Fatty Acid Composition of Propionibacterium propionicum (Arachnia propionica)

CECIL S. CUMMINS1* and C. WAYNE MOSS2

Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, and Division of Bacterial Diseases, Center for Infectious Disease, Centers for Disease Control, Atlanta, Georgia 30333

The cellular fatty acids of Arachnia propionica are largely 13-methyltetradecanoic acid (C15:0) and 12-methylltetradecanoic acid (C15:0); thus, the fatty acid pattern of this organism resembles the pattern found in propionibacteria. This finding supports the transfer of members of the genus Arachnia to the genus Propionibacterium.

Since the original description of Arachnia propionica (3, 12), the position of this organism among the microaerophilic actinomycetes has been anomalous, since it produces acetic and propionic acids as the major products of glucose fermentation and has L-L-diaminopimelic acid as the diamino acid in its peptidoglycan. Pine (11) proposed that this organism should be transferred to the genus Propionibacterium, and Allen and Linehan (1) found that Arachnia propionica produces propionic acid by the methylmalonyl-coenzyme A pathway used by other propionibacteria. More recently, Charfreitag et al. (4) have shown by using 16S RNA sequencing that this organism is closely related to propionibacteria but unrelated to members of the genus Actinomyces. In view of this, Charfreitag et al. (4) suggested that Arachnia propionica should be renamed Propionibacterium propionicum. However, the name should probably be Propionibacterium propionicum since Propionibacterium is a neuter noun (K. P. Schaal, personal communication).

The data in this paper establish another similarity between strains of P. propionicum and other propionibacteria, namely, that the fatty acid profile of P. propionicum consists largely of iso- and anteiso-methyl branched long-chain fatty acids, principally C15:0 and C15:1 acids.

Strains. The following strains of P. propionicum (Arachnia propionica) were examined: VPI 0026 (= ATCC 14157), VPI 5069, VPI 5070, and VPI 5074. In addition, two strains of Actinomyces naeslundii, strains VPI 5072 and VPI 5073, were included. (In a previous paper [5] strain VPI 5072 was described as an Arachnia propionica strain, but this is clearly wrong and must have been due to an error in numbering or proofreading in the earlier work.)

Growth of organisms. The strains were grown in a Trypticase-yeast extract-glucose medium containing 0.05% Tween 80. Generally, 250 ml of medium in flasks under an N2 atmosphere was inoculated with 20 ml of a 4-day culture grown in the same medium, and the organisms were grown for an additional 4 days at 35 to 36°C. Formalin was then added to a final concentration of 0.1%, and after standing 1 h at 25°C the organisms were recovered by centrifugation (15,000 × g, 10 min), washed twice in distilled water, and finally suspended in 5 ml of distilled water containing 0.05% NaN3. Suspensions were kept at 4°C prior to fatty acid analysis.

Fatty acid analysis. Cells were saponified and processed for total cellular fatty acid as described previously (9). The resulting fatty acid methyl ester samples were analyzed with a microbial system (Hewlett-Packard Co., Avondale, Pa.) which contained a methyl phenyl silicone fused capillary column (25 m by 0.2 mm [inside diameter]) (6, 9). Fatty acid methyl esters were tentatively identified by determining computer-calculated equivalent chain length values compared with equivalent chain length values of authentic standards; identities were confirmed by hydrogenating unsaturated acids and performing combined gas chromatography-mass spectrometry. The locations of the double bond positions of monounsaturated fatty acids were determined by a gas chromatography-mass spectrometry analysis of dimethyl disulfide derivatives of fatty acid methyl esters (8).

Propionibacteria are characterized by 12- and 13-methyltetradecanoic acids, as shown by Moss et al. (7), whereas Actinomyces species exhibit a tetradecanoic to octodecanoic pattern (13). It is evident from Table 1 that the four strains of P. propionicum which we examined are characterized by large amounts of branched-chain 15-carbon acids, with the methyl branch at the iso or anteiso position in the carbon chain, together with small amounts of C16:0 and C18:1 acids. Three of the four strains also contained an unusual monounsaturated 18-carbon acid (equivalent chain length, 17.838) in amounts ranging from 2 to 22%. This acid was identified by hydrogenation and combined gas chromatography-mass spectrometry as a monounsaturated straight-chain 18-carbon acid with the double bond at position w08 (or A10) (6, 8). In contrast to P. propionicum, the two strains of Actinomyces naeslundii contained predominantly C16:0 and C18:1 acids, and branched-chain 15-carbon acids either were not found or were present in only trace amounts.

W. E. C. Moore (Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg) also examined the fatty acid profiles of these four strains and found a similar preponderance of 15-carbon iso- and anteiso-branched acids (unpublished data) (included in the VPI Broth Library for Bacterial Identification [Microbial ID, Inc., Newark, Del.]). However, he did not find any evidence of the C19:1w8 acid but instead found up to 15% C20:1w9 (11) acid. However, the cultures which he examined were all less than 24 h old and had been grown in a pepticase medium instead of the Trypticase medium which we used, which may explain the differences in the results.

A difference in the ages of the cultures may also explain the considerable difference (2 versus 22%) in the amounts of the C19:1w8 acid found in the two preparations of strain VPI 0026; the higher amount (22%) was found in the older culture (incubated for 6 to 7 days instead of 4 days).

References in the literature to the fatty acid profiles of P. propionicum strains are scanty and somewhat contradictory.
TABLE 1. Major fatty acids of *P. propionicum* (Arachnia propionica)*a*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fatty acid composition (% of total)</th>
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<tr>
<td></td>
<td>C15:0</td>
</tr>
<tr>
<td><em>P. propionicum</em> strains</td>
<td></td>
</tr>
<tr>
<td>VPI 0026 prep 1</td>
<td>29</td>
</tr>
<tr>
<td>VPI 0026 prep 2</td>
<td>18</td>
</tr>
<tr>
<td>VPI 5069</td>
<td>26</td>
</tr>
<tr>
<td>VPI 5070</td>
<td>17</td>
</tr>
<tr>
<td>VPI 5074</td>
<td>22</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> strains</td>
<td></td>
</tr>
<tr>
<td>VPI 5072</td>
<td>ND</td>
</tr>
<tr>
<td>VPI 5073</td>
<td>ND</td>
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</tbody>
</table>

*a In addition to the fatty acid components listed, a number of other fatty acids were found inconstantly at levels of less than 5%.
*b Fatty acids with equivalent chain lengths of 17.838.
*c Two preparations of strain VPI 0026 were examined.
*d ND, Not detected.
*e Tr, Trace (less than 1%).

Amdur et al. (2) state that the fatty acids of strains of *Arachnia propionica* comprise only even-numbered n-saturated and n-unsaturated acids, and this reference is cited in Bergey's Manual of Systematic Bacteriology (14). On the other hand, Charfreitag et al. (4) state that "Arachnia propionica also resembles propionibacteria...in possessing major amounts of iso- and anteiso-methyl branched long-chain fatty acids," but the two references which they give do not appear to deal with fatty acid contents. However, O'Donnell et al. (10), who examined *Arachnia propionica* ATCC 14175 and ATCC 29326, as well as two other strains from clinical sources, found that all four strains contained 13-methyltetradecanoic and 12-methyltetradecanoic acids as major fatty acid components.

Our results are in agreement with those of O'Donnell et al. (10) and show that *P. propionicum* does indeed have a fatty acid profile which resembles that of other propionic acid bacteria. These chemical data provide additional support for the transfer of *Arachnia propionica* to the genus *Propionibacterium* as *P. propionicum*.

We thank W. E. C. Moore for allowing us to cite unpublished data and the referees for suggesting additional references.

LITERATURE CITED