Cellular Fatty Acid Composition of *Haemophilus equigenitalis*

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The cellular fatty acid composition of eight *Haemophilus equigenitalis* strains was determined by gas-liquid chromatography. All strains showed a grossly similar pattern characterized by large amounts of 18:1 and 16:0. The amounts of 16:1, 18:2, 18:0, 3-OH 14:0, 3-OH 16:0, and 3-OH 18:1 were relatively small.

*Haemophilus equigenitalis* is the causative agent of contagious equine metritis. This venereal disease of horses, whose occurrence was first reported by Crowhurst (2) in 1977 in Ireland, rapidly spread over the world, to the European countries, the United States, Australia, and Japan (5, 15, 20, 21).

Taylor et al. (23) proposed that this organism be classified within the genus *Haemophilus* on the basis of its DNA guanine-plus-cytosine contents (36.1 mol%) and its requirement of X factor as reported by Shreeve (19). Taylor et al. (23) and Sugimoto et al. (20), however, confirmed that this organism does not require X nor V factors and is positive in the porphyrin test (9). Consequently, the taxonomic position of *H. equigenitalis* is still questionable (24).

This organism is a gram-negative, microaerophilic, and nonfermentative coccobacillus and is very unreactive in the conventional biochemical tests; it is positive only in the catalase, oxidase, and phosphatase tests (23). This characteristic makes taxonomic study of this organism difficult.

The cellular fatty acid composition of bacterial cells has been successfully utilized in the identification and classification of bacteria (4, 8, 18). Braunthal et al. (1) and Jantzen et al. (7) studied the cellular fatty acid compositions of species of *Haemophilus* and revealed that they were grossly similar to those of species of *Pasteurella* and *Actinobacillus*. The present study was undertaken to determine the cellular fatty acid composition of *H. equigenitalis*.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** Eight strains of *H. equigenitalis*, including a reference strain (NCTC 11184 [23]), were used. Six strains were isolated from horses in Japan (20), and one was isolated in the United States. The strains were cultivated on Eugon agar (BBL Microbiology Systems) supplemented with 5% Fildes pepsin-digested sheep blood (Fildes agar). To examine the effects of culture condition on the cellular fatty acid composition, 5% sheep blood agar, 10% horse blood chocolate agar, and IsoVitaleX (BBL Microbiology Systems)-hemin agar prepared from Eugon agar as a basal medium were also used.

Incubation was performed for 72 h at 37°C in the presence of 10% CO₂. The bacteria were harvested and washed three times with saline before lyophilization.

**Chemical procedures and gas-liquid chromatography.** Dried bacterial cells (about 20 mg) were methanolyzed by 5% HCl in methanol as previously described (13). Gas-liquid chromatography of fatty acid methyl esters was carried out on a Hewlett-Packard 5710A gas chromatograph equipped with a flexible fused silica capillary column (0.28 mm by 30 m) coated with OV-101.

**Identification of fatty acids.** Fatty acids were primarily identified by equivalent chain length calculated using a standard mixture of fatty acid methyl esters (11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0) as previously described (13). All structural elucidations were verified by a gas chromatograph-mass spectrometer-computer system (Hewlett-Packard 5992B) equipped with a glass capillary column (0.28 mm by 30 m) coated with OV-101.

**RESULTS**

The cellular fatty acid composition of *H. equigenitalis* (NCTC 11184) cells cultivated under various culture conditions were compared. There was slight difference in the cellular fatty acid composition among cells cultivated on four different media (Table 1). The cells cultivated on blood agar contained a larger amount of 18:0 and a smaller amount of 18:1 than those cultivated on the other media. A minor component, 18:2, was not detected in the cells cultivated on IsoVitaleX-hemin agar. The cellular fatty acid composition of *H. equigenitalis*, however, was thought not to be critically influenced by the culture media. Fildes agar was used throughout the following experiments because it gave good growth of all strains tested. There was no significant difference between the cellular fatty acid compositions of 48-h-old cells and 72-h-old cells cultivated on Fildes agar.

The structures of all fatty acids detected were verified by gas chromatograph-mass spectrome-
**TABLE 1.** Cellular fatty acid composition of *H. equigenitalis* (NCTC 11184) cells cultivated under various culture conditions

<table>
<thead>
<tr>
<th>ECL</th>
<th>Fatty acid</th>
<th>% of total acids 72 h&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% of total acids 48 h&lt;sup&gt;b&lt;/sup&gt;, FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BA</td>
</tr>
<tr>
<td>13.97</td>
<td>14:0</td>
<td>1.72</td>
<td>0.53</td>
</tr>
<tr>
<td>15.42</td>
<td>3-OH 14:0</td>
<td>3.61</td>
<td>3.71</td>
</tr>
<tr>
<td>15.75</td>
<td>16:1</td>
<td>1.70</td>
<td>1.00</td>
</tr>
<tr>
<td>16.00</td>
<td>16:0</td>
<td>29.4</td>
<td>28.1</td>
</tr>
<tr>
<td>17.43</td>
<td>3-OH 16:0</td>
<td>0.28</td>
<td>0.42</td>
</tr>
<tr>
<td>17.58</td>
<td>18:2</td>
<td>0.84</td>
<td>0.20</td>
</tr>
<tr>
<td>17.76</td>
<td>18:1</td>
<td>55.3</td>
<td>41.2</td>
</tr>
<tr>
<td>17.99</td>
<td>18:0</td>
<td>4.02</td>
<td>12.8</td>
</tr>
<tr>
<td>19.16</td>
<td>3-OH 18:1</td>
<td>1.66</td>
<td>1.16</td>
</tr>
</tbody>
</table>

<sup>a</sup> ECL, Equivalent chain length.
<sup>b</sup> Culture age.
<sup>c</sup> Media: FA, Fildes agar; BA, 5% sheep blood agar; CA, 10% horse blood chocolate agar; IHA, IsoVitaleX-hemin agar; Eugonagar (BBL Microbiology Systems) was used as a basal medium.
<sup>d</sup> ND, Not detected.

**FIG. 1.** Mass spectra of two 3-hydroxy fatty acid methyl esters.
characterized by large amounts of 18:1 and 16:0 and relatively small amounts of 14:0, 16:1, 18:2, and 18:0. Amounts of 3-OH hydroxy fatty acids were not so large, but the existence of 3-OH 18:1, which had not been detected in other species thus far studied, was characteristic.

The cellular fatty acid composition of H. equigenitalis is clearly distinct from those of true members of the genus Haemophilus. Species of Haemophilus contain large amounts of C16 fatty acids (16:1, 16:0) and small amounts of C18 fatty acids (18:2, 18:1, 18:0) as reported by Jantzen et al. (7). Species of Haemophilus showed a cellular fatty acid pattern indistinguishable from those of Pasteurella and Actinobacillus species, which also share a number of phenotypic characters with Haemophilus species and whose DNA guanine-plus-cytosine contents are in the same range as the genus Haemophilus (10, 12, 24). H. equigenitalis, however, seems to be clearly separated from Actinobacillus-Haemophilus-Pasteurella group in its cellular fatty acid composition.

The cellular fatty acid composition of H. equigenitalis shows partial similarity to those of Brucella canis and species of Moraxella and Acinetobacter in its abundance of 18:1 (3, 8, 11, 16). These species contain 18:1 as a major fatty acid, but their amounts of C16 fatty acids are not so large as those of species of Haemophilus. The cellular fatty acid composition of H. equigenitalis is distinctively different from those of other gram-negative bacteria so far analyzed, including species of Francisella, Legionella, and Brucella, except Brucella canis (3, 6, 14, 22). Further studies of H. equigenitalis based on the cellular fatty acid composition is now proceeding in our laboratory to clarify the taxonomic position of this organism.

### DISCUSSION

The cellular fatty acid compositions of eight H. equigenitalis strains tested in the present study were very similar, although two of these strains were collected from geographically separate regions. Consequently, the cellular fatty acid composition well characterizes this organism whose characterization by the conventional biochemical tests is difficult because of its extreme unreactiveness.

The cellular fatty acid composition is known to be influenced by composition of culture medium, culture age of cells, temperature of incubation, and other culture conditions (4). The cellular fatty acid composition of H. equigenitalis, however, was relatively stable even if the culture conditions had changed. This fact makes it possible to compare the results in our study with those in other species.

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LITERATURE CITED


