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, C17Δ and C19Δ 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 support. 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% SP™-2100 DOH on 100/120 SUPELCOPORT™ 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 SP™-1 capillary column. The 15m thick film (1.0μm) column used to obtain Figure C provides resolution similar to a 30m x 0.25mm ID standard film (0.25μm) column, at much lower cost. Similar analyses can be obtained from equivalent 0.32mm ID columns, 30m x 0.53mm ID columns (1.5μm film) and 60m x 0.75mm ID columns (1.0μm 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

| Packing: 3%SP™-2100 DOH on 100/120 SUPELCOPORT™, 10' x 2mm ID glass |  
| Cat. No.: 12101 |  
| Col. Temp.: 150°C to 225°C at 4°C/min. |  
| Carrier: N2, 20mL/min. |  
| Det.: FID |  
| Inj.: 1µL, 250°C |  

<table>
<thead>
<tr>
<th>A1 — P. cepacia</th>
<th>A2 — P. aeruginosa</th>
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</thead>
<tbody>
<tr>
<td>14:0 16:0 18:0</td>
<td>14:0 16:0 18:0</td>
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<tr>
<td>30H 15:0 16:0</td>
<td>30H 15:0 16:0</td>
</tr>
<tr>
<td>17:0 Δ</td>
<td>17:0 Δ</td>
</tr>
<tr>
<td>19:0 Δ</td>
<td>19:0 Δ</td>
</tr>
<tr>
<td>16:1 Δ</td>
<td>16:1 Δ</td>
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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.
### Figure B. Fatty Acids in Microorganisms on a Packed Column

**Packing:**
- GP3% 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, 25µL, 250°C

**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, 285cm/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

#### 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
- 15m x 0.20mm ID, 0.20µm film
- 30m x 0.20mm ID, 0.20µm film
- 15m x 0.25mm ID, 1.0µm film
- 30m x 0.25mm ID, 0.25µm film
- 15m x 0.32mm ID, 1.0µm film
- 30m x 0.32mm ID, 0.25µm film
- 30m x 0.53mm ID, 1.5µm film

- Borosilicate Glass
- 60m x 0.75mm ID, 1.0µm film
- 23720-U
- Kit (20 x 2mL ampuls)

#### Trademarks

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- SPB — Supelco, Inc.

- SUPELCOPORT — Supelco, Inc.

Fused silica columns manufactured under HP US Pat. No. 4,293,415.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

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**Det.:** FID, 270°C

**Inj.:** 1µL, 250°C, split injection (100:1)

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### References

3. 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.
4. 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.
5. Wadsworth Anaerobic Bacteriology Manual. Direct inquiries about availability to: Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, Veterans Administration, Los Angeles, CA 90073, USA.

References not available from Supelco.