amounts of cyclopropane, hydroxy, and branched-chain fatty acids were distinguishing features of these strains. Determination of short-chain fatty acids extracted from spent growth media provided an additional means for identifying some strains. Our results show that clinical isolates of pseudomonads can be divided into eight distinct GLC groups. The procedures were especially useful for distinguishing glucose-nonoxidizing pseudomonads, which are difficult to identify by conventional criteria. Since the GLC procedures are simple, rapid, and highly reproducible, they are useful in diagnostic laboratories that process large numbers of cultures. Coupled with selected conventional tests, the analysis of short-chain and cellular fatty acids can be very useful for rapid screening of clinical isolates of *Pseudomonas* species.

The increasing importance of *Pseudomonas* species in human infections has emphasized the need for accurate and reliable criteria for their identification (6, 24). These bacteria are opportunistic agents of the infections that often occur after therapy with antibiotics and immunosuppressive drugs. They are found in association with debilitating or malignant diseases and are often contaminants of hospital equipment. Recognized as major offenders in nosocomial infections, these organisms have been isolated from clinical specimens in ever increasing numbers. Since their antibiotic susceptibility varies with the species involved in the disease process, it is important that these organisms be properly identified.

Currently, clinical laboratories use a large number of conventional biochemical tests to identify *Pseudomonas* (7-9, 19, 20, 22, 25). Some of these tests, especially those for determining the identity of glucose-nonoxidizing species, are not completely satisfactory for classification of pseudomonads (19, 20). In addition to conventional methods, more sophisticated techniques to determine substrate utilization (2, 3, 21, 23), deoxyribonucleic acid base composition (10), and deoxyribonucleic acid ribosomal ribonucleic acid homologies (17, 18) have been developed. Although they have provided valuable information concerning taxonomic relationships among various *Pseudomonas* species, these techniques are time consuming and impractical for the clinical microbiologist who must identify organisms as rapidly and accurately as possible.

Classification of microorganisms on the basis of their chemical composition was proposed in 1963 (1). The feasibility of using this approach has been significantly advanced through sensitive analytical techniques such as gas-liquid chromatography (GLC). Our laboratory has used GLC to investigate various classes of compounds obtained directly from whole bacterial cells (11, 12, 14). In preliminary studies, our results showed that fatty acids obtained from whole-cell hydrolysates were useful in distinguishing species of *Pseudomonas* isolated from clinical specimens (5, 16). Moreover, some of the species could be characterized by short-chain acids produced in a simple agar medium (4, 13, 15). Data in this report show that the combination of conventional tests with relatively simple GLC tests for fatty acids provides an effective procedure for rapid identification of medically important species of *Pseudomonas*.

MATERIALS AND METHODS

Cultures. A total of 112 strains of pseudomonads, representing 15 species, were analyzed by GLC. Ten reference strains were supplied by R. Y. Stanier, Berkeley, Calif.; 15 reference strains and 87 clinical isolates were obtained from R. E. Weaver, Center for Disease Control, Atlanta, Ga. The 25 reference strains are listed in Table 1. Cultural and biochemical tests used to establish the identity of these isolates have been described in previous publications (23, 25). Cultures were maintained by monthly

TABLE 1. *Reference strains analyzed for short-chain acids and cellular fatty acids*

Strain	No.	Other designation[a]	Source
P. aeruginosa	RYS 52	ATCC 17429	R. Y. Stanier
	RYS 45	ATCC 17423	R. Y. Stanier
	7013		R. E. Weaver, CDC
P. putida	RYS 90	ATCC 12633	R. Y. Stanier
	4413		R. E. Weaver
P. fluorescens	5826		R. E. Weaver
P. cepacia	RYS 382	ATCC 17759	R. Y. Stanier
P. (multivorans)	KC[b] 984	NCTC 10661, derived from ATCC 17759	R. E. Weaver
P. pseudomallei	7210		R. E. Weaver
P. stutzeri	RYS 220	ATCC 17587	R. Y. Stanier
	KC 404	Van Niel MD 41.1	
P. mendocina	KC 1218	ATCC 25411	R. E. Weaver
P. alcaligenes	RYS 142	ATCC 14909	R. Y. Stanier
	KC 676	Derived from ATCC 14909	R. E. Weaver
P. pseudoalcaligenes	KC 945	Pickett K99, derived from ATCC 12815	R. E. Weaver
P. vesicularis	KC 1099	Lautrop AB 102	R. E. Weaver
P. diminuta	KC 1100	Lautrop AB 236	R. E. Weaver
P. maltophilia	RYS 67	ATCC 13637	R. Y. Stanier
	2897		R. E. Weaver
P. putrefaciens	KC 988	RH 1085, derived from ATCC 8073	R. E. Weaver
P. acidovorans	RYS 14	ATCC 15668	R. Y. Stanier
	KC 939	Pickett K210	R. E. Weaver
P. testosteroni	RYS 78	ATCC 11996	R. Y. Stanier
	RYS 138	ATCC 17510	R. Y. Stanier
	KC 943	Pickett K207, derived from ATCC 15667	R. E. Weaver

[a] ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures.
[b] KC, Known culture.

transfers on semisolid motility medium (Difco, Detroit, Mich.).

Cultural conditions and derivatization. Bacteria were grown on 100- by 15-mm plates of Trypticase soy agar (Baltimore Biological Laboratory, Cockeysville, Md.) for 24 h at 37°C. After incubation, the cells for fatty acid analysis were removed from the surface of the plate and transferred to a test tube for saponification (5). For analysis of short-chain acids, approximately 1 ml of 50% H_2SO_4 (vol/vol) was added to the surface of the plate after the removal of cells and was allowed to stand for 10 min. The agar was then cut into small pieces, transferred to a test tube, and heated for 30 min at 100°C. After the melted agar was cooled to room temperature, the acids were extracted with diethyl ether and converted to butyl esters (15). Approximately 1 μl of the butyl ester samples was injected into the gas chromatograph.

The procedure for methylation of long-chain cellular fatty acids and the subsequent extraction of these esters has been previously reported (5). A major feature of this procedure is that the saponification, methylation, and extraction steps can be accomplished in a single test tube, thereby preventing loss of sample which may occur through excessive manipulation. Serious loss of methyl esters during evaporation was controlled by slowly evaporating the solvent containing the fatty acid methyl esters and never reducing the sample to complete dryness. Approximately 2 μl of the final sample volume of 100 μl was injected into the gas chromatograph.

GLC. Methyl and butyl esters were analyzed on a Perkin-Elmer gas chromatograph (model 900) equipped with a hydrogen flame detector and a disk integrator recorder. The instrument contained two coiled glass columns 3.66 m by 4.06 mm (ID). The methyl esters were analyzed on 3% OV-1 methyl silicone coated onto 80/100 mesh, acid-washed, DMCS-treated, high-performance chromosorb W (Applied Science). In preliminary studies OV-1 was found to be superior to various polar and moderately polar phases in separating the relatively wide range and variety of fatty acids present in some *Pseudomonas* species. The butyl esters were analyzed on 15% Dexsil 300 GC coated onto 80/100 mesh, acid-washed, dimethylchlorosilane-treated chromosorb W (Analabs). Highly purified nitrogen was used as carrier gas at a flow rate of 60 ml/min. The initial temperature of the OV-1 column was 155°C, and after injection of the methyl ester sample the temperature was increased to 270°C at a rate of 6°C/min. Under these conditions, fatty acid methyl esters ranging from 10 to 20 carbons in length eluted from the column within 25 min. For analysis of the more volatile short-chain acid esters, the initial temperature of the Dexsil column was lowered to 100°C. After sample injection, the temperature was increased to 230°C at a rate of 5°C/min. Butyl esters

of highly purified short-chain acid standards (C_1-C_7) eluted from the Dexsil column within 20 min; the butyl ester derivative of phenylacetic and glutaric acids eluted within 30 min.

Both long- and short-chain fatty acids from bacteria were identified by comparing the retention times of the esterified acids in the sample to those of pure standards (Applied Science, Chemical Services, Eastman Organic Chemicals, National Institutes of Health). Some standards of iso branched-chain acids were provided by Toshi Kaneda; others were obtained from Applied Science. Quantitative data were obtained on the cellular fatty acids by determination of peak areas with the disk integrator. The percentage of each acid was calculated from the ratio of the area of its peak to the total area of all peaks. Relative response factors were determined for each acid and were used in the calculation. Hydroxy acids were quantitated after acetylation, which improved their GLC resolution from other acids. A 100-μg amount of nonanoic acid ($C_{9:0}$; Analabs) was added to each sample (prior to saponification) as an internal standard. For the majority of samples, a total concentration of 2 μg of $C_{9:0}$ injected into the column at an attenuation setting of 32 and range 100 yielded a peak size of 80 to 90% of full-scale deflection. Since $C_{9:0}$ eluted well before the bacterial fatty acids, any necessary adjustment in attenuation could be made on the basis of the size of this peak. Final identification of both short-chain and cellular fatty acid esters was accomplished by a combination of techniques: GLC-mass spectrometry, hydrogenation, infrared spectroscopy, and acetylation (5, 12, 16).

RESULTS AND DISCUSSION

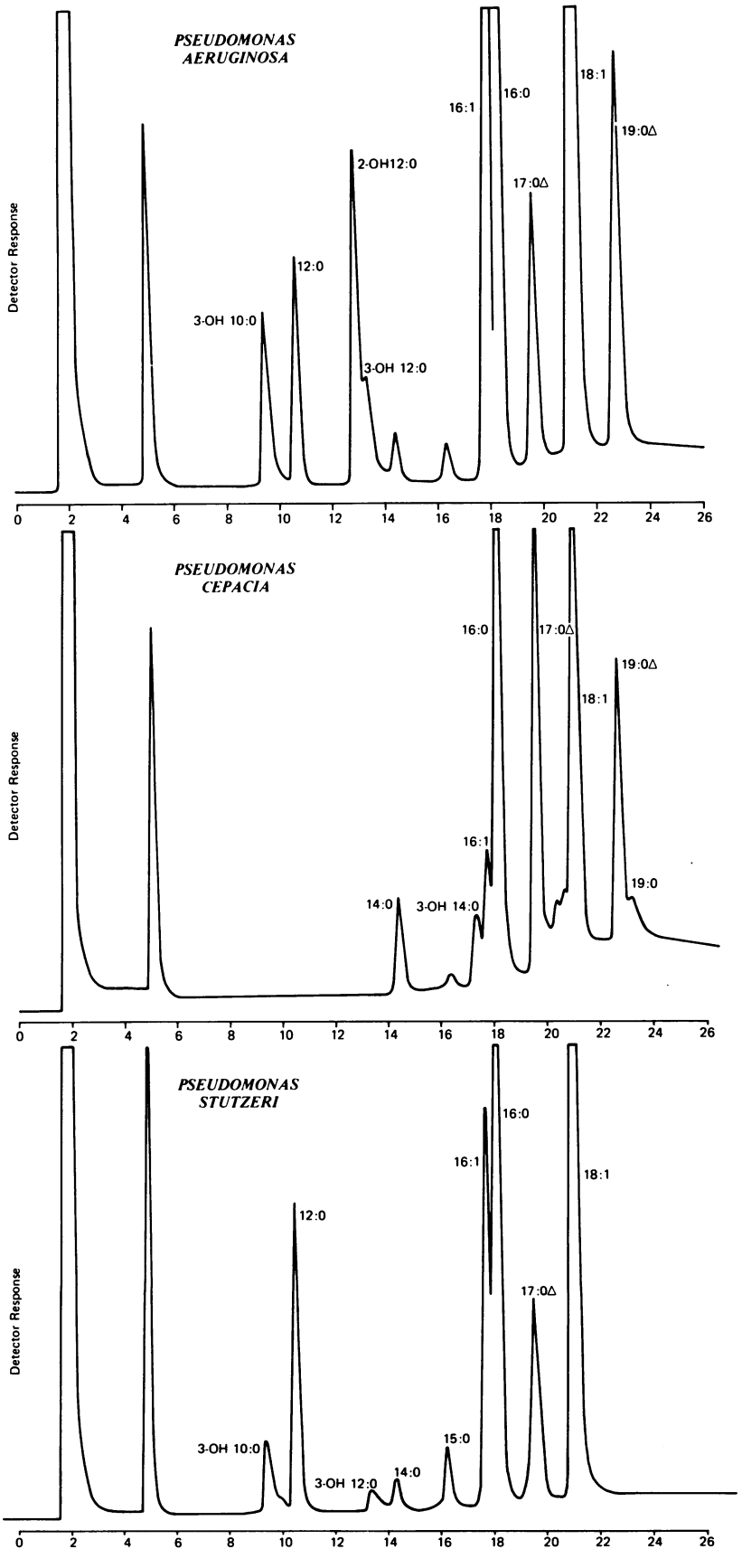
In the initial part of this investigation, reference strains of each of the 15 species of *Pseudomonas* most frequently isolated from clinical materials were studied (9, 19, 20, 22). The results showed that 14 species could be placed into one of eight distinct groups on the basis of qualitative or relatively large quantitative differences in their cellular fatty acid compositions. Chromatograms of the cellular fatty acids of representative strains of the eight groups are shown in Fig. 1 through 4.

Three fluorescent pigmented species, *P. aeruginosa*, *P. putida*, and *P. fluorescens* were placed in group 1. The top chromatogram in Fig. 1, which shows the fatty acid profile of *P. aeruginosa* strain RYS 52, is representative of strains within this group. All strains contained relatively large amounts of 16:1, 16:0, and 18:1 acids, smaller amounts of 17- and 19-cyclopropane acids (17Δ and 19Δ), and three hydroxy acids—3-hydroxy-decanoate (3-OH 10:0), 2-hydroxydodecanoate (2-OH 12:0), and 3-hydroxydodecanoate (3-OH 12:0). The presence of 19Δ in this group easily distinguished it from groups 3, 5, 6, 7, and 8, in which no 19Δ or only traces of it were detected. Group 1 organisms contained both 17Δ and 19Δ acids, whereas organisms in group 4 contained only 19Δ. Group 1 was readily distinguished from group 2 by the presence of 3-OH 10:0, 12:0, 2-OH 12:0, and 3-OH 12:0 acids. The first peak in each chromatogram (retention time of approximately 5 min) is the $C_{9:0}$ internal standard.

When clinical isolates of each of the three species in GLC group 1 were tested for cellular fatty acids, all gave chromatograms essentially identical to that shown for the reference strain of *P. aeruginosa* (Fig. 1, top). Quantitative data of the fatty acids of each species are presented in Tables 2 and 3. Values in the tables are average percentages determined from the analysis of several strains of a species. For example, the mean percentage of 19Δ acid from seven isolates of *P. aeruginosa* was 11%; the percentage for the most abundant hydroxy acid (2-OH 12:0) was 9%. The similarity of the fatty acid composition of the three species in group 1 is apparent by comparing the values in Table 2.

Two species, *P. cepacia* and *P. pseudomallei*, made up group 2. A representative profile of the fatty acids of this group is illustrated in the middle chromatogram (Fig. 1) with *P. cepacia* strain KC 984. The presence of 3-hydroxytetradecanoate (3-OH 14:0) at a retention time of approximately 17.2 min distinguished group 2 organisms from those of the other seven groups, which did not contain this acid. Although present in relatively small amounts (5 to 7%, Table 2), it was consistently present in all cultures of *P. cepacia* and *P. pseudomallei* and was absent in all other species. Other small but consistent differences between this group and others is the absence in group 2 of both lauric acid (12:0) and hydroxy acids with less than 14 carbons. The two small peaks at retention times of 20 and 20.5 min (which appear as shoulders on the leading edge of the 18:1 peak in the middle chromatogram) were identified as 2- and 3-hydroxyhexadecanoate (2-OH 16:0, 3-OH 16:0). These two hydroxy acids were present in relatively small amounts, but only in group 2 organisms (Table 2). GLC resolution of these two acids and other hydroxy acids was greatly improved by acetylation of the hydroxy group (16). Quantitative data from clinical isolates of each of the two species in group 2 are presented in Table 2.

FIG. 1. *Gas chromatograms of esterified fatty acids from saponified whole cells of P. aeruginosa, P. cepacia, and P. stutzeri. Analysis was made on a 3% OV-1 column. Numbers on horizontal axis represent minutes.*


PSEUDOMONAS AERUGINOSA
Detector Response
3-OH 10:0
12:0
2-OH12:0
3-OH 12:0
16:1
16:0
17:0Δ
18:1
19:0Δ
0 2 4 6 8 10 12 14 16 18 20 22 24 26
PSEUDOMONAS CEPACIA
Detector Response
14:0
3-OH 14:0
16:1
16:0
17:0Δ
18:1
19:0Δ
19:0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
PSEUDOMONAS STUTZERI
Detector Response
3-OH 10:0
12:0
3-OH 12:0
14:0
15:0
16:1
16:0
17:0Δ
18:1
0 2 4 6 8 10 12 14 16 18 20 22 24 26

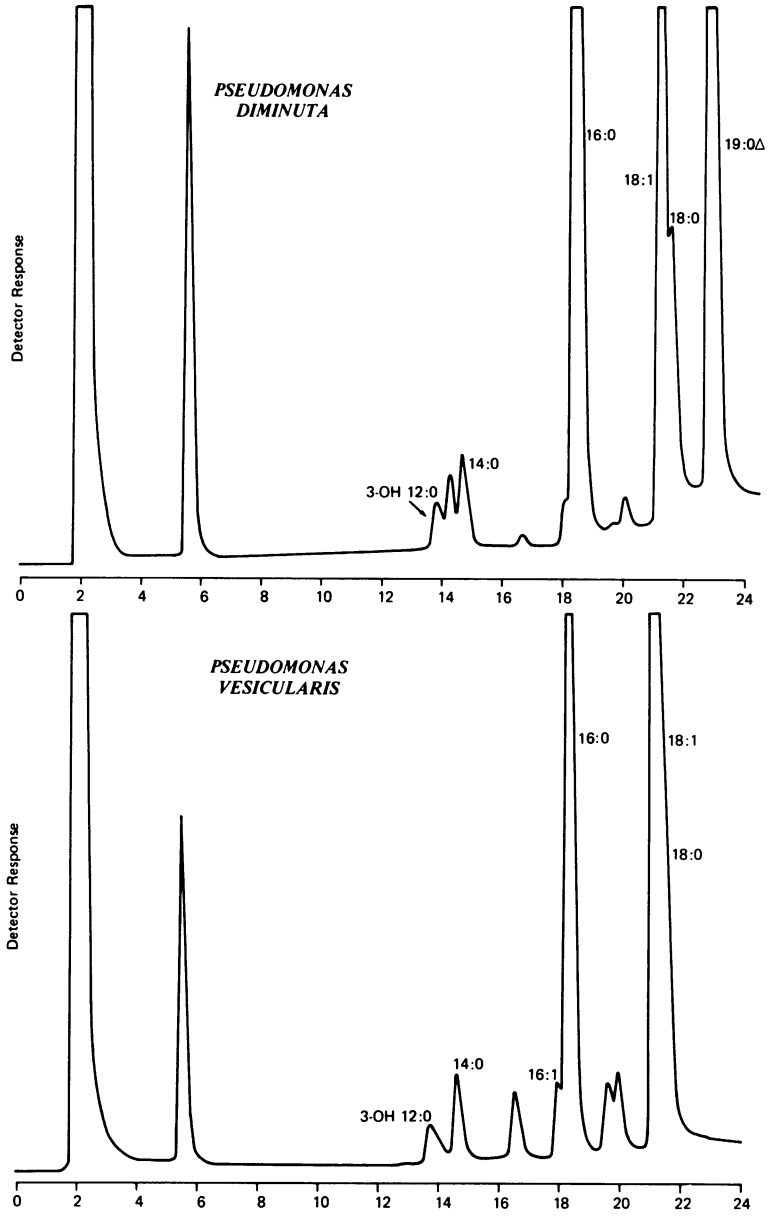


FIG. 2. *Gas chromatograms of esterified fatty acids from saponified whole cells of P. diminuta and P. vesicularis. Analysis was made on a 3% OV-1 column. Numbers on horizontal axis represent minutes.*

Three species, *P. stutzeri*, *P. mendocina*, and *P. pseudoalcaligenes*, were placed in group 3. A representative fatty acid profile of organisms of this group is illustrated with *P. stutzeri* strain RYS 220 (Fig. 1, bottom). As discussed previously, the absence of 19Δ acid and the differences in the hydroxy acids that were present clearly distinguished this group from all others except group 8, which is similar to group 3 in several respects. Chromatograms of group 3 and 8 organisms are shown in Fig. 4. A small but significant difference between the two groups was the presence of 3-OH 12:0 acid in group 3 (bottom), which was absent in all strains of group 8 (top). Another consistent difference was the relative amounts of 3-OH 10:0 and 12:0 acid; in group 8 organisms the ratio of 3-OH 10:0 to 12:0 was approximately 1:1,

whereas in group 3 organisms this ratio was 1:2 or greater for all strains (Fig. 4, Table 2). The iso-branched 17-carbon acid (i-17:0) was present in *P. pseudoalcaligenes* but not in the other two species of group 3 (Table 2, footnote *d*).

The fatty acid composition of the type strain of *P. alcaligenes* was most like that of species in group 3, but it could be distinguished from this group by the presence of moderate amounts (10%) of decanoic acid and by differences in the relative amounts of 14:0, 15:0, and 17Δ acids. However, only two of five clinical isolates of this species had profiles similar to that of the type strain. Studies are now in progress to determine the extent of heterogeneity of cellular fatty acids among strains of this species. Data from this study may provide a basis for establishing a more restrictive definition of *P. alcaligenes*.

Four groups contained only one species: *P. diminuta* was designated as group 4; *P. vesicularis*, group 5; *P. maltophilia*, group 6; and *P. putrefaciens*, group 7. Representative chromatograms of *P. diminuta* and *P. vesicularis* are shown in Fig. 2. A major difference between these two groups is the presence of relatively

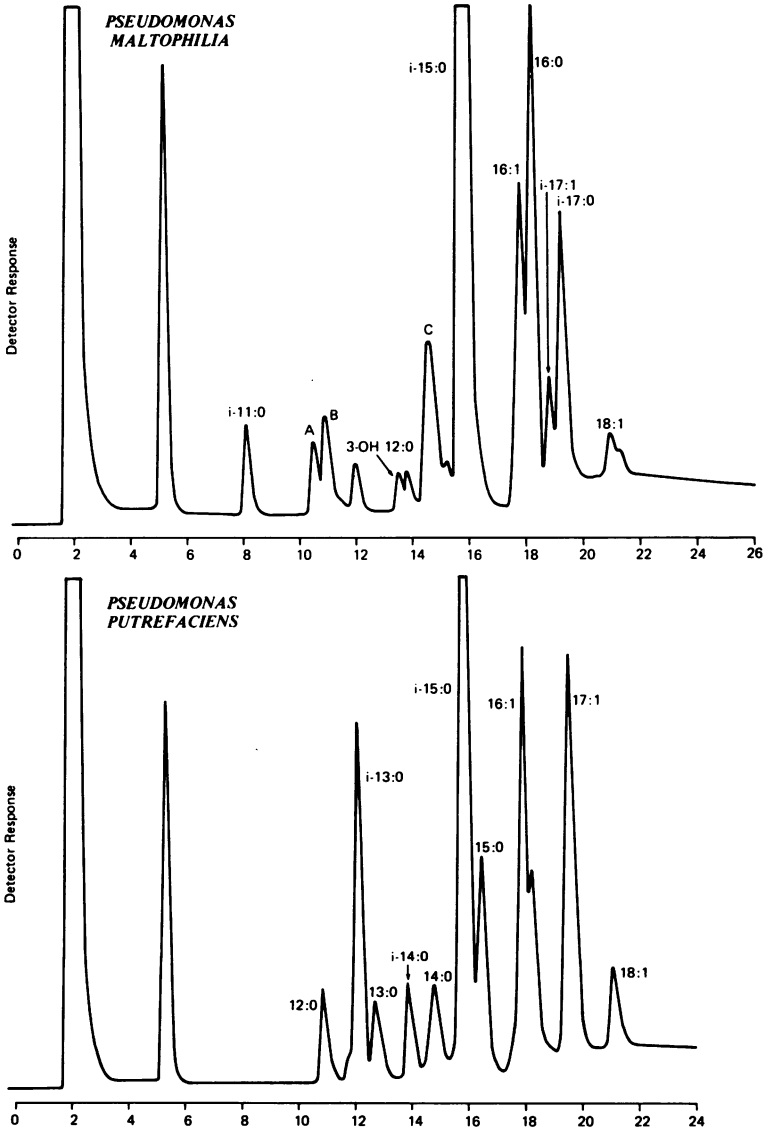


FIG. 3. *Gas chromatograms of esterified fatty acids from saponified whole cells of P. maltophilia and P. putrefaciens. Analysis was made on a 3% OV-1 column. Numbers on horizontal axis represent minutes.*

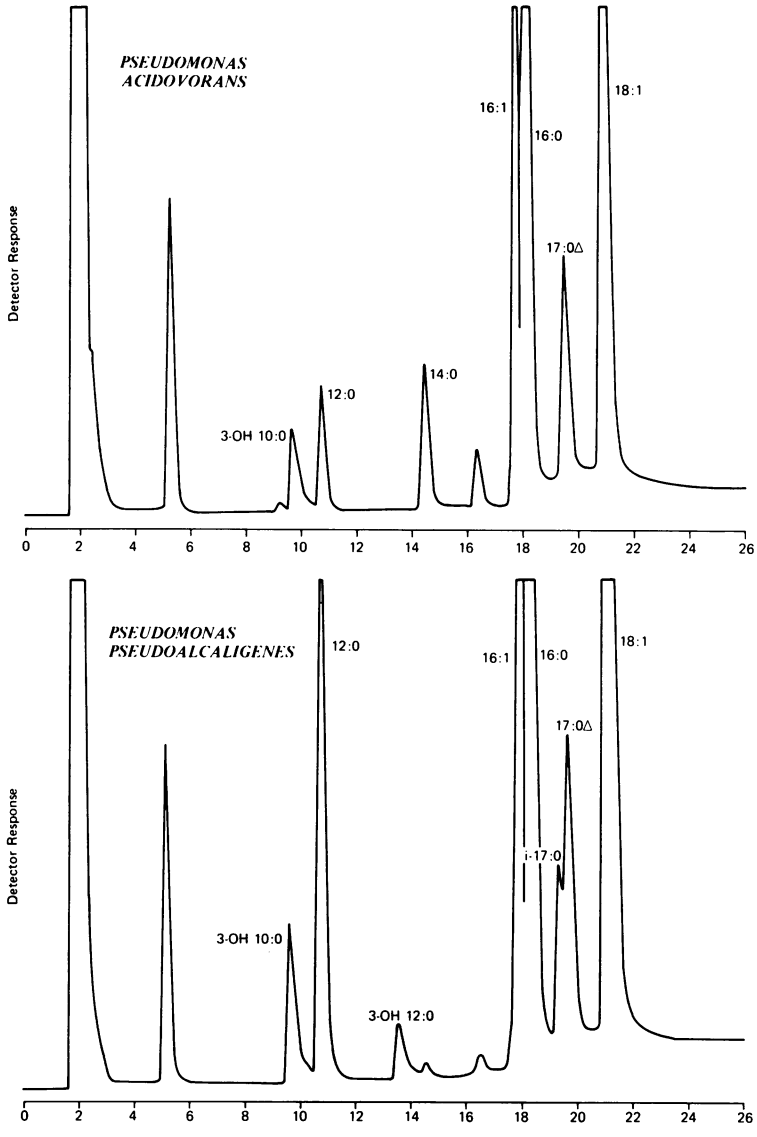


FIG. 4. *Gas chromatograms of esterified fatty acids from saponified whole cells of P. acidovorans and P. pseudoalcaligenes. Analysis was made on a 3% OV-1 column. Numbers on horizontal axis represent minutes.*

large amounts of 19Δ acid in *P. diminuta* (top), which was absent in *P. vesicularis* (bottom). This acid accounted for approximately 30% of the total fatty acids in nine isolates of *P. diminuta* but was not detected in isolates of *P. vesicularis* (Table 3). Both species contained relatively large amounts of 16:0 and 18:1 acids, which together accounted for 50 to 60% of the total acids. Neither species contained branched-chain acids, and both had only small amounts (<5%) of hydroxy acids (Table 3).

P. maltophilia (group 6) and *P. putrefaciens* (group 7) were the only species tested that contained more than trace amounts of branched-chain acids. Representative chromatograms of these two species are shown in Fig. 3. The single most abundant acid in each of these species was a branched-chain 15-carbon acid, 13-methyl tetradecanoic acid (i-15:0), which serves as a convenient marker for rapid differentiation of these two from other species (Fig. 3). This acid constituted 30% of the total acids of *P. maltophilia* and 22% of *P. putrefaciens* (Table 3). Aside from this similarity, however, there were several major differences between these two groups. *P. maltophilia* (top) contained i-

TABLE 2. *Cellular fatty acid composition of Pseudomonas species (GLC groups 1-3)*

Species	Straight-chain acids									Hydroxy acids							Cyclopropane acids	
	12:0[a]	14:0	15:0	16:1	16:0	17:0	18:1	18:0	19:0	3-OH 10:0	2-OH 12:0	3-OH 12:0	2-OH 14:0	3-OH 14:0	2-OH 16:0	3-OH 16:0	17:0	19:0
GLC group 1																		
P. aeruginosa (7)[b]	6[c]	T	T	17	20	T	25	T	T	5	9	2	–	–	–	–	5	11
P. putida (4)	5	2	2	18	20	T	25	T	T	5	8	2	–	–	–	–	5	8
P. fluorescens (4)	5	T	T	18	22	T	26	T	T	5	8	2	–	–	–	–	5	9
GLC group 2																		
P. cepacia (8)	–	5	2	7	20	T	19	2	2	–	–	–	T	5	4	5	17	12
P. pseudomallei (8)	–	6	T	8	22	T	16	T	2	–	–	–	T	7	5	3	17	14
GLC group 3																		
P. stutzeri (7)	12	3	4	15	23	2	25	2	–	5	–	2	–	–	–	–	7	–
P. mendocina (7)	12	2	3	16	24	2	25	T	–	5	–	2	–	–	–	–	9	–
P. pseudoalcaligenes[d] (8)	15	T	T	18	22	T	26	T	–	5	–	2	–	–	–	–	8	–

[a] Number to left of colon refers to number of carbon atoms; number to right refers to number of double rods; 2- and 3-OH refer to hydroxy acid.

[b] Number in parentheses gives number of strains tested.

[c] Number refers to percentage of total acids; T, less than 2%; –, not detected.

[d] Approximately 4% of total fatty acids of this species was i-17:0.

11:0, 3-OH 12:0, i-17:1, i-17:0, and components A, B, and C, which were not present in *P. putrefaciens* (bottom). In addition, a 17:1 acid was present in *P. putrefaciens*, but was not present in *P. maltophilia* or in any other species in more than trace amounts (Tables 2 and 3). Additional studies established the identity of components A, B, and C as branched-chain hydroxy acids. These acids appear to be present only in the cellular fatty acids of *P. maltophilia* (16).

In addition to cellular fatty acids, reference strains and clinical isolates of each of the 15 species were tested for short-chain acids present in the Trypticase soy agar medium after 24 h of growth. Only five species produced more than trace amounts of acids, but the production of these provided additional criteria for distinguishing some of the species (Table 4). For example, the two species in group 8 can be distinguished by the presence of phenylacetic acid, which was produced by strains of *P. testosteroni* but not by *P. acidovorans* (4, 15). Likewise, the presence of relatively large amounts of isobutyric (iC4) and isovaleric (iC5) acids clearly distinguishes *P. pseudoalcaligenes* from *P. alcaligenes*. *P. diminuta* and *P. vesicularis* differed from other species in that they produced relatively large amounts of propionic, isobutyric, and isovaleric acids and could be distinguished from each other by the production of glutaric acid by *P. diminuta* (13). The production of isovaleric acid together with smaller amounts of phenylacetic acid was a unique feature of *P. maltophilia*.

The use of a small number of conventional tests in combination with tests for cellular fatty acids and short-chain acid products provides an effective means for rapid identification of 14 of the 15 species included in this study. In addition to the minimal characters of the genus as described by Hugh and Gilardi (9), tests for arginine dihydrolase, gelatin hydrolysis, and growth at 42°C were selected for the purpose of distinguishing those species not differentiated by fatty acid data alone. These three tests were chosen from a number of possible conventional tests because of their simplicity, reproducibility, and high specificity. As shown in the flow chart in Fig. 5, the three species in GLC group 1 are easily distinguished from each other on the basis of gelatin hydrolysis and their ability to grow at 42°C. The two species in GLC group 2 differ in their ability to metabolize arginine. *P. stutzeri* and *P. mendocina*, two of the three species comprising GLC group 3, oxidize glucose, whereas *P. pseudoalcaligenes* fails to do so. The two glucose-oxidizing species are distinguished by the arginine dihydrolase reaction. The production of phenylacetic acid by *P. testosteroni* differentiates the two species in GLC group 8.

TABLE 3. *Cellular fatty acid composition of Pseudomonas species (GLC groups 4–8)*

Species	Straight-chain acids										Branched-chain acids							Hydroxy acids		Branched-chain hydroxy acids			Cyclo-propane acid	
	12:0[a]	13:0	14:0	15:0	16:1	16:0	17:1	17:0	18:1	18:0	i-11:0	i-13:0	i-14:0	i-15:0	i-16:0	i-17:1	i-17:0	3-OH 10:0	3-OH 12:0	2-OH i-11:0	3-OH i-11:0	3-OH i-13:0	17:0	19:0
GLC group 4																								
P. diminuta[b] (9)[c]	–[d]	–	4	T	3	29	–	T	22	T	–	–	–	–	–	–	–	–	3	–	–	–	T	29
GLC group 5																								
P. vesicularis (9)	–	–	6	5	6	26	–	4	34	10	–	–	–	–	–	–	–	–	4	–	–	–	5	–
GLC group 6																								
P. maltophila (9)	–	–	5	T	10	18	–	T	T	T	5	2	2	30	T	4	10	–	2	4	4	4	T	–
GLC group 7																								
P. putrefaciens (7)	3	3	4	7	16	8	16	2	4	T	–	11	4	22	–	–	–	–	–	–	–	–	T	–
GLC group 8																								
P. acidovorans (9)	4	–	5	2	21	28	–	T	26	2	–	–	–	–	–	–	–	4	–	–	–	–	8	–
P. testosteroni (9)	5	–	5	2	22	25	–	T	25	T	–	–	–	–	–	–	–	6	–	–	–	–	10	–

[a] Number to left of colon refers to number of carbon aotms; number to right refers to number of double bonds; 2- and 3-OH refer to hydroxy acid; i, iso acid.

[b] Approximately 3% of the fatty acids of this species is 14:1 (Fig. 2, peak between 3-OH 12:0 and 14:0).

[c] Number in parentheses gives number of strains tested.

[d] Number refers to percentage of total acids; T, less than 2%; –, not detected.

TABLE 4. *Short-chain acids of Pseudomonas species isolated from clinical specimens*

Species	GLC group	Acid[a]					
		C_2	C_3	iC_4	iC_5	Phenylacetic	Glutaric
P. acidovorans	8	1[b]	–	–	–	–	–
P. testosteroni	8	2	–	–	–	4	–
P. alcaligenes	3	1	–	–	–	1	–
P. pseudoalcaligenes	3	1	–	4	12	1	–
P. maltophilia	6	8	–	–	4	1	–
P. diminuta	4	12	12	12	12	1	4
P. vesicularis	5	12	12	12	12	T	–
Uninoculated TSA medium[c]		12	–	–	–	–	–

[a] C_2, Acetic acid; C_3, propionic acid; iC_4, isobutyric acid; iC_5, isovaleric or 2-methylbutyric acid.

[b] Numbers refer to relative areas of peaks. T, Peak with less than 10% of full-scale deflection; 1, 10 to 39%; 2, 40 to 69%; 3, 70 to 90%; 4, peak with full-scale deflection or greater; 8, peak with twice the area of 4; 12, peak with three times the area of 4; –, acid not detected.

[c] TSA, Trypticase soy agar.

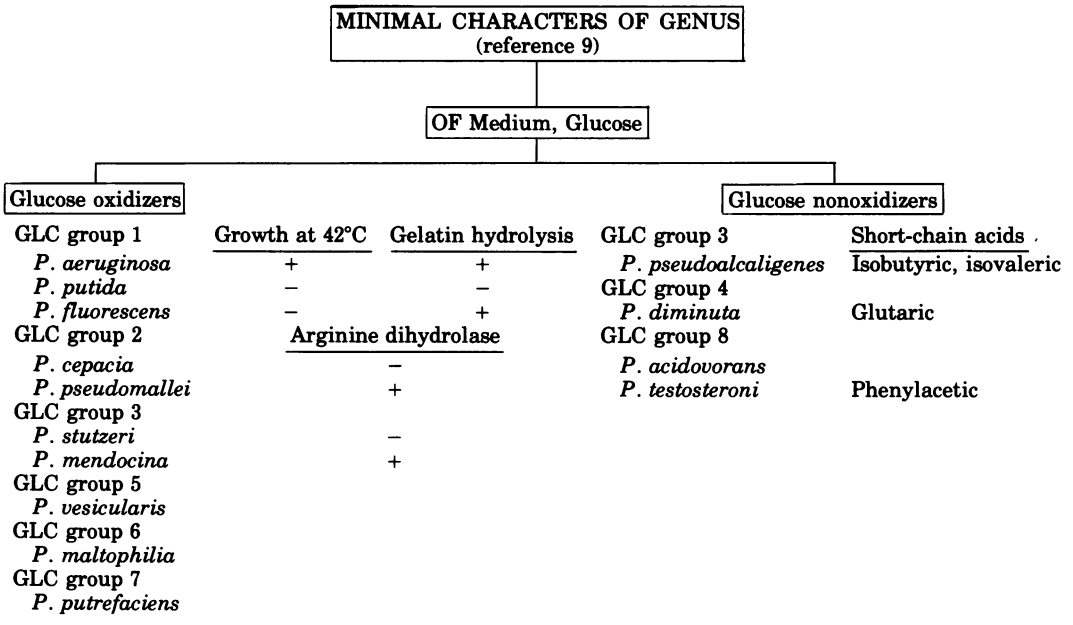


FIG. 5. *Flow chart showing useful biochemical tests for separation of species within a GLC group. OF, Oxidation-fermentation.*

Our results indicate that cellular fatty acids and short-chain acid products provide additional useful information for identification of pseudomonads. The above data, in conjunction with selected conventional tests, can be used to distinguish medically important species. The GLC procedures for measurement of these compounds are rapid and reproducible. The extraction, derivatization, and GLC steps can be accomplished without difficulty by technical personnel. The excellent temperature stability of the GLC stationary-phase materials (OV-1, Dexsil) allows for multiple analysis over a period of months without column deterioration. Highly purified fatty acid reference standards are readily available from various commercial sources.

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