The Fatty Acids of *Pseudomonas multivorans* (*Pseudomonas cepacia*) and *Pseudomonas kingii*

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SUMMARY

The fatty acid compositions of three strains of *Pseudomonas multivorans* and three of *P. kingii* were determined by gas-liquid chromatography. The major fatty acids identified were 16:0, 16:1, 18:1, 3-OH 14:0, 3-OH 16:0 and cyclopropane acids 17 Δ and 19 Δ . The fatty acid compositions of these strains were similar to the acids identified in sixteen clinical isolates of the '*P. multivorans* (*P. cepacia*)/*P. kingii* group'. These data support the current view that *P. multivorans* and *P. kingii* are identical species.

INTRODUCTION

The taxonomic relationship of *Pseudomonas cepacia* Burkholder (1950) to *P. multivorans* (Stanier, Palleroni & Doudoroff, 1966) and to *P. kingii* (Jonsson, 1970) has been the subject of recent studies. Many workers have shown that these species are culturally and biochemically similar (Ballard *et al.* 1970; Gilardi, 1970; Pickett & Pederson, 1970; Sands, Schroth & Hildebrand, 1970). Others have shown that these species are also similar in their genetic composition (Ballard *et al.* 1970; Klein & Blazevic, 1972; Palleroni, Ballard, Ralston & Doudoroff, 1972).

Chemical analysis of hydrolysates of *Pseudomonas* species has shown that some of them can be distinguished by differences in fatty acid composition (Moss, Samuels & Weaver, 1972). One species which is readily differentiated by this procedure is *Pseudomonas multivorans* (*P. cepacia*). Since strains of this species cannot be distinguished from *P. kingii*, we have determined if these organisms are similar in fatty acid composition.

METHODS

Thirteen cultures of the '*Pseudomonas multivorans* (*P. cepacia*)/*P. kingii* group' were clinical isolates received from various geographical locations by the Centre for Disease Control, Atlanta, Georgia, U.S.A. These cultures, along with the type strains of *P. multivorans* and *P. kingii* (EO-1), were identified by the Clinical Bacteriology Unit of the Centre for Disease Control according to the procedures outlined in *Laboratory Methods in Special Medical Bacteriology* (1968). The principal biochemical characteristics of the three type strains of *P. multivorans* (R. Y. Stanier, 382, ATCC 17759; KC984, NCTC 10661; KC938, ATCC 17460) and of three strains of *P. kingii* (ATCC 25608, ATCC 25609, ATCC 25610) are presented in Table 1.

Bacteria for fatty acid analysis were obtained from 24 h growth (37 °C) in 100 ml of Trypticase Soy Broth* (Baltimore Biological Laboratory, Baltimore, Maryland, U.S.A.)

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education and Welfare.

Table 1. *Principal cultural and biochemical characteristics of three type strains of Pseudomonas multivorans and three ATCC strains of P. kingii**

Oxidase	5†	Number of polar flagella ≥ 2	6
Acid production, OF‡ (unsealed)		Motility	5
Glucose	6	H ₂ S production	0
Lactose	6	Lysine decarboxylase	6
Maltose	6	Arginine dihydrolase	0
Cellobiose	6		
Glycogen	0		
Erythritol	0		
Starch	0		

* Strain ATCC 25608 was non-motile; strain ATCC 25610 was negative for oxidase.

† Numbers refer to strains producing a positive reaction.

‡ Oxidation-fermentation medium.

and on Trypticase Soy Agar (BBL) plates. Washed whole bacteria were saponified with 1 N-NaOH in 50% methanol (w/v) for 1 h at 100 °C. The fatty acids were extracted and methylated by methods described earlier (Moss & Lewis, 1967).

Fatty acid composition was determined by gas-liquid chromatography (g.l.c.) of methyl esters (Perkin-Elmer Model 900, 12 ft $\times$ 0.16 in column of 3% OV-1 on Chromosorb W, at 150 °C programmed to 255 °C at 5 °C/min, He as carrier gas). Samples were also analysed isothermally at 230 °C on an 8 ft column of 15% ethylene glycol adipate (EGA) coated on Chromosorb W with N₂ as carrier gas. Eluted compounds were tentatively identified by comparison of retention times on each column with retention times of methyl ester standards. Final identification was established by a combination of techniques including hydrogenation of unsaturated compounds (Brian & Gardner, 1968), trifluoroacetylation of hydroxy acids (Hancock, Humphreys & Meadow, 1970), and mass spectrometry (Hancock *et al.* 1970). Combined g.l.c.-mass spectrometry was carried out on an LKB 9000 instrument. The mass spectra were recorded at 70 eV with an electron energy of 60 μ A, the ion source was at 290 °C, and the molecular separator at 250 °C. The trifluoroacetylated methyl esters were separated on an 18 ft $\times$ 0.16 in glass column packed with 3% OV-1. Peak areas were determined by direct integration and the percentage of each acid was calculated from the ratio of the area of its peak to the total area of all peaks.

RESULTS

The range of fatty acids identified in *Pseudomonas multivorans* (*P. cepacia*) and *P. kingii* (EO-1) extends from 14:0 to 20:0 (Table 2) and is comparable with that found in several other *Pseudomonas* species (Moss *et al.* 1972). Three of the four most abundant acids were palmitic acid (16:0) and 16:1 and 18:1 acids. These acids are also major constituents in the lipids of other *Pseudomonas* species (Romero & Brenner, 1966; Bobo & Eagon, 1968; Edmonds & Cooney, 1969; Hancock & Meadow, 1969; Wilkinson, 1969; Kates & Wassef, 1970; Anderes, Sandine & Elliker, 1971; Cullen, Phillips & Shipley, 1971). More significant was the fourth most abundant acid, 3-hydroxytetradecanoic acid (3-OH 14:0), which has been observed only in *P. multivorans* (Fensom & Gray, 1969; Hancock *et al.* 1970; Key, Gray & Wilkinson, 1970; Moss *et al.* 1972). This hydroxy acid is also present in each culture of *P. kingii* and in each of the thirteen clinical isolates (Table 2) and accounts for approximately 18 to 29% of the total fatty acids of each strain. Other fatty acids which occurred in each strain were the cyclopropane acids, 9,10-methylene hexadecanoic acid (17 Δ) and

Table 2. Cellular fatty acid composition of thirteen clinical isolates and ATCC strains of *Pseudomonas multivorans* (*P. cepacia*) and *P. kingii*

Strain	% Fatty acid														
	2-OH 3-OH					2-OH 3-OH									
	14:0*	14:0	16:1	16:0	17 Δ	17:0	16:0	16:0	16:0	16:0	18:1	18:0	19 Δ	19:0	20:0
<i>Pseudomonas multivorans</i>															
RYS 382, ATCC 17759	9	T	20	17	12	3	2	3	10	18	T	2	4	—	—
KC 984, NCTC 10661	5	T	19	15	20	2	4	4	8	21	T	2	—	—	T
KC 938, ATCC 17460	12	T	25	7	15	10	2	3	10	12	T	2	2	T	—
<i>P. kingii</i> (EO-1)															
B 7368, ATCC 25608	6	2	26	9	20	5	2	2	10	16	T	2	—	—	T
B 7369, ATCC 25609	7	2	19	11	17	8	T	4	10	17	T	3	2	T	—
B 7370, ATCC 25610	10	2	24	12	22	2	T	T	12	14	T	T	—	—	2
Clinical isolates†															
8776	5	T	22	12	17	4	4	5	10	13	T	6	T	2	—
8786	9	T	25	13	20	3	4	3	11	12	T	T	T	—	—
B 2495	5	—	25	5	15	10	2	5	7	11	T	10	5	—	—
B 7617	7	—	18	15	29	6	T	T	2	19	T	4	—	—	T
B 7879	6	T	29	11	21	2	T	2	8	17	T	T	4	—	—
B 8851	7	—	21	10	15	10	3	4	8	14	T	4	4	T	—
B 8994	6	—	20	13	26	4	T	T	9	20	T	2	—	—	—
B 6191	8	—	22	15	13	4	4	2	10	17	T	2	3	—	—
B 6272	8	—	22	12	13	8	6	5	9	12	T	2	3	—	—
B 6282	8	—	24	15	15	3	4	3	10	12	T	2	4	T	—
B 6388	9	T	20	11	12	4	4	9	10	12	T	3	6	T	—
B 6566	4	2	23	10	26	3	T	2	10	16	T	2	2	T	—
B 7272	6	2	18	9	15	3	2	8	10	14	T	9	4	T	—

* Number to the left of the colon refers to the number of carbon atoms; number to the right refers to number of double bonds; 2-OH and 3-OH refer to hydroxy acid; Δ = symbol for cyclopropane acid; UN is unidentified.

† These 13 strains conform to the current biochemical descriptions of *Pseudomonas multivorans* (Ballard *et al.* 1970) and *P. kingii* (Jonsson, 1970).

9,10-methylene octadecanoic acid (19 Δ), which were present in small to trace amounts. Both of these acids have been found in *P. aeruginosa* (Hancock & Meadow, 1969) and *P. fluorescens* (Cullen *et al.* 1971). A second hydroxy acid, 3-hydroxyhexadecanoic acid (3-OH 16:0), was also present in small amounts (2 to 12%) in each strain. Although the percentage of each fatty acid varied somewhat among strains, the occurrence and relative proportions of these acids in each of the 19 strains produced similar profiles which were different from those of other pseudomonads we have tested (Moss *et al.* 1972).

All fatty acids were tentatively identified by comparing g.l.c. retention times of their methyl esters with standards on both polar and non-polar columns. Trifluoroacetylated hydroxy acid methyl esters had retention times identical to those of the trifluoroacetylated methyl ester standards of 3-OH 14:0 and 2-OH 16:0 acids (16.4 and 20 min, respectively). As a standard of 3-OH 16:0 acid was not available, tentative identification of this compound was made by g.l.c. retention time data. In extensive studies in our laboratory, we have found that methyl esters of 2-hydroxy acids elute from non-polar columns, such as OV-1, before methyl esters of 3-hydroxy acids of the same chain length. The same elution sequence (2-hydroxy acids before 3-hydroxy acids) is observed for trifluoroacetylated methyl esters on this column. In both instances, the peak tentatively identified as 3-OH 16:0 acid eluted just after the 2-OH 16:0 acid standard.

Identification of the methyl esters of normal, cyclopropane, and hydroxy acids was confirmed by mass spectrometry. Each acid gave a fragmentation pattern identical to that of a known standard (Ryhage & Stenhagen, 1960; McCloskey & Law, 1967). Although the 3-OH 16:0 acid could not be compared with a standard, the fragmentation pattern of the methyl ester of this acid was consistent with that of other 3-hydroxy methyl ester standards (i.e. 3-OH 10:0, 3-OH 12:0, 3-OH 14:0). The mass spectrum showed an *m/e* 103 peak, an *m/e* 74 peak, an M-50 (*m/e* 236) peak, and an M-18 (*m/e* 268) peak, which are characteristic fragments of 3-hydroxy methyl esters (Ryhage & Stenhagen, 1963).

DISCUSSION

The fatty acid compositions of *Pseudomonas multivorans* (*P. cepacia*) and *P. kingii* are similar and differ significantly from that of other pseudomonads (Moss *et al.* 1972). The occurrence of 3-OH 14:0, 3-OH 16:0, and other major fatty acids (Table 2) was consistent throughout a large number of strains from a variety of sources at diverse geographical locations. These data support recent biochemical and genetic studies which have shown that organisms of the '*P. multivorans* (*P. cepacia*)/*P. kingii* group' are identical. Ballard *et al.* (1970) demonstrated a high level of DNA homology between 18 strains of *P. cepacia* and the type strain of *P. multivorans* (ATCC 17759); he concluded that the two species are synonymous and that the correct specific designation is *Pseudomonas cepacia* Burkholder (1950) *emend.* Other investigators (Gilardi, 1970; Pickett & Pederson, 1970; Klein & Blazevic, 1972) have extended the comprehensive biochemical studies of EO-1 strains by Jonsson (1970) to show that *P. kingii* (EO-1) is identical to *P. cepacia* and *P. multivorans*. Our fatty acid data also indicate that these are identical species. Therefore, we propose that the original description of *Pseudomonas cepacia* Burkholder (1950) be amended to include not only *P. multivorans* (Stanier *et al.* 1966) as suggested by Ballard *et al.* (1970) but also *P. kingii* (Jonsson, 1970). This would simplify the classification of clinical and phytopathogenic isolates of these species.

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REFERENCES

- ANDERES, E. A., SANDINE, W. E. & ELLIKER, P. R. (1971). Lipids of antibiotic-sensitive and -resistant strains of *Pseudomonas aeruginosa*. *Canadian Journal of Microbiology* **17**, 1357-1365.
- BALLARD, R. W., PALLERONI, N. J., DOUDOROFF, M., STANIER, R. Y. & MANDEL, M. (1970). Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia*, *P. marginata*, *P. allucada*, and *P. caryophylli*. *Journal of General Microbiology* **60**, 199-214.
- BOBO, R. A. & EAGON, R. G. (1968). Lipids of cell walls of *Pseudomonas aeruginosa* and *Brucella abortus*. *Canadian Journal of Microbiology* **14**, 503-513.
- BRIAN, B. L. & GARDNER, E. W. (1968). A simple procedure for detecting the presence of cyclopropane fatty acids in bacterial lipids. *Applied Microbiology* **16**, 549-552.
- BURKHOLDER, W. H. (1950). Sour skin, a bacterial rot of onion bulbs. *Phytopathology* **40**, 115-117.
- CULLEN, J., PHILLIPS, M. C. & SHIPLEY, G. G. (1971). The effects of temperature on the composition and physical properties of the lipids of *Pseudomonas fluorescens*. *Biochemical Journal* **125**, 733-742.
- EDMONDS, P. & COONEY, J. J. (1969). Lipids of *Pseudomonas aeruginosa* cells grown on hydrocarbons and on trypticase broth. *Journal of Bacteriology* **98**, 16-22.
- FENSOM, A. H. & GRAY, G. W. (1969). The chemical composition of the lipopolysaccharide of *Pseudomonas aeruginosa*. *Biochemical Journal* **114**, 185-196.
- GILARDI, G. L. (1970). Characterization of EO-1 strains (*Pseudomonas kingii*) isolated from clinical specimens and the hospital environment. *Applied Microbiology* **20**, 521-522.
- HANCOCK, I. C., HUMPHREYS, G. O. & MEADOW, P. M. (1970). Characterization of the hydroxy acids of *Pseudomonas aeruginosa* 8602. *Biochimica et biophysica acta* **202**, 389-391.
- HANCOCK, I. C. & MEADOW, P. M. (1969). The extractable lipids of *Pseudomonas aeruginosa*. *Biochimica et biophysica acta* **187**, 366-379.
- JONSSON, V. (1970). Proposal of a new species *Pseudomonas kingii*. *International Journal of Systematic Bacteriology* **20**, 255-257.
- KATES, M. & WASSEF, M. K. (1970). Lipid chemistry. *Annual Review of Biochemistry* **39**, 323-358.
- KEY, B. A., GRAY, G. W. & WILKINSON, S. G. (1970). The purification and chemical composition of the lipopolysaccharide of *Pseudomonas alcaligenes*. *Biochemical Journal* **120**, 559-566.
- KLEIN, M. M. & BLAZEVIC, D. J. (1972). Nucleic acid enzyme studies of nonfermentative Gram-negative bacteria using thin-layer chromatography. *Applied Microbiology* **23**, 276-280.
- Laboratory Methods in Special Medical Bacteriology* (8390-C) (1968). Atlanta, Georgia: Centre for Disease Control.
- MCCLOSKEY, J. A. & LAW, J. H. (1967). Ring location in cyclopropane fatty acid esters by a mass spectrometric method. *Lipids* **2**, 225-230.
- MOSS, C. W. & LEWIS, V. J. (1967). Characterization of clostridia by gas chromatography. I. Differentiation of species by cellular fatty acids. *Applied Microbiology* **15**, 390-397.
- MOSS, C. W., SAMUELS, S. B. & WEAVER, R. E. (1972). Cellular fatty acid composition of selected *Pseudomonas* species. *Applied Microbiology* (in the Press).
- PALLERONI, N. J., BALLARD, R. W., RALSTON, E. & DOUDOROFF, M. (1972). Deoxyribonucleic acid homologies among some *Pseudomonas* species. *Journal of Bacteriology* **110**, 1-11.
- PICKETT, M. J. & PEDERSON, M. M. (1970). Characterization of saccharolytic nonfermentative bacteria associated with man. *Canadian Journal of Microbiology* **16**, 351-362.
- ROMERO, E. M. & BRENNER, R. R. (1966). Fatty acids synthesized from hexadecane by *Pseudomonas aeruginosa*. *Journal of Bacteriology* **91**, 183-188.
- RYHAGE, R. & STENHAGEN, E. (1960). Mass spectrometry in lipid research. *Journal of Lipid Research* **1**, 361-390.
- RYHAGE, R. & STENHAGEN, E. (1963). Mass spectrometry of long-chain esters. In *Mass Spectrometry of Organic Ions*, pp. 399-452. Edited by F. W. McLafferty. New York: Academic Press.
- SANDS, D. C., SCHROTH, M. N. & HILDEBRAND, D. C. (1970). Taxonomy of phytopathogenic pseudomonads. *Journal of Bacteriology* **101**, 9-23.
- STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: A taxonomic study. *Journal of General Microbiology* **43**, 159-271.
- WILKINSON, S. G. (1969). Lipids of *Pseudomonas diminuta*. *Biochimica et biophysica acta* **187**, 492-500.