Cellular Fatty Acid Composition of Selected *Pseudomonas* Species

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Received for publication 15 June 1972

The cellular fatty acid composition of 10 reference strains representing eight species of *Pseudomonas* was determined by gas-liquid chromatography. A variety of acids were detected in these organisms, including branched and straight-chained acids, cyclopropane, and hydroxy acids. Comparison of the presence and relative amounts of these acids among strains was useful for distinguishing various *Pseudomonas* species.

Recent studies on the distribution of lipids in *Pseudomonas* species have revealed interesting information about their content of hydroxy fatty acids (2-4). The most striking feature is the absence of 3-hydroxymyristic (3-OH 14:0) acid, a characteristic fatty acid of the lipopolysaccharide fraction of other gram-negative bacteria (5). The hydroxy fatty acids reported for *Pseudomonas* include 3-hydroxydecanoic acid (3-OH 10:0), 2-hydroxydecanoic acid (2-OH 12:0), and 3-hydroxydecanoic acid (3-OH 12:0). These three acids have been found in the extractable lipopolysaccharide fraction of *Pseudomonas aeruginosa* (2, 3); 3-OH 10:0 and 3-OH 12:0 were reported in *P. alcaligenes* (4). In recent studies, we have investigated the cellular fatty acid composition of whole cells of reference or type strains of several *Pseudomonas* species. Data from these studies show that one or more hydroxy fatty acids are present in each strain; comparison of the relative amounts of these and other cellular fatty acids is useful for distinguishing various species of *Pseudomonas*.

**MATERIALS AND METHODS**

The 10 reference strains of *Pseudomonas* were supplied by R. Y. Stanier of the University of California, Berkeley. A detailed description of the cultural and biochemical characteristics of these strains was published (7). Cells for fatty acid analysis were obtained from 24-hr growth (37°C) in 100 ml of Trypticase soy broth (BBL). Washed whole cells were saponified with 1.0 N NaOH in 50% methanol for 1 hr at 100°C. The fatty acids were extracted and methylated by methods described previously (6).

Fatty acid composition was determined by gas-liquid chromatography (GLC) analysis of methyl esters. The GLC analysis was done on a Perkin-Elmer model 900 gas chromatograph (Perkin-Elmer, Norwalk, Conn.) equipped with a hydrogen flame detector and a disc integrator recorder. The instrument contained a 0.16 inch (4.03 mm, inside diameter) by 12 ft (3.66 m) coiled glass column which was packed with 3% OV-1 methyl silicone coated on 80/100 mesh, acid-washed, dimethylchlorosilane (DMCS)-treated, high-performance Chromosorb W (Applied Science Laboratories, State College, Pa.). Samples were also analyzed on an 8 ft (2.4 m) column of 15% ethylene glycol succinate (EGS) coated on 80/100 mesh Chromosorb W.

For routine analysis, 3 µl of the methyl ester sample was injected into the OV-1 and EGS column and analyzed for 42 min. This time interval allowed the detection of normal, saturated, fatty acid methyl esters of carbon chains from 8 to 23 carbons in length. Fatty acid methyl ester peaks were tentatively identified by comparison of retention times on each column (OV-1, EGS) with retention times of highly purified methyl ester standards (Applied Science Laboratories and National Institutes of Health). Final identification was established by a combination of techniques, including mass spectrometry, hydrogenation of unsaturated acids (1), and trifluoroacetylation of hydroxy acids (3). The trifluoroacetylated methyl ester samples were also analyzed by GLC on both columns (OV-1, EGS). Peak areas were determined by an integrator (Disc Instruments Co.), 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 AND DISCUSSION**

The cellular fatty acid composition of the 10 reference strains of *Pseudomonas* is presented in Table 1. The fatty acids of *P. aeruginosa* (two strains) were similar to those of *P. putida*. The most abundant straight-chain fatty acids in these three strains were hexadecanoic acid...
(16:0), hexadecenoic acid (16:1), and octadecenoic acid (18:1). Each of these strains also contained relatively large amounts of 3-OH 10:0, 2-OH 12:0, and 3-OH 12:0. None of the three strains contained 3-hydroxytetradecanoic acid (3-OH 14:0), which is consistent with data of other workers who reported its absence in lipopolysaccharide preparations from *P. aeruginosa* (2, 3). The presence and relative amounts of 3-OH 10:0, 2-OH 12:0, and 3-OH 12:0 in *P. aeruginosa* and *P. putida* distinguish these organisms from other *Pseudomonas* tested (Table 1). Even though each of the three hydroxy acids was also present in *P. maltophilia*, the relative amounts in this species were much smaller than in *P. aeruginosa* and *P. putida*.

The cellular fatty acids of *P. acidovorans* and *P. testosteroni* were strikingly similar and were characterized by relatively large amounts of palmitic, hexadecenoic, and octadecenoic acids, and by smaller amounts of 3-OH 10:0. The presence of 3-OH 10:0 as the only hydroxy acid in whole cell hydrolysates of these two species distinguishes them from other *Pseudomonas*, since all the other strains contained two or more hydroxy acids (Table 1). Recent studies with 11 additional strains of each of these two species have shown that 3-OH 10:0 is the only hydroxy acid present in more than trace amounts (unpublished data). The fatty acids of *P. alcaligenes* and *P. stutzeri* were similar; they differed from *P. acidovorans* and *P. testosteroni* by the pres-

**Table 1. Percentages of total cellular fatty acids for each of eight Pseudomonas species**

| Strain               | Straight-chain acids | Hydroxy acids | Branched-chain acids | Cyclo-propane acids |
|----------------------|----------------------|---------------|----------------------|---------------------|
|                      | 12:0                 | 14:0          | 16:0                 | 17:0                |
|                      |                      | 18:0          | 18:1                 | 19:0                | 2-OH 10:0 | 3-OH 10:0 | 2-OH 12:0 | 3-OH 12:0 | 3-OH 14:0 | 2-OH 16:0 | 3-OH 16:0 | i-11:0 | i-15:0 | 17:0 |
| *P. aeruginosa*      | RYS 45               | 7             | 4                    | 11                   | 17                   | 17        | 16           | 15         | 15        | 6            | 9        | 12        | 12 |
|                      | RYS 52               | 7             | 3                    | 10                   | 18                   | 22        | 16           | 15         | 15        | 6            | 9        | 12        | 12 |
| *P. putida*          | RYS 90a              | 4             | 2                    | 22                   | 21                   | 17        | 11           | 12         | 12        | 5            | 9        | 15        | 15 |
| *P. acidovorans*     | RYS 14b              | 4             | 2                    | 4                    | 27                   | 27        | 19           | 17         | 17        | 12           | 12       | 15        | 15 |
| *P. testosteroni*    | RYS 78c              | 5             | 6                    | 28                   | 28                   | 18        | 10           | 10         | 10        | 10           | 10       | 10        | 10 |
|                      | RYS 138              | 3             | 3                    | 28                   | 28                   | 18        | 11           | 11         | 11        | 11           | 11       | 11        | 11 |
| *P. stutzeri*        | RYS 220              | 13            | 4                    | 23                   | 19                   | 23        | 10           | 10         | 10        | 6            | 6        | 11        | 11 |
| *P. alcaligenes*     | RYS 142c             | 6             | 11                   | 15                   | 23                   | 23        | 11           | 11         | 11        | 4            | 4        | 9         | 9 |
| *P. maltophilia*     | RYS 67c              | 7             | 7                    | 9                    | 3                    | 3         | 14           | 12         | 12        | 10           | 10       | 10        | 10 |
| *P. multivorans*     | RYS 382c             | 8             | 14                   | 22                   | 2                    | 18        | 14           | 12         | 12        | 10           | 10       | 10        | 10 |

a Number to left of colon refers to number of carbon atoms; number to right refers to number of double bonds; 2-OH and 3-OH refer to hydroxy acid; i = iso acid; Un is unidentified.

b Value includes 18:2 when present. This acid was detected in trace amounts only in strains RYS 138 and RYS 220.

c Number refers to percentage of total acids; T = less than 2%; blank spaces = not detected.

* American Type Culture Collection type strain.
The difference, with 3-OH 12:0.

The fatty acid profiles of *P. maltophilia* and *P. multivorans* were markedly different from each other and from other *Pseudomonas* (Table 1). The most striking feature of *P. maltophilia* was the presence of relatively large amounts (26%) of a branched-chain 15 carbon acid (13-methyl tetradecanoic acid) which was not found in other species. In addition, this organism contained three unidentified hydroxy acids which were not found in other species. *P. multivorans* were easily distinguished from other *Pseudomonas* species by the presence of 3-OH 14:0. To our knowledge, this is the only *Pseudomonas* species examined to date which contains 3-OH, 14:0.

The fatty acid composition of additional strains of *P. aeruginosa*, *P. maltophilia*, and *P. multivorans* is presented in Table 2. Each of these cultures was isolated from clinical material at various geographical locations. The data from each of these cultures show a fatty acid composition similar to that obtained for the reference strain of these species. These data clearly indicate that cellular fatty acids are a useful additional criterion for classifying certain *Pseudomonas* species.

### Table 2. Cellular fatty acids of clinical isolates of *Pseudomonas aeruginosa*, *P. maltophilia* and *P. multivorans*

| Strain   | Straight-chain acids                      | Hydroxy acids | Branched-chain acids | Cyclopropane acids |
|----------|-------------------------------------------|---------------|----------------------|-------------------|
|          | 12:0  | 14:0 | 16:1 | 18:0 | 18:1 | 19:0 | 3-OH 10:0 | Un | 3-OH 12:0 | Un | 3-OH 14:0 | Un | 3-OH 16:0 | i-11:0 | i-15:0 | 17:0 | 19:0 |
| *P. aeruginosa* |        |      |      |      |      |      |           |    |           |    |           |    |           |      |      |      |      |
| B9894    | 3     | 2    | 11   | 22   | 26   | 2    | 13        | 13 | 8         |    |           |    |           |      |      |      | T    |
| 1615     | T     | 4    | 12   | 13   | 25   | 2    | 12        | 10 | 4         |    |           |    |           |      |      |      | 2    |
| *P. maltophilia* |        |      |      |      |      |      |           |    |           |    |           |    |           |      |      |      |      |
| B3545    | 7     | 5    | 5    | 4    |      |      | 9         | 10 | 2         | 7  | 11        |    | 10        | 23   |      |      |      |
| B3588    | 7     | 5    | 9    | 5    | 5    |      | 8         | 12 | 2         | 9  | 10        |    | 8         | 20   |      |      |      |
| A2897    | 5     | 6    | 14   | 9    | 8    |      | 7         | 9  | T         | 5  | 8         |    | 8         | 22   |      |      |      |
| B3643    | 6     | 5    | 9    | 5    | 4    |      | 9         | 13 | 2         | 6  | 10        |    | 9         | 22   |      |      |      |
| B3731B   | 6     | 6    | 13   | 4    | 3    |      | 9         | 13 | 2         | 8  | 8         |    | 9         | 19   |      |      |      |
| *P. multivorans* |        |      |      |      |      |      |           |    |           |    |           |    |           |      |      |      |      |
| KC984    | 5     | 19   | 20   | 4    | 21   | 5    | T         | 10 | 4         | 8  | 2         |    | 2         |      |      |      |      |
| KC938    | 13    | 11   | 21   | 4    | 11   | 7    | 6         | 10 | 4         | 7  | 4         |    | 2         |      |      |      |      |
| B7368    | 6     | 9    | 20   | 2    | 16   | 5    | 7         | 21 | 4         | 10 | 5         |    | 2         |      |      |      |      |
| B7369    | 7     | 13   | 21   | T    | 19   | 5    | T         | 21 | 4         | 8  | 2         |    | 2         |      |      |      |      |
| B7370    | 10    | 12   | 22   | T    | 16   | 4    | T         | 24 | 2         | 8  | 2         |    | 2         |      |      |      |      |

* See footnotes, Table 1.
* CDC identification number.

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