

Identifying Bacteria by Analyzing Their Cellular Fatty Acids

Just as anaerobic bacteria are routinely identified by analyzing their short chain fatty acids, differences in long chain fatty acids can be used to differentiate between closely related bacteria species. (ChromFax: 394050)

Key Words:

- anaerobic bacteria
- cellular fatty acids

Many hospital and other laboratories routinely identify anaerobic bacteria by analyzing short chain fatty acids extracted from the medium in which the cultures are grown. Gas chromatography columns are used in these analyses. Reviews (1,2) and manuals (3-5) are available that describe the details of the procedures.

Similarly, studies have shown that even closely related species of microorganisms can be differentiated by qualitative or quantitative differences in cellular long chain fatty acids. Figure A, for example, shows the cellular fatty acid profiles of *Pseudomonas cepacia* and *P. aeruginosa*. It is clear from these chromatograms that there are major differences between the two species. *P. aeruginosa* contains four acids (3-OH 10:0, 12:0, 2-OH 12:0, 3-OH 12:0) absent from *P. cepacia*. *P. cepacia* contains three acids (3-OH 14:0, 2-OH 16:0, 3-OH 16:0) absent from *P. aeruginosa*. *P. cepacia* contains large amounts of 17:0 Δ and 19:0 Δ cyclopropane acids, compared to *P. aeruginosa*. The fatty acid profiles of other strains of each of the two species were essentially the same as those shown in Figure A. Thus, these closely related species can be differentiated by quantitative differences in several acids.

The fatty acids of other *Pseudomonas* species, and of many other microorganisms, have also been studied. The long chain fatty acids that may be present in a bacterial cell consist of C10 to C20 saturated acids (both odd and even carbon numbers), C16:1 and C18:1 monosaturated acids, iso (i) and anteiso (a) branched chain acids, C17 Δ and C19 Δ cyclopropane acids, and C10, C12, C14, and C16 α (2-hydroxy) and β (3-hydroxy) acids (38). For each group or species, the chromatogram will have a distinct pattern.

The general procedure for analyzing cellular fatty acids involves culturing the bacteria (broth and plate), collecting the cell mass, and saponifying the cells with NaOH in methanol. The liberated fatty acids are converted to the methyl esters with BCl₃-methanol, and the methyl esters are analyzed by gas chromatography (6).

Methyl esters of bacterial cellular fatty acids can be analyzed by using either a packed column or a capillary column. The most effective packing for this analysis is a 3% methyl silicone coated on an acid washed dimethylchlorosilane (DMCS) treated sup-

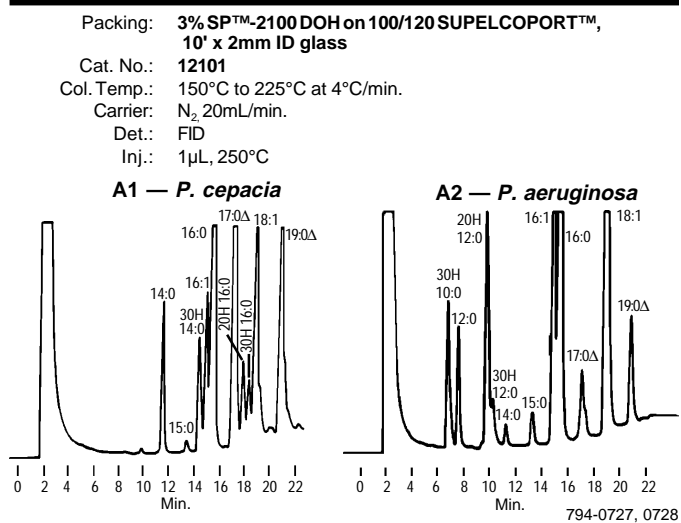
port. If the support is not adequately deactivated, however, the hydroxy acid peaks will tail. To ensure reliable and reproducible results with minimal peak tailing, we developed 3% SPTM-2100 DOH on 100/120 SUPELCOTM packing specifically for these acids. To ensure consistent performance, each batch of this packing is tested with a standard of bacterial cell fatty acid methyl esters (Figure B).

These methyl esters can also be analyzed by using an SPBTM-1 capillary column. The 15m thick film (1.0 μ m) column used to obtain Figure C provides resolution similar to a 30m x 0.25mm ID standard film (0.25 μ m) column, at much lower cost. Similar analyses can be obtained from equivalent 0.32mm ID columns, 30m x 0.53mm ID columns (1.5 μ m film) and 60m x 0.75mm ID columns (1.0 μ m film). Alternatively a 0.20mm ID column will afford greater resolution without changing the peak elution pattern or retention times.

The 0.53mm and 0.75mm ID columns can be used in packed column injectors and detectors. In a packed column system, a 0.53 or 0.75mm ID capillary column will resolve bacterial acid methyl esters faster than a packed column, or with greater resolution. For more information on wide bore capillary columns, request **Bulletin 814**.

- Columns specifically for analyzing short chain fatty acids are available from Supelco. See our catalog, or request **Bulletin 855 and 856**.

Figure A — Closely Related Species of Bacteria Distinguished by Different Fatty Acid Profiles



Acknowledgment: Dr. C. Wayne Moss of the Centers for Disease Control, Atlanta, GA, USA provided the chromatograms shown in Figure A, and provided many helpful suggestions with regard to the information in this application note.

Peak Identification for Figures B and C

S	Component	Shorthand Designation
	Solvent	
1.	Me undecanoate	11:0
2.	Me 2-hydroxyundecanoate	12-OH 10:0
3.	Me dodecanoate	12:0
4.	Me tridecanoate	13:0
5.	Me 2-hydroxytridecanoate	2-OH 12:0
6.	Me 3-hydroxytridecanoate	3-OH 12:0
7.	Me tetradecanoate	14:0
8.	Me 12-methyltetradecanoate	α -15:0
9.	Me pentadecanoate	15:0
10.	Me 2-hydroxypentadecanoate	2-OH 14:0
11.	Me 3-hydroxypentadecanoate	3-OH 14:0
12.	Me cis-9-hexadecenoate	16:1 ⁹
13.	Me hexadecanoate	16:0
14.	Me 14-methylhexadecanoate	α -17:0
15.	Me cis-9,10-methylenehexadecanoate	17:0 Δ
16.	Me heptadecanoate	17:0
17.	Me 2-hydroxyheptadecanoate	2-OH 16:0
18.	Me cis-9-octadecenoate	18:1 ⁹
19.	Me trans-9-octadecenoate	18:1 ⁹
20.	Me octadecanoate	18:0
21.	Me cis-9,10-methyleneoctadecanoate	19:0 Δ
22.	Me nonadecanoate	19:0
23.	Me eicosanoate	20:0

Figure B. Fatty Acids in Microorganisms on a Packed Column

Packing: **GP 3% SP-2100 DOH on 100/120 SUPELCOPORT™ 10' x 2mm ID glass**
 Cat. No. **12101**
 Col. Temp.: 150°C to 230°C at 4°C/min.
 Carrier: N₂, 20mL/min.
 Det.: FID
 Inj.: 2.5µL, 250°C

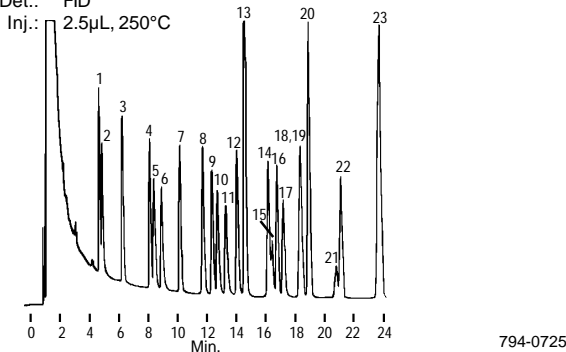
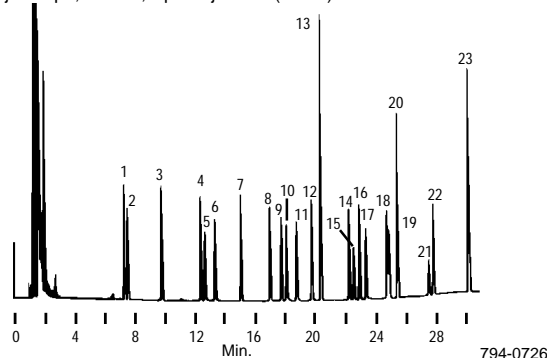


Figure C. Bacterial Fatty Acid Methyl Esters on a Capillary Column

Column: **SPB-1 fused silica, 15m x 0.25mm ID, 1.0µm film**
 Cat. No. **24026**
 Col. Temp.: 150°C (4 min.) then to 250°C at 4°C/min. (4 min.)
 Carrier: He, 25cm/sec.
 Det.: FID, 270°C
 Inj.: 1µL, 250°C, split injection (100:1)



Ordering Information:

Column Packing

GP 3% SP-2100 DOH on
 100/120 SUPELCOPORT, 20g

12101

Packed Glass Columns

We can prepare for your instrument a 10' x 1/4" OD x 2mm ID glass column filled with GP 3% SP-2100 DOH on 100/120 SUPELCOPORT. Refer to our latest catalog, or call our Technical Service Department.

SPB-1 Capillary Columns

Fused Silica	Cat. No.
15m x 0.20mm ID, 0.20µm film	24162
30m x 0.20mm ID, 0.20µm film	24163
15m x 0.25mm ID, 1.0µm film	24027
30m x 0.25mm ID, 0.25µm film	24028
15m x 0.32mm ID, 1.0µm film	24098-U
30m x 0.32mm ID, 0.25µm film	24044
30m x 0.53mm ID, 1.5µm film	25303
Borosilicate Glass	
60m x 0.75mm ID, 1.0µm film	23720-U
BCl ₃ -Methanol (12% w/w)	
Kit (20 x 2mL ampuls)	33089-U
Pint	33033

GP — Indicates packing has been tested for specific analysis shown in this bulletin.

References

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 - Mitruka, B.M., Gas Chromatographic Applications in Microbiology and Medicine, John Wiley & Sons, New York, (1975).
 - Anaerobe Laboratory Manual, VPI Anaerobe Laboratory, VPI and SU, Blacksburg, Virginia. Direct inquiries about availability to : Virginia Polytechnic Institute and State University, Department of Anaerobe Microbiology, Research Division, Blacksburg, VA 24061, USA.
 - Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual, CDC, Atlanta, Georgia. Request this publication from: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA 30333, USA.
 - Wadsworth Anaerobic Bacteriology Manual. Direct inquiries about availability to: Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, Veterans Administration, Los Angeles, CA 90073, USA.
 - Moss, C.W., *et al.*, Appl. Microbiol., 28, 80-85 (1974).
- References not available from Supelco.

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