Production of Glutaric Acid: a Useful Criterion for Differentiating *Pseudomonas diminuta* from *Pseudomonas vesiculare*

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A gas-liquid chromatographic procedure was used to determine short-chain acids produced by *Pseudomonas diminuta* and *P. vesiculare* after growth on Trypticase soy agar. Each of nine strains of *P. diminuta* produced glutaric acid, whereas none of the strains of *P. vesiculare* produced this acid.

Identification of pseudomonads by morphological and physiological characteristics is often a difficult, cumbersome, and time-consuming task (7, 10). For these reasons, a search for additional criteria which might lead to the development of rapid, specific, and reliable tests for distinguishing various *Pseudomonas* species was begun in this laboratory. Previous reports have shown that examination of cellular components and metabolic products provides useful new approaches to the taxonomy of certain of these organisms (2, 5, 9). This report describes the short-chain acids produced by *Pseudomonas diminuta* and *P. vesiculare* and shows that their analysis by gas-liquid chromatography (GLC) may provide a rapid, sensitive method for distinguishing the two species.

Two strains of *P. diminuta* (ATCC 11568, ATCC 13184) and one strain of *P. vesiculare* (ATCC 11426) were obtained from R. Y. Stanier of the University of California, Berkeley. Seven additional clinical isolates of each species were also tested. Conventional cultural and biochemical tests were performed on all cultures according to published procedures (3a). Trypticase soy agar (TSA; BBL) supplemented with 0.1% yeast extract (Difco) was used as the primary medium to test for short-chain acid production. Comparative studies were made with heart infusion agar (HIA; Difco) and nutrient agar (Difco).

Approximately 0.1 ml of an actively growing culture in Trypticase soy broth (BBL) was spread over the surface of a 15- by 85-mm agar plate containing 10 ml of medium. After 24 h of incubation at 35 C, the acids were extracted from the agar medium (2) and converted to butyl ester derivatives (4). The esters were analyzed by GLC on 15% Dexsil 300 GC as described previously (4) and on a Resoflex column (Burrell Corp., Pittsburgh, Pa.). The Resoflex column, a polar-phase GLC material that we have found to be quite useful for analysis of short-chain acids, was heated at 120 C for 3 min, and then the temperature was increased to 155 C at a rate of 5 C/min. The acids were identified by comparing GLC retention times of both free and esterified acids in the sample to those of highly purified standards (Eastman Organic Chemicals, Rochester, N.Y.).

Combined GLC-mass spectrometry of esterified acids was carried out on an LKB 9000 instrument. The mass spectra were recorded at an electron energy of 70 eV, a trap current of 60 μA, an ion source temperature of 290 C, and a molecular separator temperature of 250 C. The esters were separated on a 12-ft by ⅛-inch (approximately 3.66-m by 0.64-cm) glass column packed with 15% Dexsil 300 GC. The column was conditioned for 72 h at 250 C prior to use.

Chromatograms of the short-chain acids produced by type strains of the two species are presented in Fig. 1. Peaks which are designated "M" are media components. The relative size of these peaks remained essentially constant for uninoculated control TSA plates which were processed daily along with cultures. The identity of the media peak at a retention time of 6.5 min was established by GLC and mass spectrometry as acetic acid. The other two major peaks at retention times of 24.2 and 31 min were not identified, but, on the basis of their solubility characteristics and their conversion to esters under normal esterification procedures, they appear also to be acids. Whatever their nature, it is interesting to note that some of the cultures apparently metabolized one or both of these

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compounds, as evidenced by a marked decrease in peak sizes in comparison with those of the controls. The size of the acetic acid peak was essentially the same for both control media and cultures, which indicates there was no significant production or utilization of this compound by the cultures.

All of the cultures studied produced relatively large amounts of propionic, isobutyric, and isovaleric acids (Fig. 1). Some but not all strains of both species produced relatively large amounts of an unknown compound which eluted from the Dexsil column at 22.5 min (top chromatogram). No extensive efforts were made to identify this component since it was not a consistent characteristic of either species. A major difference in chromatograms from the two species was the presence of a peak at 37 min in *P. diminuta* (bottom) that was not detected in *P. vesiculare* (top). This component was present in relatively large amounts in each of nine strains of *P. diminuta* and was totally absent in eight strains of *P. vesiculare*. The peak was not present in chromatograms of unesterified samples. This fact, along with its extraction characteristics, indicated that the component was an acid. Additional studies were made to identify this component since it appeared to be a valuable marker to distinguish the two species.

An esterified sample of short-chain acids from *P. diminuta* was analyzed by combined GLC-mass spectrometry, and the mass spectrum of the peak at 37 min was recorded. Close examination and study of the fragmentation pattern indicated that the component was the dibutyl ester of glutaric acid. A reference standard of this acid was obtained from a commercial source (Eastman Organic Chemicals), esterified, and analyzed by GLC and mass spectrometry. The reference standard and the unknown produced identical GLC retention times on two columns. The fragmentation patterns were also identical and showed major ion peaks at m/e 244, 189, 171, 142, 129, 116, and 115. Identical mass spectra and matching retention times on two columns firmly established the identity of the 37-min peak as glutaric acid.

The production of glutaric acid by *P. diminuta* was observed in both TSA and HIA but not in nutrient agar. The nutritional factors contributing to formation of this acid were not determined. Other workers have reported glutaric acid as an intermediate in lysine metabolism by *P. fluorescens* (6), *P. putida* (8), and an unidentified *Pseudomonas* isolated from soil (3). It is known that *P. diminuta* cannot utilize lysine as a sole carbon and energy source in defined media (1), nor does it decarboxylate this amino acid in conventional laboratory tests (3a). Additional studies are needed to determine the source(s) and mechanism of glutaric acid formation by *P. diminuta* grown in relatively complex media such as TSA and HIA.

The production of glutaric acid by *P. diminuta* provides a useful marker to distinguish this organism from *P. vesiculare*. This acid can be detected with accuracy, speed, and sensitivity by the GLC procedure outlined in this report. In addition to glutaric acid, at least 12 other short-chain acids can be determined simultaneously within 37 min on the 15% Dexsil
If analysis for only glutaric acid is desired, the GLC method can be shortened considerably by omitting the acylation step in the derivative procedure and by increasing the GLC column temperature. The excellent stability of the Dexsil column indicates its usefulness for multiple analysis of short-chain acids from microorganisms.

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LITERATURE CITED

1. Ballard, R. W., M. Doudoroff, R. Y. Stanier, and M. Mandel. 1968. Taxonomy of the aerobic pseudomonads. *Pseudomonas diminuta* and *P. vesiculare*. J. Gen. Microbiol. 53:349–361.

2. Brooks, J. B., R. E. Weaver, H. W. Tatum, and S. A. Billingsley. 1972. Differentiation between *Pseudomonas testosteroni* and *P. acidovorans* by gas chromatography. Can. J. Microbiol. 18:1477–1482.

3. Ichihara, A., and E. A. Ichihara. 1961. Metabolism of L-lysine by bacterial enzymes. V. Glutaric semialdehyde dehydrogenase. J. Biol. Chem. 49:154–157.

4. Lambert, M. A., and C. W. Moss. 1972. Gas-liquid chromatography of short-chain fatty acids on Dexsil 300 GC. J. Chromatogr. 74:335–338.

5. Moss, C. W., S. B. Samuels, and R. E. Weaver. 1972. Cellular fatty acid composition of selected *Pseudomonas* species. Appl. Microbiol. 24:596–598.

6. Numa, S., Y. Ishimura, T. Nakazawa, T. Okazaki, and O. Hayashi. 1964. Enzymatic studies on the metabolism of glutarate in *Pseudomonas*. J. Biol. Chem. 239:3915–3926.

7. Pickett, M. J., and C. R. Maclark. 1970. Nonfermentative bacilli associated with man. I. Nomenclature. Amer. J. Clin. Pathol. 54:155–163.

8. Reitz, M., and V. W. Rodwell. 1970. Delta-aminovaleramidase of *Pseudomonas putida*. J. Biol. Chem. 245:3091–3096.

9. Samuels, S. B., C. W. Moss, and R. E. Weaver. 1973. The fatty acids of *Pseudomonas multivorans* (*Pseudomonas cepacia*) and *Pseudomonas kingii*. J. Gen. Microbiol. 74:275–279.

10. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43:159–271.