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Grouping of Plant-Pathogenic and Some Other *Pseudomonas* spp. by Using Cellular Fatty Acid Profiles

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Approximately 500 fatty acid profiles were prepared for 340 strains of plant-pathogenic and other bacteria currently or recently classified in the genus *Pseudomonas* Migula 1984. Strains representing some infraspecific taxa were included. The fatty acid profiles were stable and reproducible provided that cultural and chemical techniques were standardized. The 2- and 3-hydroxy fatty acids were found to be useful in grouping strains into six major groups, several of which were further differentiated into subgroups. Group 1 contained strains of the following species and subspecies: *Pseudomonas aeruginosa*, *P. agarici*, *P. asplenii*, *P. aureofaciens*, *P. caricapapayae*, *P. chlororaphis*, *P. cichorii*, *P. ficuserectae*, *P. fluorescens*, *P. fuscovaginae*, "*P. gingeri*," *P. marginalis*, *P. meliae*, *P. putida*, "*P. reactans*," *P. syringae*, *P. tolaasii*, and *P. viridiflava* (subgroup 1a); *P. corrugata* (subgroup 1b); *P. rubrisubalbicans* (subgroup 1c); *P. alcaligenes*, *P. pseudoalcaligenes* subsp. *pseudoalcaligenes*, and *P. stutzeri* (subgroup 1d); *P. amygdali* (subgroup 1e); and *P. cattleyae* NCPPB 1874 (subgroup 1f). All group 1 strains contained 10:0 3-OH and 12:0 3-OH, and most group 1 strains also contained 12:0 2-OH. Group 2 contained strains belonging to the following taxa: *P. andropogonis*, *P. caryophylli*, *P. cepacia*, *P. gladioli*, *P. plantarii*, and *P. glumae* (in part) (subgroup 2a); *P. glumae* (in part) (subgroup 2b); and *P. solanacearum*, *P. syzygii*, and the banana blood disease bacterium (subgroup 2c). All of the group 2 strains contained 14:0 3-OH, 16:0 3-OH, and 18:1 2-OH; most also contained 16:1 2-OH and 16:0 2-OH. Group 3 contained strains belonging to the following taxa: *Comamonas acidovorans*, *P. avenae*, *P. cattleyae* NCPPB 961, *P. pseudoalcaligenes* subsp. *citrulli*, *P. pseudoalcaligenes* subsp. *konjaci*, *P. rubrilineans* (subgroup 3a); and *Comamonas testosteroni* (subgroup 3b). All of the group 3 strains contained 10:0 3-OH. The group 4 strains were members of *Sphingomonas paucimobilis*, and all contained only 14:0 2-OH. The group 5 strains were members of *P. flectens* and contained 12:0 2-OH, 14:0 2-OH, and 14:0 3-OH. The group 6 strains were *P. betle*, *P. cissicola*, *P. hibiscicola*, *Xanthomonas maltophilia*, and *Xanthomonas campestris* pv. campestris strains, and all contained 12:0 3-OH, 11:0 iso 3-OH, and 13:0 iso 3-OH. Within each group or subgroup, qualitative and quantitative differences in profiles occurred for most species. Differences were also found at the infraspecific level for some taxa. My results support genomic and other data which show that the plant-pathogenic and other pseudomonads tested should be placed in at least six genera.

A total of 86 species were included in the genus *Pseudomonas* Migula 1894 on the Approved Lists of Bacterial Names (25). Bradbury (1) listed 32 of these species that contain plant pathogens. DNA-DNA and DNA-rRNA homology studies have been useful in classifying some of these species, and Palleroni and coworkers (17–19) have proposed five rRNA subgroups, which center around the following species: *Pseudomonas aeruginosa*, *P. solanacearum*, *P. acidovorans*, *P. diminuta*, and *P. maltophilia*. This proposal has been supported by the results of other independent work, which has grouped these bacteria into similar subdivisions (4, 5, 7) by using rRNA cistron similarity data. These groupings have also been supported by the results of studies in which enzyme techniques were used (2, 32). More recently, cellular fatty acid profiles have been used to classify some *Pseudomonas* spp. (8, 16). The results of these studies have indicated that each of the major rRNA subgroups has a characteristic fatty acid profile in which the hydroxy acids are of particular taxonomic value.

Members of two of the rRNA subgroups have been reclassified in other genera. *P. maltophilia* (rRNA group V) has been reclassified as *Xanthomonas maltophilia* (29). *P. acidovorans* and *P. testosteroni* (rRNA group III) have recently been reclassified in the genus *Comamonas* (6) as *Comamonas acidovorans* and *Comamonas testosteroni*, respectively (30). In addition, *P. paucimobilis* has been reclassified as *Sphingomonas paucimobilis* (35).

In this study I wanted to determine whether the plant-pathogenic pseudomonads can be classified on the basis of fatty acid profiles. I hoped that a classification based on fatty acid profiles which allowed differentiation of taxa at least at the specific level might form the basis of a future rapid, accurate identification method.

All of the bacteria used in this study fall under the definition of the genus *Pseudomonas* Migula 1894. Recent nomenclatural changes were taken into account where appropriate. Some infraspecific taxa and some recently described and invalidly named *Pseudomonas* species were also included.

MATERIALS AND METHODS

Bacterial cultures. All of the cultures were obtained from the National Collection of Plant Pathogenic Bacteria (NCPPB) and from recent diagnoses unless stated otherwise. Information on the strains used is shown in Table 1. All of the strains except the *P. amygdali*, and *P. syzygii* strains and the banana blood disease bacterium strains were grown aerobically on Trypticase soy agar (Trypticase soy broth [BBL] containing Bacto Agar [Difco]) at 28°C for 24 ± 1 h. *P. amygdali* strains were grown on nutrient dextrose agar (CM3 nutrient agar [Oxoid] containing 1% glucose) for 7 days at 28°C. *P. syzygii* strains were grown on casein salts agar (7.5 g of acid casein hydrolysate [Oxoid], 2.0 g of

TABLE 1. Strains used

Bacterium (synonym[s])	No. of GC runs	Strain(s)[a]
C. acidovorans (den Dooren de Jong 1926) Tamaoka et al. 1987 (*P. acidovorans* den Dooren de Jong 1926, *P. desmolytica* Gray and Thornton 1928)[b]	3	NCPPB 1967, NCPPB 1968
C. testosteroni (Marcus and Talalay 1956) Tamaoka et al. 1987 (*P. testosteroni* Marcus and Talalay 1956)[b]	5	NCPPB 1969^T, NCPPB 1976, H24D, H29C
P. aeruginosa (Schroeter 1872) Migula 1900 (*P. polycolor* Clara 1930)[c]	14	NCPPB 249, NCPPB 288, NCPPB 292, NCPPB 1224, NCPPB 1896, NCPPB 1965^T, NCPPB 2195, NCPPB 2650, NCPPB 2653, IHRL Mia, JT2042a
P. agarici Young 1970	16	NCPPB 1996, NCPPB 1999, NCPPB 2289^T, NCPPB 2290, NCPPB 2304, NCPPB 2471, NCPPB 2472
P. alcaligenes Monias 1928[b]	4	NCPPB 1970
P. amygdali Psallidas and Panagopoulos 1975	5	NCPPB 2607^T, NCPPB 2608, NCPPB 2610
P. andropogonis (Smith 1911) Stapp 1928 (*P. stizolobii* (Wolf) Stapp 1935, *P. woodsii* (Smith 1911) Stevens 1925)	11	NCPPB 450, NCPPB 934^T, NCPPB 968, NCPPB 1024, NCPPB 1130, NCPPB 2157, NCPPB 2179, NCPPB 2386, NCPPB 2868
P. asplenii (Ark and Tompkins 1946) Savulescu 1947	2	NCPPB 959, NCPPB 1947^T
P. aureofaciens Kluyver 1956 (*P. fluorescens* biotype E Migula 1895)	3	NCPPB 1800, NCPPB 1801
P. avenae Manns 1909 (*P. alboprecipitans* Rosen 1922 *P. setariae* (Okabe 1934) Savulescu 1947)	15	NCPPB 1011^T, NCPPB 1392, NCPPB 2398, NCPPB 2399, NCPPB 2400, NCPPB 2401, NCPPB 2402, NCPPB 2403, NCPPB 3354, NCPPB 3355, NCPPB 3357
P. betle (Ragunathan 1928) Savulescu 1947	3	NCPPB 323^T
P. caricapapayae Robbs 1956	4	NCPPB 1872, NCPPB 1873^T, NCPPB 3439
P. caryophylli (Burkholder 1942) Starr and Burkholder 1942	5	NCPPB 349, NCPPB 353, NCPPB 609, NCPPB 2151^T
P. cattleyae (Pavarino 1911) Savulescu 1947	7	NCPPB 961^T, NCPPB 1874
P. cepacia (ex Burkholder 1950) Palleroni and Holmes 1981	13	NCPPB 945, NCPPB 946, NCPPB 1962, NCPPB 2993^T, NCPPB 3025, NCPPB 3480, A3228a, A3328b
P. chlororaphis (Guignard and Sauvageau 1894) Bergey et al. 1930 (*P. fluorescens* biotype D Migula 1895)	8	NCPPB 1598, NCPPB 1798, NCPPB 1799, NCPPB 2466
P. cichorii (Swingle 1925) Stapp 1928	14	NCPPB 285, NCPPB 906, NCPPB 907, NCPPB 908, NCPPB 943^T, NCPPB 950, NCPPB 1022, NCPPB 1511, NCPPB 2379
P. cissicola (Takimoto 1939) Burkholder 1948	3	NCPPB 2982, ICMP 8561
P. corrugata (ex Scarlett et al. 1978) Roberts and Scarlett 1981	12	NCPPB 2445^T, NCPPB 2447, NCPPB 2448, NCPPB 2455, NCPPB 2456, A4227a, A4227b, A4379/98
P. ficuserectae Goto (1983)	3	ICMP 7848, ICMP 7849, ICMP 7850
P. flectens Johnson 1956	6	NCPPB 538, ICMP 127, ICMP 745
P. fluorescens Migula 1895 biovar 1 (*P. fluorescens* biotype A Migula 1895)[b]	7	NCPPB 1793, NCPPB 1964^T
P. fluorescens Migula 1895 biovar 2 (*P. fluorescens* biotype B Migula 1895)[c]	3	NCPPB 1795
P. fluorescens Migula 1895 biovar 3 (*P. fluorescens* biotype C Migula 1895)[b]	3	NCPPB 1796, NCPPB 1797
P. fluorescens Migula 1895 biovar 4 (*P. fluorescens* biotype F Migula 1895)[b]	2	NCPPB 1803
P. fluorescens Migula 1895 biovar 5 (*P. fluorescens* biotype G Migula 1895)[b]	2	NCPPB 1805
P. fuscovaginae Miyajima et al. 1983	6	NCPPB 3085^T, NCPPB 3598, NCPPB 3599, NCPPB 3600, NCPPB 3601, NCPPB 3602
"*P. gingeri*" Preece and Wong 1982	4	NCPPB 3146, IHRL PgSA, IHRL PgSB
P. gladioli pv. gladioli Severini 1913 (*P. marginata* (MuCulloch 1921) Stapp 1928)	12	NCPPB 644, NCPPB 1051, NCPPB 1887, NCPPB 1890, NCPPB 1891^T, NCPPB 3265, NCPPB 3266
P. gladioli pv. alliicola (Burkholder 1942) Young et al. 1978 (*P. alliicola* (Burkholder 1942) Stapp and Burkholder 1942)	44	NCPPB 947^T, NCPPB 2478, NCPPB 2940, NCPPB 2942, NCPPB 3307, NCPPB 3308, JT1159, JT1180D, JT1228H, JT122C, JT1870A, JT1898C, JT1899B, JT1900E, JT1900K, JT1960A, JT1963A, JT1970, JT1972, JT1974, JT2140D, JT2142A, JT2161A
P. glumae Kurita and Tabei 1967	7	NCPPB 2391, NCPPB 2981^T, A4111, ICMP 3728, ICMP 3729
P. hibiscicola Moniz 1963	2	NCPPB 1683^T
P. marginalis pv. marginalis (Brown 1918) Stevens 1925	20	NCPPB 667^T, NCPPB 679, NCPPB 808, NCPPB 1187, NCPPB 1307, NCPPB 1557, NCPPB 1558, NCPPB 1559, NCPPB 1603, NCPPB 1606, NCPPB 1676, NCPPB 1677, NCPPB 1678, NCPPB 1690, NCPPB 2380

Continued on following page

TABLE 1—*Continued*

Bacterium (synonym[s])	No. of GC runs	Strain(s)[a]
P. marginalis pv. alfalfae Shinde and Lukezic 1974	3	NCPPB 2644^T, NCPPB 2645, NCPPB 2646
P. marginalis pv. pastinacae (Burkholder 1960) Young et al. 1978	6	NCPPB 804, NCPPB 805, NCPPB 806^T, NCPPB 807, NCPPB 949, NCPPB 951
P. meliae Ogimi 1981	3	NCPPB 3033^T
P. plantarii Azegami et al. 1987	5	NCPPB 3590, A4109, ICMP 9425, ICMP 9426, ICMP 9429
P. pseudoalcaligenes subsp. *pseudoalcaligenes* Stanier 1966[b]	2	NCPPB 1959, NCPPB 1971^T
P. pseudoalcaligenes subsp. *citrulli* Schaad et al. 1978	6	NCPPB 3055, NCPPB 3244, ICMP 6522, ICMP 7500^T, ICMP 7713
P. pseudoalcaligenes subsp. *konjaci* Goto 1983	3	ICMP 7733^T, ICMP 7734, ICMP 7851
P. putida biovar A (Trevisan 1889) Migula 1895[b]	5	NCPPB 244, NCPPB 1806, NCPPB 1807, IHRL 201
P. putida biovar B (Trevisan 1889) Migula 1895[b]	3	NCPPB 1809
"*P. reactans*" Preece and Wong 1982	3	NCPPB 2946, NCPPB 2947, IHRL 14
P. rubrilineans (Lee et al. 1925) Stapp 1928	16	NCPPB 359, NCPPB 360, NCPPB 522, NCPPB 920^T, NCPPB 921, NCPPB 931, NCPPB 2890, NCPPB 2891, NCPPB 3029, NCPPB 3107, NCPPB 3108, NCPPB 3111, NCPPB 3112, NCPPB 3113, NCPPB 3234
P. rubrisubalbicans (Christopher and Edgerton 1930) Krassilnikov 1949	6	NCPPB 932, NCPPB 1026, NCPPB 1027^T, NCPPB 1673, NCPPB 2994
P. solanacearum (Smith 1896) Smith 1914	49	NCPPB 325^T, NCPPB 339, NCPPB 500, NCPPB 613, NCPPB 616, NCPPB 643, NCPPB 789, NCPPB 790, NCPPB 909, NCPPB 1019, NCPPB 1225, NCPPB 1323, NCPPB 1331, NCPPB 1400, NCPPB 1483, NCPPB 1489, NCPPB 1579, NCPPB 1702, NCPPB 2088, NCPPB 2199, NCPPB 2201, NCPPB 2203, NCPPB 2314, NCPPB 2315, NCPPB 2797, NCPPB 2937, NCPPB 3190, NCPPB 3205, NCPPB 3362, N30, N40, N455, A3918, A3920, A3926, A3927, A3929, A3930, A3932, A4141, A4198, A4209
P. stutzeri (Lehmann and Neumann 1896) Sijderius 1946[b]	2	NCPPB 1972, NCPPB 1973^T
P. syringae pv. syringae van Hall 1902	19	NCPPB 93, NCPPB 281^T, NCPPB 310, NCPPB 524, NCPPB 1070, NCPPB 1071, NCPPB 1072, NCPPB 1073, NCPPB 1087, NCPPB 1088, NCPPB 1091, NCPPB 1282, NCPPB 2843, NCPPB 2844
P. syringae pv. phaseolicola (Burkholder 1926) Young et al. 1978 (*P. phaseolicola* (Burkholder 1926) Dowson 1943)	19	NCPPB 52^T, NCPPB 380, NCPPB 604, NCPPB 1057, NCPPB 1098, NCPPB 1104, NCPPB 1321, NCPPB 1372, NCPPB 2571, NCPPB 3523, NCPPB 3524
P. syringae pv. tomato (Okabe 1933) Young et al. 1978 (*P. tomato* (Okabe 1933) Alstaff 1944)	8	NCPPB 1008, NCPPB 1106^T, NCPPB 1107, NCPPB 1367, NCPPB 1369
P. syzygii Roberts et al. 1990	5	S107X, T326X, T328X, T329X, T394X
P. tolaasii Pain 1919	21	NCPPB 387, NCPPB 741, NCPPB 1116, NCPPB 1311, NCPPB 1957, NCPPB 2192^T, NCPPB 2193, NCPPB 2194, NCPPB 2325, NCPPB 2412, NCPPB 2413, NCPPB 3148
P. viridiflava Burkholder (1930) Dowson 1939	8	NCPPB 389, NCPPB 635^T, NCPPB 636, NCPPB 1248, NCPPB 1810, NCPPB 2012, NCPPB 2502
S. paucimobilis (Holmes et al. 1977) Yabuuchi et al. 1991 (*P. paucimobilis* Holmes et al. 1977)[b]	6	NCPPB 2439, A4082/2, A4083/1, A4083/2
X. campestris pv. campestris (Pammel 1895) Dowson 1939	6	NCPPB 403, NCPPB 528^T, NCPPB 1043, NCPPB 1129, NCPPB 1144, NCPPB 1645
X. maltophilia (Hugh 1981) Swings et al. 1983 (*P. maltophilia* Hugh 1981)	10	NCPPB 1974, NCPPB 2923, IHRL MC55, H34bc, H7C, A4138
Banana blood disease bacterium	5	T334X, T379X, T389X, T391X, T394X

[a] All strains were obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom (NCPPB), except those with the following prefixes: A, diagnostic specimens from the Central Science Laboratory, Harpenden, United Kingdom; H, I. Harris, Royal Holloway and New College, London, United Kingdom; ICMP, International Collection of Microorganisms from Plants, Auckland, New Zealand; IHRL, Horticulture Research International, Littlehampton, United Kingdom; JT, J. Taylor, Horticulture Research International, Wellesbourne, United Kingdom; N, J. Neto, Instituto Biologico, Campinas, Brazil; S, T, and U, S. Eden-Green, Institute of Arable Crop Research, Rothamsted, Harpenden, Hertfordshire, United Kingdom.

[b] Nonpathogens.

[c] Opportunistic pathogens.

sucrose, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of K_2HPO_4, 0.25 g of ferric ammonium citrate, 15 g of agar [Difco], 1 liter of distilled water) for 6 days at 28°C. Banana blood disease bacterium strains were grown on Trypticase soy agar at 28°C for 48 ± 2 h.

Preparation of fatty acid profiles. Cells were harvested from confluent growth in the second and third quadrants of one or more steak plates by using a small sterile aluminum spatula. Portions (approximately 50 mg, fresh weight) of cells were transferred to Teflon-capped test tubes. Cellular

TABLE 2. Fatty acids found in *Pseudomonas* spp.

Shorthand name	Equivalent chain length	Systematic name	Trivial name
Saturated acids			
10:0	10.000	Decanoic acid	Capric acid
12:0	12.000	Dodecanoic acid	Lauric acid
14:0	14.000	Tetradecanoic acid	Myristic acid
15:0	15.000	Pentadecanoic acid	
16:0	16.000	Hexadecanoic acid	Palmitic acid
17:0	17.000	Heptadecanoic acid	Margaric acid
18:0	18.000	Octadecanoic acid	Stearic acid
Unsaturated acids			
15:1 B	14.856	Pentadecenoic acid isomer B	
16:1 B	15.774	Hexadecenoic acid isomer B	
16:1 C	15.908	Hexadecenoic acid isomer C	
16:1 *cis* 9	15.817	*cis*-9-Hexadecenoic acid	Palmitoleic acid
16:1 *trans* 9	15.856	*trans*-9-Hexadecenoic acid	Palmitelaidic acid
17:1 B	16.792	Heptadecenoic acid isomer B	
17:1 C	16.862	Heptadecenoic acid isomer C	
18:1 *cis* 9	17.769	*cis*-9-Octadecenoic acid	Oleic acid
18:1 *cis* 11	17.822	*cis*-11-Octadecenoic acid	*cis*-Vaccenic acid
18:1 B	17.919	Octadecenoic acid isomer B	
Branched acids			
11:0 iso	10.605	9-Methyl decanoic acid	
13:0 iso	12.612	11-Methyl dodecanoic acid	
14:0 iso	13.618	12-Methyl tridecanoic acid	Isomyristic acid
15:1 iso F	14.414	13-Methyl tetradecenoic acid isomer F	
15:0 iso	14.621	13-Methyl tetradecanoic acid	
15:0 anteiso	14.711	12-Methyl tetradecanoic acid	
16:0 iso	15.626	14-Methyl pentadecanoic acid	Isopalmitic acid
17:1 iso F	16.416	15-Methyl hexadecenoic acid isomer F	
17:0 iso	16.629	15-Methyl hexadecanoic acid	Isomargaric acid
17:0 anteiso	16.722	14-Methyl hexadecanoic acid	Anteisomargaric acid
Cyclopropane acids			
17:0 cyclo	16.888	*cis*-9,10-Methylene hexadecanoic acid	
19:0 cyclo 9-10	18.860	*cis*-9,10-Methylene octadecanoic acid	Dihydrosterulic acid
19:0 cyclo 11-12	18.900	*cis*-11,12-Methylene octadecanoic acid	Lactobacillic acid
Hydroxy acids			
10:0 3-OH	11.423	3-Hydroxydecanoic acid	3-Hydroxycapric acid
11:0 iso 3-OH	12.090	3-Hydroxy-9-methyl decanoic acid	
12:0 2-OH	13.178	2-Hydroxydodecanoic acid	2-Hydroxylauric acid
12:1 3-OH	13.289	3-Hydroxydodecenoic acid	
12:0 3-OH	13.455	3-Hydroxydodecanoic acid	3-Hydroxylauric acid
13:0 iso 3-OH	14.110	3-Hydroxy-11-methyl dodecanoic acid	
13:0 2-OH	14.191	2-Hydroxytridecanoic acid	
14:0 2-OH	15.205	2-Hydroxytetradecanoic acid	2-Hydroxymyristic acid
14:0 3-OH	15.490	3-Hydroxytetradecanoic acid	3-Hydroxymyristic acid
15:0 2-OH	16.217	2-Hydroxypentadecanoic acid	
16:1 2-OH	17.047	2-Hydroxyhexadecenoic acid	
16:0 2-OH	17.235	2-Hydroxyhexadecanoic acid	2-Hydroxypalmitic acid
16:0 3-OH	15.520	3-Hydroxyhexadecanoic acid	2-Hydroxypalmitic acid
18:1 2-OH	19.088	2-Hydroxyoctadecenoic acid	
Unknown fatty acids			
UNK 9.521	9.521		
UNK 11.798	11.798		
UNK 13.961	13.961		
UNK 14.503	14.503		
UNK 19.368	19.368		

lipids were saponified, and the fatty acids were methylated. Fatty acid methyl esters (FAMEs) were extracted and purified. The methods which I used have been described elsewhere (26–28). Profiles were obtained after gas chromatography (GC) (Hewlett-Packard model 5890 gas chromatograph) through a 25-m Hewlett-Packard fused silica capillary column lined with methyl phenyl silicone by using split injection (1 μl) and hydrogen as the carrier gas (55 ml/min). The injector temperature was 250°C. The column temperature was increased from 170 to 270°C at a rate of 5°C/min. Detection was by flame ionization. Peaks were integrated by using a Hewlett-Packard model 3392A integrator. Individual FAMEs were quantified and identified by using the peak-naming table component of the Microbial Identification System software package (Microbial ID, Inc., Newark, Del.). Quantities were expressed as percentages of the total named FAME peak area. Identification of FAMEs was based on calculation of equivalent chain lengths.

TABLE 3. Fatty acid profiles for *P. syringae* and its close relatives (subgroup 1a)

Fatty acid	% in profile (SD)							
	P. syringae pv. syringae	*P. syringae* pv. tomato	*P. syringae* pv. phaseolicola	*P. caricapapayae*	*P. cichorii*	*P. ficuserectae*	*P. meliae*	*P. viridiflava*
10:0	tr[a]	tr	tr	tr	0.1 (0.1)		tr	tr
10:0 3-OH	2.4 (0.3)	3.0 (0.4)	3.6 (1.4)	2.6 (0.3)	2.6 (0.3)	2.1 (1.8)	3.0 (0.2)	2.3 (0.3)
12:0	4.8 (0.3)	4.7 (0.3)	4.2 (1.4)	4.6 (0.3)	4.8 (0.2)	4.3 (0.2)	4.7 (0.4)	5.1 (0.2)
12:0 2-OH	2.9 (0.2)	2.6 (0.1)	2.5 (0.8)	2.7 (0.1)	2.6 (0.2)	3.0 (0.2)	2.9 (0.3)	2.4 (0.2)
12:1 3-OH					0.2 (0.4)			
12:0 3-OH	4.1 (0.3)	4.0 (0.2)	3.6 (1.2)	3.0 (1.1)	4.1 (0.3)	3.7 (0.3)	4.1 (0.7)	4.0 (0.1)
14:0	0.2 (0.1)	0.2 (0.1)	0.6 (1.1)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.3 (0.1)	tr
15:0	tr		tr					
14:0 3-OH			0.7 (2.2)					
16:1 *cis* 9	38.6 (1.6)	40.5 (1.8)	34.9 (2.1)	35.6 (2.4)	36.0 (2.1)	36.4 (0.1)	34.3 (1.1)	37.2 (0.8)
16:0	26.4 (0.15)	26.0 (1.4)	26.0 (1.8)	24.8 (1.5)	26.1 (1.6)	24.4 (1.2)	30.8 (0.1)	26.1 (1.3)
16:0 3-OH			0.7 (0.7)	tr			0.4 (0.1)	
17:0 iso	0.2 (0.2)	tr	0.3 (0.2)	0.2 (0.1)				0.2 (0.2)
17:0 cyclo	0.3 (0.6)		2.0 (1.8)	1.8 (1.9)	tr	1.3 (0.2)	0.7 (0.1)	0.4 (0.3)
17:0	tr		tr	0.2 (0.1)				
18:1 *cis* 11	18.4 (1.5)	17.8 (1.1)	19.0 (2.1)	22.9 (1.8)	21.5 (1.4)	23.6 (0.4)	17.7 (1.0)	20.8 (1.6)
18:0	1.2 (0.8)	0.9 (0.2)	0.4 (0.1)	1.0 (0.1)	1.2 (0.2)	1.1 (0.1)	0.9 (0.1)	1.3 (0.1)
19:0 cyclo 9-10	0.2 (0.2)		0.2 (0.2)	0.2 (0.2)	0.2 (0.1)		tr	tr
19:0 cyclo 11-12		tr	tr					

[a] tr, trace amounts are present in some strains.

For each taxon, duplicate fatty acid profiles of at least one strain were prepared. All profiles for each taxon were pooled, and the mean and standard deviation for each FAME were determined. Groupings were based on differences in the types and amounts of FAMEs present. FAMEs are referred to below as the parent fatty acids.

RESULTS

For each taxon, the mean percentages for all of the major FAMEs (mean percentage, >0.2%) were used for further analysis. Minor FAMEs, which were present at lower values or which were found only in occasional strains of a taxon, were omitted. The reproducibility of profiles was good. Standard deviations were generally lower for taxa when multiple runs of a single strain were examined than when single runs of several strains were examined. Most strains of a given taxon had profiles which were qualitatively and quantitatively similar. Variation between strains was greatest for the cyclopropane acids. The percentages of these acids tended to increase with the age of the culture (26).

In many cases fatty acids which accounted for less than 1% of the total named peak area were consistently found in all strains of a particular taxon, further illustrating the reproducibility and specificity of the profiles. However, fatty acids present at levels less than 0.2% were often not detected in some repeat runs or in runs of other strains, usually as a result of reduced total peak area caused by use of a lower fresh weight of cells.

For all gas chromatographic runs included in this analysis, more than 85% of the total peak area consisted of peaks that were named. For most runs the value was 98 to 100%. A total of 45 fatty acids were identified and used in this analysis; an additional 5 fatty acids were present in substantial quantities (>0.2%) in some strains. An equivalent chain length was calculated for each unidentified fatty acid, and the resulting values were used as interim names (for example, the unknown with an equivalent chain length of 14.503 was designated UNK 14.503). Mass spectra of some of these fatty acids were prepared, and the results are discussed below.

The 50 fatty acids which I identified or characterized are classified and listed along with their systematic and trivial names (where known) in Table 2. Shorthand chemical symbols (e.g., 10:0 3-OH) are used below.

All of the strains contained 16:0, 16:1 *cis* 9, and 18:1 *cis* 11, and the FAMEs of these acids were often the most abundant FAMEs. Most strains also contained 14:0. Six discrete groups of strains were identified on the basis of the types of hydroxy acids found (referred to as the core hydroxy fatty acids), although these fatty acids usually accounted for less than 10% of the total peak area in a profile. These hydroxy fatty acids were of three types, 2-hydroxy, 3-hydroxy, and iso-branched 3-hydroxy. Several of the groups were further subdivided on the basis of additional hydroxy acids (referred to as minor hydroxy fatty acids). Each subgroup contained one or more taxa. A few taxa contained strains that belonged to more than one group or subgroup. The profiles for groups 1 through 6 are shown in Tables 3 through 8. Most taxa exhibited great uniformity in the profiles of their strains. The few taxa which exhibited great variation are described below. Most taxa at and above subspecific rank could be differentiated on the basis of quantitative differences in one or more fatty acids. There were few qualitative differences among the profiles of taxa included in the same subgroup.

Group 1. All members of group 1 contained the hydroxy acids 10:0 3-OH and 12:0 3-OH, as well as 12:0, 16:1 *cis* 9, 16:0, and 18:1 *cis* 11. Most also contained 14:0, 17:0 cyclo, and 18:0. The compositions of subgroups were determined on the basis of the presence of other hydroxy acids and in some cases on the basis of the relative amounts of these other hydroxy acids. Six subgroups were differentiated. Strains belonging to subgroup 1a, 1b, 1c, 1e, and 1f profiles all contained 10:0 3-OH, 12:0 2-OH, and 12:0 3-OH. Differentiation was based primarily on the relative amounts of 10:0 3-OH. Subgroup 1a contained 27 taxa, whereas subgroups 1b, 1c, 1e, and 1f each contained a single taxon. Subgroup 1b profiles also contained 14:0 3-OH and 16:0 3-OH, whereas

TABLE 4. Fatty acid profiles for *P. fluorescens* and its close relatives (subgroup 1a)

Fatty acid	% in profile (SD)								
	P. fluorescens biovar 1	*P. fluorescens* biovar 2	*P. fluorescens* biovar 3	*P. fluorescens* biovar 4	*P. fluorescens* biovar 5	*P. aeruginosa*	*P. agarici*	*P. asplenii*	*P. aureofaciens*
10:0	tr[a]	tr	tr	0.5 (0.2)	tr	tr			0.2 (0.1)
10.0 3-OH	3.1 (0.2)	3.0 (0.4)	3.6 (0.6)	5.2 (1.0)	3.7 (0.6)	2.9 (0.6)	3.0 (0.5)	3.1 (0.1)	4.7 (0.5)
12:0	2.4 (0.3)	2.9 (0.4)	1.8 (0.6)	6.6 (0.2)	2.7 (0.1)	3.1 (0.4)	2.0 (0.5)	1.1 (0.1)	1.6 (0.2)
12:0 2-OH	4.7 (0.4)	3.8 (0.4)	5.1 (0.4)	2.2 (0.2)	4.7 (0.3)	4.1 (0.4)	5.1 (0.5)	5.5 (0.1)	5.6 (0.2)
12:1 3-OH				1.9 (0.8)		tr			0.6 (0.2)
12:0 3-OH	4.2 (0.3)	3.9 (0.1)	4.0 (0.2)	4.2 (0.7)	4.2 (0.1)	4.1 (0.3)	4.3 (0.4)	4.3 (0.3)	4.9 (0.1)
14:0	0.4 (0.1)	0.4 (0.1)	0.4 (0)	0.3 (0)	0.3 (0)	0.8 (0.2)	0.9 (0.3)	0.3 (0.1)	0.5 (0.1)
15:0	0.4 (0.4)	0.1 (0.1)	0.3 (0)	0.2 (0)	0.2 (0)	0.4 (0)	0.4 (0.2)	tr	0.3 (0.1)
14:0 3-OH				0.2 (0.1)					0.4 (0.1)
16:1 *trans* 9						1.3 (2.0)	tr		
16:1 *cis* 9	28.8 (1.4)	27.5 (2.7)	33.7 (2.0)	29.3 (0.9)	29.1 (2.0)	14.2 (2.1)	27.7 (4.2)	34.9 (0.4)	22.9 (6.1)
16:0	29.8 (1.3)	32.1 (2.1)	33.1 (2.2)	26.4 (0.1)	30.3 (1.2)	25.0 (1.6)	36.1 (2.8)	29.8 (2.8)	29.5 (2.2)
17:0 iso	0.1 (0.1)		0.2 (0)	0.2 (0.1)	0.2 (0)			0.2 (0.1)	tr
17:0 cyclo	7.5 (1.3)	7.6 (1.8)	5.2 (0.8)	6.4 (0.9)	6.6 (0.6)	0.8 (0.7)	9.8 (3.4)	2.6 (1.5)	13.6 (5.5)
17:0	0.4 (0.5)	0.1 (0.1)	0.4 (0.1)	0.2 (0)	0.2 (0)	tr	0.2 (0.2)	0.2 (0.1)	0.2 (0.1)
18:1 *cis* 11	16.7 (1.4)	16.6 (1.0)	11.0 (1.3)	14.6 (2.0)	16.6 (0.7)	40.6 (2.2)	9.0 (2.6)	17.0 (3.8)	13.3 (1.1)
18:0	0.8 (0.2)	1.1 (0.2)	0.9 (0.4)	0.4 (0.1)	0.6 (0.2)	0.6 (0.1)	0.8 (0.3)	0.9 (0.1)	0.4 (0.1)
19:0 cyclo 11-12	0.3 (0.2)	0.8 (0.2)	0.1 (0.1)	0.3 (0.1)	0.4 (0.1)	1.2 (1.2)			0.7 (0.4)

[a] tr, trace amounts are present in some strains.

subgroup 1c profiles contained the 2-hydroxy analogs 14:0 2-OH and 16:0 2-OH. Subgroup 1d profiles contained only the two root hydroxy acids for group 1, 10:0 3-OH and 12:0 3-OH. The profiles of occasional strains or occasional runs of a particular strain contained trace amounts of other hydroxy acids. Subgroup 1e and 1f were differentiated from subgroup 1a by quantitative differences in the core hydroxy acids.

The nonhydroxy acids were often important in differentiating among taxa at specific and infraspecific levels, but they did not have the same apparent value in classifying strains at the generic level. Qualitative and quantitative differences in many of these acids were found.

(i) **Subgroup 1a.** Subgroup 1a contained *P. agarici*, *P. aeruginosa*, *P. asplenii*, *P. aureofaciens*, *P. caricapapayae*, *P. chlororaphis*, *P. cichorii*, *P. ficuserectae*, *P. fluorescens* biovar 1, *P. fluorescens* biovar 2, *P. fluorescens* biovar 3, *P. fluorescens* biovar 4, *P. fluorescens* biovar 5, *P. fuscovaginae*, "*P. gingeri*," *P. marginalis* pv. alfalfae, *P. marginalis* pv. marginalis, *P. marginalis* pv. pastinacae, *P. meliae*, *P. putida* biovar A, *P. putida* biovar B, "*P. reactans*," *P. syringae* pv. phaseolicola, *P. syringae* pv. syringae, *P. syringae* pv. tomato, *P. tolaasii*, and *P. viridiflava*. All of the fluorescent pseudomonads tested fell into this subgroup. Subgroup 1a is perhaps most closely related to subgroups 1e and 1f, whose profiles contained the same three hydroxy acids (10:0 3-OH, 12:0 2-OH, and 12:0 3-OH). Subgroup 1a was differentiated on the basis of having less than 5 to 6% 10:0 3-OH (Tables 3 and 4). The members of subgroups 1e and 1f contained significantly more of this acid.

(ii) **Subgroup 1b.** Subgroup 1b contained *P. corrugata*. This species was differentiated on the basis of having significant amounts of 12:1 3-OH, 14:0 3-OH, and 16:0 3-OH in addition to the three core hydroxy acids (10:0 3-OH, 12:0 2-OH, and 12:0 3-OH) (Table 5). All of the strains also contained two other acids which were not named (equivalent chain lengths of 13.961 and 14.503); each of these acids was present at a level of 0.5 to 2.0%. No other group 1 strain contained significant amounts of these acids. Mass spectra of the compounds with equivalent chain lengths of 13.961 and 14.503 indicated that they were branched hydroxy acids containing 13 and 14 carbon atoms, respectively; for both, the hydroxy groups were not in the 2 or 3 position.

(iii) **Subgroup 1c.** Subgroup 1c contained *P. rubrisubalbicans*. This species was differentiated on the basis of having significant amounts of 14:0 2-OH and smaller amounts of 16:0 2-OH (Table 5). All of the other acids were qualitatively typical of subgroup 1a, although the quantities of 10:0 3-OH and 12:0 2-OH were significantly lower than the quantities in subgroup 1a strains.

(iv) **Subgroup 1d.** Subgroup 1d contained *P. alcaligenes*, *P. pseudoalcaligenes* subsp. *pseudoalcaligenes*, and *P. stutzeri*. Subgroup 1d was characterized by the relative lack of 12:0 2-OH (Table 5), which was found only in trace amounts in some strains. This subgroup differed significantly from the other subgroups not only in the hydroxy acids but also in some of the other acids (notably, the presence in some strains of 16:1 *trans* 9 and the presence of significantly higher amounts of 12:0 and 18:1 *cis* 11 and significantly lower amounts of 16:0).

(v) **Subgroup 1e.** Subgroup 1e contained *P. amygdali*. The subgroup 1e profile was qualitatively similar to that of subgroup 1a in that except for a trace of 14:0 3-OH the only hydroxy acids were the core acids 10:0 3-OH, 12:0 2-OH, and 12:0 3-OH. The major difference was that the relative amounts of the hydroxy acids were much greater (26 to 32%, compared with less than 15% for subgroup 1a members). 10:0 3-OH was by far the most abundant acid in the whole profile (>20%). *P. amygdali* was the only bacterium in which a hydroxy acid was more abundant than 16:1 *cis* 9, 16:0, or 18:1 *cis* 11. Very few minor acids were present in the profile. *P. amygdali* was the only bacterium other than *P. syringae* and its close relatives (Table 3) that contained 19:0 cyclo 9-10. It was also unusual that it also contained 19:0 cyclo 11-12. Another unusual feature was the presence of an unknown fatty acid (UNK 19.368) in significant quantities (4.7%). This fatty acid occurred in trace amounts in some *P. gladioli* pv. alliicola strains (group 2) (data not shown).

(vi) **Subgroup 1f.** Subgroup 1f contained *P. cattleyae* NCPPB 1874. This strain formed a separate subgroup in

TABLE 4—*Continued*

% in profile (SD)									
P. chlororaphis	*P. fuscovaginae*	"*P. gingeri*"	*P. marginalis* pv. marginalis	*P. marginalis* pv. alfalfae	*P. marginalis* pv. pastinaceae	*P. putida* biovar A	*P. putida* biovar B	"*P. reactans*"	*P. tolaasii*
		tr	tr	tr	tr	0.4 (0.4)			tr
4.0 (0.2)	4.1 (0.4)	3.3 (0.7)	3.5 (0.4)	3.8 (0.3)	3.0 (0.3)	5.3 (1.1)	3.3 (0.6)	3.4 (1.1)	3.2 (0.6)
1.9 (0.8)	1.4 (0.4)	1.7 (0.8)	2.9 (0.5)	2.7 (0.8)	2.5 (0.1)	2.1 (0.5)	1.8 (0.7)	3.2 (1.0)	2.0 (0.5)
5.1 (0.6)	5.8 (0.2)	5.7 (1.6)	4.2 (0.3)	4.7 (0.4)	4.5 (0.2)	5.1 (1.1)	3.6 (3.1)	4.8 (1.2)	5.0 (0.4)
tr						0.7 (0.3)			
4.4 (0.3)	4.6 (0.5)	4.4 (0.6)	3.9 (0.5)	4.3 (0.1)	3.9 (0.3)	4.8 (0.5)	2.7 (2.3)	5.1 (1.3)	4.2 (0.4)
0.5 (0.1)	0.3 (0.2)	0.4 (0.2)	0.4 (0.3)	0.8 (0.5)	0.3 (0)	0.7 (0.4)	0.6 (0.2)	0.2 (0.2)	0.3 (0.1)
tr	0.1 (0.1)	tr	0.3 (0.2)	0.2 (0.2)	0.2 (0)	0.2 (0.2)	0.4 (0.1)	tr	0.2 (0.1)
						2.2 (3.0)			
32.1 (3.2)	36.2 (2.5)	25.8 (1.6)	31.1 (3.0)	35.3 (2.4)	31.2 (2.3)	24.1 (3.7)	26.2 (7.7)	29.4 (3.3)	28.8 (5.0)
31.2 (2.7)	27.9 (1.5)	31.1 (1.6)	30.7 (1.8)	32.9 (3.7)	31.1 (0.5)	28.9 (2.0)	31.5 (2.4)	30.0 (0.2)	29.4 (3.3)
	tr	tr	tr	tr	tr			tr	tr
4.9 (2.6)	1.9 (1.9)	10.1 (4.8)	6.2 (2.3)	4.6 (2.0)	6.7 (2.7)	5.9 (2.3)	13.9 (2.9)	7.5 (4.7)	8.1 (4.4)
tr	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.2 (0)	tr	0.2 (0.1)	tr	0.3 (0.2)
14.1 (2.2)	16.2 (2.4)	12.8 (4.4)	15.4 (2.3)	10.0 (5.3)	15.2 (1.2)	18.5 (1.1)	15.4 (1.9)	15.1 (0.3)	16.7 (2.8)
0.6 (0.4)	0.7 (0.1)	0.7 (0.1)	0.7 (0.2)	0.5 (0.4)	0.7 (0.1)	0.8 (0.3)	0.4 (0.2)	0.9 (0.2)	0.8 (0.3)
tr		0.5 (0.5)	0.2 (0.2)	tr	0.3 (0.2)	tr		0.6 (0.6)	0.5 (0.4)

group 1, intermediate between subgroups 1a and 1e in that the major hydroxy acid was 10:0 3-OH (ca. 10%) (Table 5). The profile also contained significant amounts of 12:1 3-OH, as well as a wide range of minor acids.

Group 2. Group 2 was readily differentiated from the other groups on the basis of hydroxy fatty acid composition. The core hydroxy fatty acids of group 1, 10:0 3-OH, 12:0 2-OH, and 12:0 3-OH, were not represented apart from an occasional trace of 10:0 3-OH (Table 6) except in two strains of *P. glumae*. In many profiles the smallest acid was 14:0. For this

TABLE 5. Fatty acid profiles for *Pseudomonas* subgroups 1b through 1f

Fatty acid	% in profile (SD)						
	Fatty acid subgroup 1b: *P. corrugata*	Fatty acid subgroup 1c: *P. rubrisubalbicans*	Fatty acid subgroup 1d			Fatty acid subgroup 1e: *P. amygdali*	Fatty acid subgroup 1f: *P. cattleyae* NCPPB 1874
			P. alcaligenes	*P. pseudoalcaligenes* subsp. *pseudoalcaligenes*	*P. stutzeri*		
10:0	0.5 (0.4)	0.3 (0.1)	2.3 (0.1)	tr[a]	0.2 (0.1)	tr	1.2 (1.6)
10:0 3-OH	4.6 (0.6)	3.3 (0.1)	3.7 (0.2)	3.0 (0.2)	2.9 (0.1)	14.3 (4.5)	10.1 (0.9)
12:0	3.6 (0.3)	3.6 (0.1)	5.2 (0.1)	7.9 (0.3)	8.3 (0.3)	5.1 (0.4)	1.3 (0.1)
12:0 2-OH	3.8 (0.2)	0.4 (0.1)	tr		tr	2.0 (0.6)	4.9 (0.4)
12:1 3-OH	1.1 (0.5)					tr	1.3 (0.1)
12:0 3-OH	4.4 (0.4)	2.9 (0.3)	3.2 (0.3)	3.8 (0.4)	2.6 (0.3)	4.4 (1.0)	4.0 (0.3)
UNK 13.961	0.8 (0.4)						
14:0	tr	0.5 (0.1)	3.6 (0.1)	0.3 (0)	0.8 (0.4)	0.2 (0.1)	0.4 (0)
UNK 14.503	0.5 (0.3)						
15:0	tr		2.0 (0.2)	0.7 (0.2)			0.2 (0)
14:0 2-OH		2.1 (0.6)					
14:0 3-OH	1.0 (0.3)					0.2 (0.1)	tr
16:1 *cis* 9	29.1 (3.7)	36.5 (2.2)	19.8 (1.4)	19.0 (2.9)	26.6 (1.8)	14.1 (6.4)	20.4 (0.1)
16:1 *trans* 9			6.8 (1.5)	5.0 (0.7)			1.1 (1.4)
16:0	26.4 (1.6)	25.1 (2.2)	11.9 (0.2)	13.1 (1.1)	17.3 (2.7)	17.2 (4.3)	26.7 (0.5)
16:1 2-OH		0.3 (0.2)					
16:0 3-OH	0.5 (0.3)					7.9 (2.6)	
17:0 iso				1.0 (0.2)	0.5 (0.1)		
17:0 cyclo	5.0 (2.6)	2.3 (1.2)		1.6 (0.3)	0.5 (0.1)	2.6 (0.6)	10.6 (3.5)
17:1 B			1.3 (0.1)	0.8 (0.4)			
17:1 C			1.1 (0.2)				
17:0			0.3 (0)	0.6 (0.3)			tr
18:1 *cis* 11	17.1 (1.4)	22.4 (2.9)	35.6 (1.1)	41.6 (2.7)	38.4 (0.5)	22.7 (2.8)	16.2 (0.4)
18:0	0.6 (0.2)	1.1 (0.1)	0.2 (0.1)	0.4 (0)	0.4 (0.1)	0.5 (0.1)	0.5 (0)
19:0 cyclo 9-10						0.4 (0.3)	
19:0 cyclo 11-12	0.5 (0.4)			0.3 (0.5)	0.9 (0)	0.2 (0.2)	0.2 (0.1)
UNK 19.368						4.7 (2.4)	

[a] tr, trace amounts are present in some strains.

TABLE 6. Fatty acid profiles for *Pseudomonas* group 2

Fatty acid	Mean % in profile (SD)										
	Fatty acid subgroup 2a							Fatty acid subgroup 2b: *P. glumae* GC subgroup B	Fatty acid subgroup 2c		
	P. andropogonis	*P. caryophylli*	*P. cepacia*	*P. gladioli* pv. gladioli	*P. gladioli* pv. alliicola	*P. glumae* GC subgroup A	*P. plantarii*		*P. solanacearum*	*P. syzygii*	Banana blood disease bacterium
10:0				tr[a]	0.2 (0.8)			0.9 (0.1)			
10:0 3-OH	tr			tr	0.9 (1.0)			1.8 (0.4)			
12:0 3-OH								3.2 (0.1)	4.5 (0.3)	3.0 (0.6)	4.0 (0.1)
14:0	4.1 (0.2)	3.6 (1.8)	3.9 (0.3)	4.0 (0.3)	4.0 (0.2)	3.7 (0.3)	4.4 (0.1)	3.2 (0.5)			
UNK 14.503								18.6 (1.0)	7.2 (0.5)	6.9 (1.7)	9.6 (0.5)
14:0 3-OH	3.5 (0.6)	4.6 (0.3)	4.8 (0.2)	4.2 (0.4)	4.6 (0.4)	5.1 (0.8)	5.1 (0.5)		0.2 (0.2)	0.3 (0.4)	
15:0			tr		tr				28.8 (1.5)	25.9 (6.7)	27.5 (1.8)
16:1 *cis* 9	17.2 (1.7)	14.1 (0.9)	14.9 (5.8)	11.4 (3.2)	8.4 (4.7)	15.5 (3.4)	5.0 (1.8)	6.2 (1.9)	25.1 (1.3)	19.2 (5.8)	24.7 (1.5)
16:0	15.6 (1.6)	21.7 (1.8)	22.4 (4.6)	23.1 (2.3)	24.0 (1.9)	19.9 (1.1)	24.3 (1.6)	16.4 (0.6)	4.5 (1.1)	5.7 (3.0)	4.0 (1.1)
17:0 cyclo	3.8 (2.4)	2.4 (0.5)	9.1 (4.6)	13.0 (3.3)	14.6 (3.8)	3.0 (2.6)	21.6 (3.6)	8.7 (1.3)	0.2 (0.3)	0.4 (0.5)	
17:0		tr	tr	0.3 (0.2)	0.2 (0.2)				3.6 (1.0)	0.4 (0.6)	1.8 (0.3)
16:1 2-OH	2.6 (0.6)	1.4 (0.3)	1.1 (0.3)	1.2 (0.2)	1.4 (0.2)	1.6 (0.3)	0.7 (0.2)	1.0 (0.2)	0.7 (0.2)	0.3 (0.2)	0.7 (0.1)
16:0 2-OH	4.1 (0.7)	1.4 (0.3)	1.0 (0.3)	1.1 (0.2)	1.4 (0.4)	1.1 (0.3)	0.4 (0.3)	0.9 (0.3)			
16:0 3-OH	3.7 (1.0)	3.9 (1.1)	4.5 (0.2)	4.0 (0.8)	4.3 (0.8)	3.9 (1.1)	5.3 (0.2)	3.7 (1.1)			
18:1 *cis* 11	32.1 (6.7)	41.8 (1.9)	28.6 (6.5)	25.0 (4.8)	20.0 (7.0)	38.7 (2.9)	18.0 (5.0)	24.1 (1.9)	19.9 (1.6)	23.4 (7.5)	23.7 (1.6)
18:0	0.3 (0.2)	1.1 (0.1)	1.1 (0.4)	1.1 (0.2)	1.0 (0.1)	0.8 (0.1)	1.2 (0.2)	0.8 (0.1)	0.5 (0.3)	0.6 (0.2)	0.3 (0.2)
19:0 cyclo 11-12	10.6 (5.6)	1.9 (0.3)	5.6 (5.3)	8.5 (2.5)	12.1 (5.8)	2.4 (1.5)	11.9 (2.5)	5.9 (2.0)	0.2 (0.2)	0.8 (0.6)	tr
18:1 2-OH	1.1 (0.4)	1.6 (0.3)	2.3 (0.9)	1.8 (0.4)	2.0 (0.5)	3.5 (1.4)	0.9 (2.0)	4.3 (0.3)	4.6 (0.5)	2.8 (1.7)	3.5 (0.8)

[a] tr, trace amounts are present in some strains.

group the core hydroxy acids were 14:0 3-OH, 16:1 2-OH, and 18:1 2-OH. In addition, most strains contained 16:0 2-OH, and all strains except strains of *P. solanacearum* and its close relatives also contained 16:0 3-OH.

Ten taxa were included in group 2. Three subgroups were differentiated. *P. glumae* strains produced two different profiles, both belonging to group 2.

The nonhydroxy acids were similar to those found in group 1 profiles. The major differences were the absence of 12:0 and relatively greater amounts of 14:0 and 19:0 cyclo 11-12.

(i) **Subgroup 2a.** Subgroup 2a contained *P. andropogonis*, *P. caryophylli*, *P. cepacia*, *P. gladioli* pv. gladioli, *P. gladioli* pv. alliicola, *P. plantarii*, and some strains of *P. glumae*, including strain NCPPB 2981^T (T = type strain). An unusual feature of subgroup 2a was the variation in the amounts of several fatty acids (notably, 16:1 *cis* 9, 18:1 *cis* 11, 17:0 cyclo, and 19:0 cyclo 11-12). All of the taxa except *P. caryophylli* showed this variation. The variation was greatest for *P. gladioli* pv. alliicola. For example, in *P. gladioli* the values for 16:1 *cis* 9 ranged from 3.2 to 11.4%, the values for 17:0 cyclo ranged from 3.3 to 14.6%, and the values for 18:1 *cis* 11 ranged from 4.8 to 25.0%. The profiles of most of the taxa were qualitatively similar; the exceptions were some *P. gladioli* pv. alliicola strains, which contained 10:0 and 10:0 3-OH.

The *P. glumae* strains included in subgroup 2a were the type strain (strain NCPPB 2981) and strains ICMP 3728 and ICMP 3729. The profiles of these strains were quite similar to those of *P. cepacia*.

(ii) **Subgroup 2b.** Subgroup 2b contained *P. glumae* NCPPB 2391 and NCPPB 3591. The two strains in this subgroup were unusual in that they contained significant amounts of 10:0 3-OH and were unique in group 2 because they contained 12:0 3-OH. The quantities of 14:0 3-OH were much higher than the quantity of 14:0 3-OH for any other member of group 2. These strains also contained an unknown branched hydroxy acid (equivalent chain length, 14.503) at levels of 2 to 4%.

(iii) **Subgroup 2c.** Subgroup 2c contained *P. solanacearum*, *P. syzygii*, and the banana blood disease bacterium. This subgroup was readily differentiated by the absence of 16:0 3-OH. Some strains also lacked 16:0 2-OH. The 49 strains of *P. solanacearum* which were studied included strains belonging to all three races and biovars I, II, III, and IV. All of the strains in subgroup 2c had very similar profiles.

Group 3. Species in group 3 were differentiated by having only a single core hydroxy acid, 10:0 3-OH (Table 7), although some strains contained small amounts of other hydroxy acids, including 12:1 3-OH, 12:0 3-OH, 14:0 2-OH, 14:0 3-OH, 15:0 2-OH, 16:1 2-OH, and 16:0 2-OH. The nonhydroxy fatty acids were very similar to those of group 1, although the levels of 14:0 and 16:0 were usually higher in the group 3 strains.

Two subgroups were recognized, and they were differentiated by their hydroxy fatty acids. Subgroup 3b strain profiles contained several 2-hydroxy acids in addition to the core acid, 10:0 3-OH.

(i) **Subgroup 3a.** Subgroup 3a contained *C. acidovorans*, *P. avenae*, *P. rubrilineans*, *P. pseudoalcaligenes* subsp. *citrulli*, *P. pseudoalcaligenes* subsp. *konjaci*, and type strain NCPPB 961 of *P. cattleyae*. The major hydroxy acid was 10:0 3-OH. Small amounts of other 3-hydroxy acids occurred in most strains, but no 2-hydroxy acids were found.

P. pseudoalcaligenes subsp. *citrulli* and *P. pseudoalcaligenes* subsp. *konjaci* had a group 3 profile similar to that of

TABLE 7. Fatty acid profiles for *Pseudomonas* group 3 species, including *Comamonas* spp.

Fatty acid	Mean % in profile (SD)						
	Fatty acid subgroup 3a						Fatty acid subgroup 3b: *C. testosteroni*
	C. acidovorans	*P. avenae*	*P. cattleyae* NCPPB 961[T]	*P. pseudoalcaligenes* subsp. *citrulli*	*P. pseudoalcaligenes* subsp. *konjaci*	*P. rubrilineans*	
10:0		0.2 (0.2)	tr[a]		tr	0.2 (0.1)	tr
10:0 3-OH	2.8 (0.1)	3.1 (1.0)	3.7 (0.2)	3.4 (0.3)	3.2 (0.2)	3.3 (0.4)	3.5 (0.7)
12:0	2.6 (0.2)	2.5 (0.7)	2.3 (0.1)	2.4 (0.1)	2.7 (0.1)	2.3 (0.2)	2.3 (0.2)
12:1 3-OH			0.6 (0.1)	tr			
14:0	0.7 (0.2)	2.4 (0.8)	1.7 (0.1)	1.6 (0.2)	3.1 (0.1)	2.1 (0.2)	0.2 (0.1)
14:0 2-OH							0.3 (0.1)
14:0 3-OH		0.9 (1.8)				0.4 (0.5)	
15:1 B				1.5 (0.7)			
15:0	1.9 (1.8)	0.3 (0.4)	0.5 (0.1)	5.2 (1.8)	tr	0.3 (0.1)	0.8 (0.4)
15:0 2-OH							0.2 (0.1)
16:1 *cis* 9	36.4 (4.3)	41.4 (3.8)	40.6 (0.3)	42.1 (0.5)	43.9 (0.3)	43.2 (1.9)	32.5 (2.4)
16:0	32.0 (1.2)	31.9 (2.0)	34.9 (0.4)	33.1 (2.5)	28.3 (1.5)	32.2 (1.4)	29.1 (2.4)
17:0 cyclo	6.3 (3.6)	0.8 (1.1)	0.5 (0.2)			0.2 (0.2)	4.4 (1.9)
16:1 2-OH							1.2 (0.9)
16:0 2-OH							2.7 (0.2)
17:0	0.7 (0.9)	tr	0.2 (0.2)	1.3 (0.4)		tr	1.2 (0.7)
18:1 *cis* 11	16.0 (2.0)	15.5 (2.0)	14.4 (0.6)	9.1 (1.5)	18.3 (1.4)	15.0 (1.8)	19.9 (1.8)
18:0	tr	0.2 (0.1)	0.2 (0.2)			0.3 (0.1)	0.4 (0.1)
19:0 cyclo 11-12							0.7 (0.1)

[a] tr, trace amounts are present in some strains.

P. acidovorans (*Pseudomonas* section III, rRNA superfamily III), whereas *P. pseudoalcaligenes* subsp. *pseudoalcaligenes* had a group 1 profile (*Pseudomonas* section I, rRNA superfamily II).

(ii) Subgroup 3b. Subgroup 3b contained *C. testosteroni*. Only two strains, including the type strain, were available. These two strains produced very similar profiles, which included small amounts of several 2-hydroxy acids (14:0 2-OH, 16:1 2-OH, and 16:0 2-OH). One strain repeatedly produced small amounts of 15:0 2-OH.

Group 4. Group 4 contained *S. paucimobilis*. The only hydroxy acid present in group 4 was 14:0 2-OH (Table 8). The profile was relatively simple, primarily consisting of 16:1 *cis* 9, 16:0, 17:0 cyclo, and 18:1 *cis* 11, which was by far the dominant acid (>60%).

S. paucimobilis is common on plant surfaces but is not regarded as a plant pathogen.

Group 5. Group 5 contained *P. flectens*. The group 5 profile contained three hydroxy acids, 12:0 2-OH, 14:0 2-OH, and 14:0 3-OH (Table 8). The other major acids included 12:0, 14:0, 16:1 *cis* 9, 16:0, 17:0 cyclo, and 18:1 *cis* 11. The type strain of *P. flectens* (strain NCPPB 539) had a profile which contained many iso- and anteiso-branched acids. However, examination of the same strain obtained from the International Collection of Microorganisms from Plants (ICMP) (strain ICMP 745) showed that it had the same profile type as strains NCPPB 538 and ICMP 127.

Group 6. Group 6 contained *P. betle*, *P. cissicola*, *P. hibiscicola*, *P. maltophilia*, and *Xanthomonas campestris* pv. campestris. The group 6 profiles were very different from those of groups 1 through 5 and are shown in Table 8. The major feature that differentiated this group was the presence of large amounts of iso- and anteiso-branched acids in the profiles, in particular 15:0 iso and 15:0 anteiso. Many strains contained 10:0 3-OH and 12:0 3-OH, the two core acids of group 1, and all strains contained 11:0 iso 3-OH and 13:0 iso 3-OH. Trace amounts of several other hydroxy acids were also present. Although there were obvious differences in the profiles of some of these taxa, there were no obvious subgroupings based on quantitative differences in the hydroxy acids, although there were quantitative differences for *P. cissicola*.

DISCUSSION

In this study I demonstrated the taxonomic value of the 2- and 3-hydroxy and branched 3-hydroxy fatty acids for differentiating plant-pathogenic and other bacteria belonging to the genus *Pseudomonas*. Most of these acids were present at levels less than 5% of the total fatty acid peak area, and although three or four hydroxy acids were often present in a profile, the total was usually less than 10% of the total peak area. The single exception was *P. amygdali*, which contained more than 20% hydroxy fatty acids, the majority of which was 10:0 3-OH. The classification of the strains which I studied into six clearly defined groups is shown in Table 9. The nonhydroxy fatty acids, although much more abundant, were of value for differentiating species, but they were generally of less value for differentiating major groups (Tables 3 through 8). All of the strains included in this study contained 16:0, 16:1 *cis*, and 18:1 *cis* 11. These were often the most abundant fatty acids present in profiles and are known to be major inner membrane constituents of most if not all gram-negative bacteria (21). Nevertheless, quantitative differences in these and other nonhydroxy fatty acids were of value for differentiating between taxa within groups. All of the fluorescent pseudomonads fell into group 1, which had two core hydroxy acids (10:0 3-OH and 12:0 3-OH). In addition, all of the strains except the subgroup 1d strains contained 12:0 2-OH. Group 3 strains had the most similar profiles, differing primarily in the absence of 12:0 2-OH and 12:0 3-OH. Group 2 strains were completely different since all of the hydroxy acids present contained 14, 16, and 18 carbon atoms. Occasional strains contained additional minor hydroxy fatty acids. Group 4 strains contained only 14:0 2-OH, whereas group 5 strains also contained 12:0 2-OH and

TABLE 8. Fatty acid profiles for *Pseudomonas* group 4, 5, and 6 species and *X. campestris* pv. campestris

Fatty acid	Mean % in profile (SD)						
	Fatty acid group 4: *S. paucimobilis*	Fatty acid group 5: *P. flectens*	Fatty acid group 6				
			P. betle	*P. cissicola*	*P. hibiscicola*	*X. campestris* pv. campestris	*X. maltophilia*
UNK 9.521			0.5 (0.2)		0.2 (0.3)		tr[a]
10:0		0.1 (0.1)	0.9 (0.4)		0.5 (0)	1.0 (0.1)	0.6 (0.1)
11:0 iso			4.6 (2.1)	0.9 (0.2)	3.3 (0.1)	3.1 (0.2)	3.2 (0.2)
10:0 3-OH			tr	1.7 (0.4)	tr	0.2 (0.1)	tr
UNK 11.798			1.6 (0.6)	1.8 (0.3)	1.4 (0)	1.4 (0.1)	1.4 (0.1)
11:0 iso 3-OH			1.6 (0.1)	2.7 (0.4)	1.6 (0)	2.0 (0.2)	1.5 (0.1)
13:0 iso			0.7 (0.3)	0.2 (0.2)	0.5 (0.1)	tr	0.5 (0.1)
12:0 2-OH		1.1 (0.1)		tr			
12:0 3-OH			2.5 (0.1)	1.0 (0.1)	2.5 (0.1)	3.6 (0.6)	2.9 (0.4)
14:0 iso			1.0 (0.1)	tr	0.5 (0.1)	tr	1.4 (0.8)
UNK 13.961		0.8 (0.4)					
14:0	0.6 (0.2)	2.6 (0.2)	5.2 (0.2)	2.5 (0.2)	3.3 (0.4)	1.4 (0.2)	4.1 (0.8)
13:0 iso 3-OH			2.5 (0.1)	1.8 (0.2)	3.1 (0.2)	2.8 (0.2)	2.8 (0.7)
13:0 2-OH			0.2 (0.2)	tr	0.3 (0.1)	tr	0.4 (0.3)
15:1 iso F			1.3 (0.3)	0.4 (0.4)	1.1 (0.1)	0.3 (0.3)	1.1 (0.1)
UNK 14.503		0.8 (0.2)					
15:0 iso			35.1 (1.1)	32.3 (5.5)	39.2 (2.6)	21.2 (1.6)	33.9 (3.3)
15:0 anteiso			12.9 (0.6)	5.8 (0.8)	10.0 (2.1)	12.1 (0.3)	13.2 (3.2)
15:0	tr		0.5 (0.1)	1.0 (0.2)	0.4 (0)	1.2 (0.2)	0.8 (0.4)
14:0 2-OH	7.7 (1.3)	3.5 (0.3)					
14:0 3-OH		8.8 (1.4)					
16:0 iso			1.1 (0.2)	0.8 (0.2)	0.9 (0.3)	1.9 (0.4)	1.6 (0.6)
16:1 B			3.4 (0.1)	2.0 (0.5)	3.1 (0.1)	2.8 (0.4)	3.3 (0.3)
16:1 *cis* 9	8.2 (1.3)	34.2 (2.2)	12.0 (0.1)	25.1 (3.0)	12.4 (0.1)	18.3 (0.8)	12.1 (1.0)
16:1 C	0.5 (0.1)	0.2 (0.1)		0.1 (0.3)			
16:0	14.0 (1.8)	37.6 (1.7)	4.8 (1.1)	8.3 (4.5)	5.2 (0.3)	8.2 (0.7)	5.8 (0.7)
17:1 iso F			3.9 (0.6)	4.2 (1.4)	4.5 (0.2)	6.3 (0.7)	3.7 (1.1)
17:0 iso			1.7 (0.6)	4.6 (1.5)	3.1 (0.1)	7.4 (0.7)	2.4 (0.6)
17:0 anteiso						0.8 (0.1)	0.2 (0.1)
17:1 B			tr	0.3 (0.3)	0.2 (0)	1.1 (0.2)	0.2 (0.1)
17:1 C	1.0 (0.7)					tr	
17:0 cyclo	tr	3.3 (2.4)					tr
18:1 *cis* 9			0.7 (0.2)	0.2 (0.3)	1.2 (0.1)	0.8 (0.1)	1.1 (0.2)
18:1 *cis* 11	65.2 (1.0)	4.1 (1.0)	0.3 (0.3)	1.8 (2.8)	0.8 (0.1)	0.8 (0.2)	0.7 (0.3)
18:1 B	1.1 (0.2)						
18:0	0.8 (0.2)	0.1 (0.1)		0.1 (0.2)			tr

[a] tr, trace amounts are present in some strains.

14:0 3-OH. In most other respects the profiles of groups 1, 3, and 4 were qualitatively similar. The dominant fatty acids were 16:0, 16:1 *cis* 9, and 18:1 *cis* 11. Group 1 and 3 strains contained significant amounts of 12:0, whereas group 2, 4, and 5 strains contained few if any nonhydroxy fatty acids that contained less than 14 carbon atoms.

Group 6 strains had a completely different profile type. Not only was this group the only group to contain branched hydroxy fatty acids (11:0 iso 3-OH and 13:0 iso 3-OH) in addition to 12:0 3-OH, but all of the group 6 strains also contained more than 50% branched nonhydroxy fatty acids (11, 13, 15, and 17 carbon atoms).

Groups 1, 2, and 3 were further subdivided on the basis of the presence of other more minor hydroxy fatty acids (subgroups 1a, 1b, 1c, 1e, 2a, 2b, and 3b) or on the basis of quantitative differences in the core hydroxy fatty acids (subgroup 1f).

Some nonhydroxy fatty acids were of value for differentiating some of the groups. The most obvious examples were the branched acids, in particular 15:0 iso, 15:0 anteiso, 17:0 iso F, and 17:0 iso in group 6. These fatty acids were not found in any of the other groups except for small amounts of 17:0 iso in some group 1 taxa. Most group 2 strains had no fatty acids with less than 14 carbon atoms. Most of the unsaturated fatty acids were *cis* isomers, although a few group 1 taxa contained significant amounts of 16:1 *trans* 9 (for example, *P. aeruginosa*, *P. putida* biovar A, *P. alcaligenes*, and *P. pseudoalcaligenes* subsp. *pseudoalcaligenes*).

The cyclopropane fatty acids also were of some value for differentiating species. Within subgroup 1a, *P. fluorescens* and its close relatives (Table 4) had significantly higher levels of 17:0 cyclo than *P. syringae* and its close relatives (Table 3). Likewise, 19:0 cyclo 11-12 was much more abundant in subgroup 2a and 2b taxa than in group 1 and subgroup 2c taxa. This fatty acid also differentiated subgroup 3a from subgroup 3b. It was not found in any group 4, 5, or 6 taxa.

Differentiation of taxa at specific and infraspecific levels. The results in Tables 3 through 8 show that many species have unique profiles. Some infraspecific taxa (subspecies, biovar, pathovar) also have unique profiles. Differences at and below the specific level were usually quantitative and were sometimes found for several acids in the profile. Occasionally, qualitative differences were found; for example, of the *P. syringae* strains tested, only *P. syringae* pv. phaseolicola strains contained 16:0 3-OH. Thus, within each subgroup most taxa could be differentiated.

TABLE 9. Grouping of strains according to the presence of hydroxy fatty acids in their profiles

Fatty acid group or subgroup	Presence of the following hydroxy fatty acids:										
	10:0 3-OH	12:0 2-OH	12:0 3-OH	14:0 2-OH	14:0 3-OH	16:1 2-OH	16:0 2-OH	16:0 3-OH	18:1 2-OH	11:0 iso 3-OH	13:0 iso 3-OH
1a[a]	+[b]	+	+		(+)			(+)			
1b	+	+	+		+			+			
1c	+	+	+	+		+					
1d	+		+								
1e	+	+	+		(+)			+			
1f[a]	+	+	+								
2a					+	+	+	+	+		
2b	+		+		+	+	+	+	+		
2c					+	(+)		+	+		
3a	+										
3b	+			+		+	+				
4				+							
5		+		+	+						
6	(+)		+							+	+

[a] Differentiation of subgroups 1a and 1f was not based on qualitative differences in hydroxy acids.
[b] +, present; (+), some taxa or strains contain small quantities.

Group 1. Subgroup 1a was by far the largest subgroup. All of the other subgroups except subgroup 1d contained single taxa. Subgroup 1a contained all of the fluorescent pseudomonads. The profiles of the strains belonging to each taxon showed little variation. However, alteration of the cultural conditions increased variation. For example, 17:0 cyclo levels increased with increasing age (26).

The data for all of the subgroup 1a taxa with a mean value for 12:0 2-OH of less than 3% are shown in Table 3, and the data for most of the subgroup 1a taxa with a mean value for 12:0 2-OH of more than 4% are shown in Table 4 (the single exception is *P. fluorescens* biovar 4 [mean value, 2.2%]). Further analysis demonstrated other differences between the bacteria listed in Tables 3 and 4. Most of the taxa included in Table 3 contained significantly higher levels of the unsaturated acids 16:1 *cis* 9 and 18:1 *cis* 11 (52 to 60%) than the taxa included in Table 4 (36 to 47%); the exceptions were *P. aeruginosa* (54.8%) and the two plant pathogens *P. asplenii* (51.9%) and *P. fuscovaginae* (52.4%). This subdivision also parallelled the subdivision based on the ratio of 16:0 to 16:1 *cis* 9. This ratio was less than 0.9 for all bacteria included in Table 3 plus the plant pathogens *P. asplenii* and *P. fuscovaginae* and greater than 0.9 for all of the other bacteria included in Table 4.

P. aeruginosa had the highest ratio by far. Likewise, *P. syringae* and its close relatives included in Table 3 usually contained small but consistent amounts of 19:0 cyclo 9-10, whereas none of the bacteria included in Table 4 contained this fatty acid. Most of these bacteria contained a different isomer, 19:0 cyclo 11-12. Only occasional strains of *P. syringae* pv. phaseolicola contained both isomers. In addition, the bacteria listed in Tables 3 and 4 could be further differentiated on the basis of the amounts of another cyclopropane fatty acid, 17:0 cyclo. *P. syringae* and its relatives contained 0 to 2% 17:0 cyclo, whereas *P. fluorescens* and its relatives listed in Table 4 contained 1.9 to 13.9%. *P. aeruginosa* contained only trace amounts of this fatty acid.

Thus, subgroup 1a comprised three well-defined profile types, represented by *P. aeruginosa*, *P. syringae*, and *P. fluorescens*. All of the plant-pathogenic species tended to have profiles very similar to the profile of *P. syringae* pv. syringae (e.g., 12:0 2-OH, <3%; 16:1 *cis* 9 + 18:1 *cis* 11, >52%; ratio of 16:0 to 16:1 *cis* 9, <0.9).

Within subgroup 1a, the largest subgroup, there were some obvious differences among some taxa even at pathovar and biovar levels for *P. syringae* and *P. fluorescens*, respectively. For example, *P. syringae* pv. tomato had a much simpler profile with fewer fatty acids than *P. syringae* pv. phaseolicola. *P. fluorescens* biovar 4 had a profile that was readily distinguished from the profiles of the other *P. fluorescens* biovars. Fatty acids with obvious diagnostic value included 12:0, 12:0 2-OH, 16:1 *cis* 9, 16:0, 17:0 cyclo, 18:1 *cis* 11, 19:0 cyclo 9-10, and 19:0 cyclo 11-12.

For some taxa within subgroup 1a, no significant differences were found. For example, *P. fluorescens* biovar 1, *P. fluorescens* biovar 2, *P. fluorescens* biovar 5, *P. marginalis* pv. marginalis, *P. marginalis* pv. alfalfae, and *P. marginalis* pv. pastinacae had very similar profiles. *P. syringae* pv. tomato and *P. viridiflava* also had very similar profiles. Further studies with larger numbers of strains and analysis under different cultural conditions will be required to determine whether these taxa can be differentiated by their fatty acid profiles.

Subgroup 1b contained a single species, *P. corrugata*. Most strains contained a series of additional minor hydroxy fatty acids (namely, 12:1 3-OH, 14:0 3-OH, 16:0 3-OH, and unknown fatty acids with equivalent chain lengths of 13.961 and 14.503). The values for these fatty acids never exceeded 2.0% of the total fatty acids. The two unknown fatty acids were confirmed as hydroxy acids by mass spectrometry. They contained 13 and 14 carbon atoms, respectively. For both, the hydroxy group was not in the 2 or 3 position.

Subgroup 1c also contained a single species, *P. rubrisubalbicans*, which was differentiated by the presence of 14:0 2-OH and 16:0 2-OH. 14:0 2-OH is not commonly found in bacteria; the only other bacteria in this study which contained it were *C. testosteroni*, *S. paucimobilis*, and *P. flectens*.

Subgroup 1d contained three taxa, *P. alcaligenes*, *P. pseudoalcaligenes* subsp. *pseudoalcaligenes*, and *P. stutzeri*. The subgroup 1d strains contained no or just trace amounts of 12:0 2-OH, which differentiated them from all other group 1 strains. The three taxa were readily differentiated from each other. *P. alcaligenes* contained significantly more 10:0 and 17:1 C than the other two taxa. *P. stutzeri* did not contain 16:1 *trans* 9, 15:0, 17:1 B, or 17:0.

Subgroup 1e contained *P. amygdali*, which was differen-

tiated from subgroup 1a strains solely on the basis of quantitative differences in hydroxy fatty acids. *P. amygdali* is a very slowly growing bacterium, and in this study 7-day cultures were required to obtain enough cells for analysis. Further study will be required to determine to what extent the profile differences between this organism and other bacteria in subgroup 1a are due to cultural conditions, such as culture age. Preliminary results (data not shown) have indicated that culturing *P. syringae* for 7 days does not dramatically increase the percentage of hydroxy fatty acids.

Subgroup 1f contained a single strain, *P. cattleyae* NCPPB 1874. The *P. cattleyae* type strain (strain NCPPB 961) was placed in group 3 and is discussed below.

Group 2. One of the most interesting features of group 2 was the variation found in the profiles of most subgroup 2a taxa. For *P. gladioli* pv. alliicola this variation was linked to colony morphology. Smooth colonies produced profiles which contained no 10:0 or 10:0 3-OH, whereas colonies with rippled surfaces consistently produced these acids. Other acids for which variation occurred in *P. gladioli* included 16:1 *cis* 9, 18:1 *cis* 11, and 17:0 cyclo. This variation is an unusual phenomenon since there appeared to be a continuum across the ranges of values rather than two or more discrete GC groups. This variation made it more difficult to identify obvious differences in the taxa belonging to subgroup 2a. *P. andropogonis*, *P. caryophylli*, *P. glumae* GC subgroup A, and *P. plantarii* were readily differentiated, whereas differences among *P. cepacia*, *P. gladioli* pv. gladioli, and *P. gladioli* pv. alliicola were less obvious. There were no differences in the profiles of the strains received as *P. woodsii*, *P. stizolobii*, and *P. andropogonis*, which supported a previous proposal of synonymy with *P. andropogonis* (14).

Subgroup 2b comprised two strains of *P. glumae*, which contained significant amounts of 10:0 3-OH and 12:0 3-OH. These two strains were unique in having the core hydroxy acids of both groups 1 and 2. They also contained an unknown branched hydroxy fatty acid with an equivalent chain length of 14.503. This fatty acid also occurs in *P. corrugata* (subgroup 1b) and in many members of the *Enterobacteriaceae* (unpublished data). All of the cultures of *P. glumae* which were tested were isolated from rice in Japan. At the present time no further phenetic studies have been carried out to substantiate the differences shown in the fatty acid profiles.

The absence of 16:0 3-OH clearly differentiated *P. solanacearum*, *P. syzygii*, and the banana blood disease bacterium from other group 2 members. As with *P. amygdali*, *P. syzygii* and the banana blood disease bacterium are slow growers and were taken from 6- and 2-day cultures, respectively. *P. syzygii* in particular exhibited great quantitative variation in its profiles, and this made it difficult to differentiate it from the other two taxa. Considering the great differences in the growth rates, the different culture media, and the phenotypic differences between *P. syzygii* and *P. solanacearum* (22), the profiles were remarkably similar. Further studies will be required to determine whether fatty acid profiles can differentiate any of these three taxa. The *P. solanacearum* strains selected also included all three known races and four of the five known biovars. No differences in the profiles were detected under the cultural conditions which I used.

Group 3. Most group 3 strains were unusual in that they contained only a single hydroxy fatty acid (10:0 3-OH), which accounted for only 2 to 4% of the total fatty acid peak area. Only the absence of 12:0 2-OH and 12:0 3-OH differentiated these strains from most group 1 strains. The nonhydroxy fatty acid components of the profiles were even quantitatively similar to those of group 1. Some *P. avenae* and *P. rubrilineans* strains contained small amounts of 14:0 3-OH. *P. cattleyae* NCPPB 961^T contained small amounts of 12:1 3-OH. In addition to 10:0 3-OH, *C. testosteroni* contained significant amounts of 16:1 2-OH and 16:0 2-OH and on this basis was placed in a separate subgroup. Some subgroup 3a taxa were readily differentiated. *P. pseudoalcaligenes* subsp. *citrulli* and *P. pseudoalcaligenes* subsp. *konjaci* were clearly differentiated from *P. pseudoalcaligenes* subsp. *pseudoalcaligenes*, which was placed in subgroup 1d. Confusion between *P. pseudoalcaligenes* and some bacteria now included in the family *Comamonadaceae* (33) is common because of a lack of convenient differential phenotypic characters (34). This perhaps accounts for the misnaming of these two plant pathogens, which clearly exhibit greater similarity to the other strains of the *Comamonadaceae* (34) included in this study. They differ from the two *Comamonas* spp. in their significantly greater amounts of 14:0 and 16:1 *cis* 9. They are readily differentiated from each other by the absence in *P. pseudoalcaligenes* subsp. *konjaci* of 15:0, 15:1 B, and 17:0 and in the higher amounts of 14:0 and 18:1 *cis* 11. In contrast, *P. avenae* and *P. rubrilineans* could not be differentiated, supporting recent evidence of synonymy (3, 20), since no major differences in nutritional, physiological, serological, or host assays were detected. The results of recent studies on the genus *Comamonas* (34) suggest that although the plant-pathogenic strains belonging to group 3 belong to the family *Comamonadaceae*, they are not members of the genus *Comamonas*.

Group 4. Group 4 contained a single species, which has been reclassified as *S. paucimobilis* (35). Other workers (7, 15) have found that this organism is not closely related to other *Pseudomonas* spp. but is closely related to the misnamed organism *Flavobacterium capsulatum*, which has also been reclassified in the genus *Sphingomonas* (35). *S. paucimobilis* is unusual in having 14:0 2-OH as the sole hydroxy fatty acid. Few gram-negative bacteria completely lack 3-hydroxy fatty acids. Other recent evidence also indicates that *S. paucimobilis* has a fatty acid profile similar to that of *Rhizomonas suberifaciens*, a newly described taxon that causes corky root of tomato (31).

Group 5. The type strain of *P. flectens* (strain NCPPB 539) had a profile which contained many branched acids and was different from the profiles of the other strains tested. It proved to be a gram-positive bacterium. However, examination of the same strain obtained as strain ICMP 745 showed that it had the same profile as the other *P. flectens* strains. Therefore, it must be assumed that contamination of strain NCPPB 539^T occurred. The only other bacteria which I know of which contain 12:0 3-OH, 14:0 2-OH, and 14:0 3-OH are some *Alcaligenes* and *Serratia* strains. The nonhydroxy part of the profile was most consistent with the profile of the genus *Alcaligenes* (unpublished data).

Group 6. All group 6 strains produced large amounts of branched acids and were the only bacteria in this study to do so. All of the strains also contained three core hydroxy fatty acids, 12:0 3-OH, 11:0 iso 3-OH, and 13:0 iso 3-OH. The inclusion of *P. cissicola* NCPPB 2982^T in group 6 along with *X. campestris* pv. campestris and *X. maltophilia* indicates that these bacteria are closely related. However, I obtained more strains in an attempt to substantiate this, and two strains (strains ICMP 4290 and ICMP 4291) produced profiles which contained no branched fatty acids but which were typical of *Agrobacterium* sp. (data not shown). However,

TABLE 10. Comparison of groupings of *Pseudomonas* spp. determined in this study with groupings obtained in other studies

Taxon	Fatty acid group or subgroup in this study	Fatty acid profile group of Oyaizu and Komagata[a]	RNA homology group[b]	rRNA branch[c]	rRNA branch[d]	Tyrosine biosynthesis group[e]	DAHP group[f]
P. aeruginosa	1a	I	I	1			
P. agarici	1a				1	I	I
P. asplenii	1a				1	I	I
P. aureofaciens	1a	I	I	1		I	I
P. caricapapayae	1a				1		
P. chlororaphis	1a	I	I	1		I	I
P. cichorii	1a		I	1	1	I	I
P. ficuserectae	1a						
P. fluorescens	1a	I	I	1	1	I	
P. fuscovaginae	1a				1		
"*P. gingeri*"	1a						
P. marginalis	1a				1	I	
P. meliae	1a				1		
P. putida	1a	I	I	1		I	
"*P. reactans*"	1a						
P. syringae	1a		I	1	1	I	I
P. tolaasii	1a				1		
P. viridiflava	1a		I		1		
P. corrugata	1b				1		
P. rubrisubalbicans	1c				2		
P. alcaligenes	1d	I	I	1		I	I
P. pseudoalcaligenes subsp. *pseudoalcaligenes*	1d		I	1			
P. stutzeri	1d	I	I	1		I	
P. amygdali	1e				1		
P. cattleyae	1f				1		
P. andropogonis	2a				2	III	III
P. caryophylli	2a		II	2	2	II	II
P. cepacia	2a		II	2	2	II	II
P. gladioli	2a	II	II	2	2	II	II
P. plantarii	2a						
P. glumae GC subgroup A	2a				2		
P. glumae GC subgroup B	2b				2		
P. solanacearum	2c		II	2	2	II	
P. syzygii	2c						
Banana blood disease bacterium	2c						
C. acidovorans	3a	III	III	3	3	III	III
P. avenae	3a	IX			3	III	III
P. cattleyae (type strain)	3a				3		
P. pseudoalcaligenes subsp. *citrulli*	3a				3		
P. pseudoalcaligenes subsp. *konjaci*	3a						
P. rubrilineans	3a				3	III	
C. testosteroni	3b	III	III	3	3		
S. paucimobilis	4	VI					
P. flectens	5				?		
P. betle	6				4		
P. cissicola	6				?		
P. hibiscicola	6				4	?	V
X. maltophilia	6	IV	V	4		V	V
X. campestris pv. campestris	6		V		4	V	V

[a] See reference 16.
[b] See reference 19.
[c] See reference 4.
[d] See reference 5. 1, *P. fluorescens* rRNA branch; 2, *P. solanacearum* rRNA branch; 3, *C. acidovorans* rRNA branch; 4, *Xanthomonas* rRNA branch.
[e] See reference 2.
[f] See reference 32. DAHP, 3-deoxy-D-arabinoheptulosonic acid 7-phosphate.

strain ICMP 8561^T had a profile that was very similar to that of strain NCPPB 2982^T. The original ICMP type strain, strain ICMP 4289, was replaced in 1977 by type strain ICMP 8561, which was also derived from the original strain PC1 culture obtained from M. Goto. Thus, of the three Goto strains available, only the type strains (strains NCPPB 2982 and ICMP 8561) had profiles containing branched fatty acids. The true taxonomic position of the other two strains will require further study. Since the fatty acid profiles of the *X. campestris* pv. campestris and *X. maltophilia* strains used in this study are typical of all other *Xanthomonas* spp. and *X. campestris* pathovars, it is highly likely that *P. betle*, *P. hibiscola*, and *P. cissicola* NCPPB 2982^T also belong to the genus *Xanthomonas*. All contain three hydroxy fatty acids (12:0 3-OH, 11:0 iso 3-OH, and 13:0 iso 3-OH). No bacteria other than those included in this study or included in the

genus *Xanthomonas* are known to contain these three hydroxy fatty acids (unpublished data). Branched, hydroxy fatty acids are not common in bacteria. However, some members of the *Cytophaga-Flavobacterium* complex contain 15:0 iso 3-OH and 17:0 iso 3-OH along with a large percentage of branched nonhydroxy fatty acids, as in the genus *Xanthomonas* (unpublished data). It is interesting that members of both genera are yellow-pigmented bacteria that often reside on the surfaces of plants. Palleroni (17) has suggested that *P. hibiscicola* is synonymous with *X. maltophilia*. Bradbury (1) cited DNA-rRNA homology studies which also indicated that *P. betle* is a *Xanthomonas* sp. These results also support the transfer of *P. maltophilia* to the genus *Xanthomonas* as *X. maltophilia* (29). It has long been known that *P. maltophilia* contains branched fatty acids (12). Thus, group 6 strains fell into three profile types representing *P. cissicola*, *X. campestris* pv. campestris, and *X. maltophilia*. The latter could not be differentiated from *P. hibiscicola* and *P. betle*, but was readily differentiated from *X. campestris* pv. campestris by the amounts of 14:0, 15:0 iso, 16:1 *cis* 9, 16:0, 17:1 iso F, and 17:0 iso. There were no major qualitative differences between the profiles of *P. cissicola* and the other xanthomonads, but these profiles were readily differentiated by the amounts of 10:0 3-OH, 12:0 3-OH, 15:0 anteiso, and 16:1 *cis* 9.

Comparison of groupings based on fatty acid profiles with groupings based on the results of other techniques. Several taxonomic studies on pseudomonads have been carried out recently (2, 4, 5, 7, 16, 18, 19, 32). There is generally good correlation among the groupings made on the basis of these techniques, including DNA-DNA and DNA-rRNA hybridization studies (4, 5, 7, 17, 18, 24), enzyme studies (2, 32), and another fatty acid profiling study (16). Table 10 compares the groups based on fatty acid profiles with the groups based on the results of the techniques mentioned above.

DNA-DNA and DNA-rRNA groupings. There was excellent correlation between groupings based on the results of DNA-DNA and DNA-rRNA techniques and groupings based on fatty acid profiles. Four major rRNA groups within the genus *Pseudomonas* have been proposed (17). These groups center around four species, *P. aeruginosa*, *P. solanacearum*, *C. acidovorans*, and *X. maltophilia*. The groups based on fatty acid profiles were identical to the nucleic acid homology groups in all but one case. *P. rubrisubalbicans* was placed in rRNA group II with *P. solanacearum* (5); fatty acid profiling indicated much closer similarity to taxa included in rRNA group I.

There was also agreement that the type strain of *P. cattleyae* is closely related to *C. acidovorans*, whereas the other strain available (strain NCPPB 1874) is closely related to *P. fluorescens* (5). Whereas *P. pseudoalcaligenes* subsp. *pseudoalcaligenes* belongs to fatty acid group 1, *P. pseudoalcaligenes* subsp. *citrulli* and *P. pseudoalcaligenes* subsp. *konjaci* do not. They belong to fatty acid group 3 along with *C. acidovorans* and *P. avenae*.

The results of the fatty acid study also indicated that there are subgroups within groups 1, 2, and 3. For group 2 there was a good correlation with DNA-DNA hybridization subgroups (17).

Enzyme studies. Several different enzyme systems have been used to group pseudomonads (2, 32). Again there was good correlation (Table 10) between the groups based on the results of enzyme studies and the groups based on fatty acid profiles. One exception was *P. andropogonis*, which was placed in group 2 in this study but in the *acidovorans* group on the basis of the results of enzymological patterning in tyrosine biosynthesis (2) and comparative allostery of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthetase (32). DNA-rRNA hybridization studies (5) placed *P. andropogonis* in rRNA group II along with *P. caryophylli* and *P. solanacearum*, as in this study.

Other fatty acid profile studies. The importance of fatty acid profiles in classification and rapid identification of some *Pseudomonas* spp. has been recognized for some time (8–13, 16, 23, 24, 26). A few plant-pathogenic species were included in two previous studies (16, 26). One of these studies (16) also compared groups based on fatty acid profiles with rRNA groups and showed fairly good correlation between them. The results also parallelled those presented here except for both strains of *P. avenae*, which were placed in a separate group. In my study all *P. avenae* strains belonged to group 3 along with *C. acidovorans* and *C. testosteroni*. Oyaizu and Komagata (16) used a different method of saponification and methylation; they also separated some FAMEs by thin-layer chromatography. Although these authors obtained the same FAMEs in their profiles, no quantitative comparison could be made. They placed great emphasis on the value of 3-hydroxy acids in grouping pseudomonads. My results also showed the value of 2-hydroxy acids. This emphasizes the need for standardization of the methods used for saponification, methylation, and extraction of the fatty acids.

Thus, apart from a few differences in grouping between the results of fatty acid profiling and the results of other techniques reported previously, there was generally excellent agreement.

In a future paper I will look at the potential of fatty acid profiling for rapid, accurate identification of the same group of bacteria by using a commercially available computerized pattern recognition algorithm to compare individual profiles with libraries of profiles derived from a range of strains for each taxon.

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