Fatty Acids of *Leptotrichia buccalis*: Taxonomic Implications

By TOR HOFSTAD¹* AND ERIK JANTZEN²

¹ Department of Microbiology and Immunology, The Gade Institute, University of Bergen, N-5016 Haukeland sykehus, Bergen, Norway
² National Institute of Public Health, Oslo, Norway

(Received 7 April 1981)

Fatty acids of five strains of *Leptotrichia buccalis* were examined by gas–liquid chromatography. The strains showed identical patterns, characterized by the presence of n-hexadecanoate, octadecenoate and 3-hydroxytetradecanoate as major acids. The general fatty acid pattern showed a distinct similarity to that of *Fusobacterium* species.

INTRODUCTION

The Gram-negative oral organism *Leptotrichia buccalis* is currently classified as a member of the family *Bacteroidaceae* (Rogosa, 1974). With respect to morphology and biochemical properties the organism seems to form a well circumscribed species. However, some intraspecies heterogeneity does exist. Several strains can grow in air in the presence of CO₂, whereas others are obligately anaerobic. Also, analyses of cell wall lipopolysaccharides showed differences amongst the strains examined (Birkeland & Hofstad, unpublished observations).

Gas–liquid chromatography (g.l.c.) of cellular fatty acids has proved useful in differentiating facultative as well as anaerobic bacteria (Lechevalier, 1977; Shaw, 1974). We have recently examined the fatty acid composition of several species of *Fusobacterium* by means of g.l.c. (Jantzen & Hofstad, 1981). The same technique has now been used in a study of *Leptotrichia buccalis*. A comparison is made with *Fusobacterium nucleatum*, since a phylogenetic relationship between these two organisms has been suggested (Page & Krywolap, 1977).

METHODS

Strains and cultivation. *Leptotrichia buccalis* strains ATCC 14201 and ATCC 19616 were received from W. Kondo, Niigata, Japan. Strains L11, L92 and L97 were isolated from saliva of healthy human individuals using blood agar (Hofstad, 1967), or a selective medium (Walker et al., 1979), and anaerobic incubation. Strains ATCC 14201 and L97 are obligately anaerobic; the other three strains grow in air in a CO₂ incubator. In other respects, the strains have similar cultural and physiological properties.

The bacteria were cultivated on blood agar plates (Difco Tryptone agar supplemented with 0.001% (w/v) menadione and 7% (v/v) human blood) at 37 °C for 72 h in a deoxygenated CO₂ atmosphere (BBL GasPak jars). Bacterial growth was washed from the blood agar using a right-angled glass rod and sterile water. Biomass was sedimented by centrifugation at 3000 g for 20 min, washed twice with distilled water, freeze-dried and stored under nitrogen in closed vials at −20 °C.

Chemicals, chemical procedures and gas–liquid chromatography. Solvents of analytical grade were distilled before use. The 2 M-HCl in methanol was obtained by bubbling dry HCl gas (Fluka, Buchs, Switzerland) into dried methanol until saturated, and subsequent dilution. Fatty acid methyl ester standards were obtained from Applied Science Laboratories, State College, Pa., U.S.A.

Dried bacterial cells (1–10 mg) were treated with 2 M-HCl in methanol, and the dimethyl acetals were removed as previously described (Jantzen & Hofstad, 1981). After trifluoroacetylation of hydroxylated fatty acids (Jantzen & Hofstad, 1981) the fatty acid methyl esters were analysed on a Hewlett-Packard 5710 gas chromatograph.
Table 1. Fatty acid composition of Leptotrichia buccalis

<table>
<thead>
<tr>
<th>Strain</th>
<th>12:0</th>
<th>14:0</th>
<th>14:0</th>
<th>15:0</th>
<th>16:1</th>
<th>16:0</th>
<th>X†</th>
<th>18:1</th>
<th>18:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 14201</td>
<td>0.4</td>
<td>5.1</td>
<td>15.1</td>
<td>0.6</td>
<td>2.8</td>
<td>35.2</td>
<td>2.0</td>
<td>35.2</td>
<td>3.7</td>
</tr>
<tr>
<td>ATCC 19616</td>
<td>0.7</td>
<td>4.7</td>
<td>15.5</td>
<td>0.4</td>
<td>2.3</td>
<td>41.1</td>
<td>1.0</td>
<td>32.1</td>
<td>2.2</td>
</tr>
<tr>
<td>L11</td>
<td>1.6</td>
<td>6.0</td>
<td>12.7</td>
<td>0.4</td>
<td>2.8</td>
<td>42.5</td>
<td>1.4</td>
<td>30.6</td>
<td>2.7</td>
</tr>
<tr>
<td>L92</td>
<td>tr</td>
<td>4.1</td>
<td>17.1</td>
<td>0.6</td>
<td>0.9</td>
<td>42.5</td>
<td>1.5</td>
<td>29.5</td>
<td>4.0</td>
</tr>
<tr>
<td>L97</td>
<td>0.6</td>
<td>6.5</td>
<td>18.8</td>
<td>0.9</td>
<td>0.9</td>
<td>2.9</td>
<td>36.3</td>
<td>1.1</td>
<td>26.8</td>
</tr>
</tbody>
</table>

tr, less than 0.1%.

* The figure before the colon indicates the number of carbon atoms in the chain, that after the colon denotes the number of double bonds (the position of the double bonds has not been determined); 3-OH indicates the presence of a 3-hydroxy group.
† A compound which co-chromatographed with octadecadienoate.

RESULTS

All five test strains of Leptotrichia buccalis exhibited a very similar fatty acid composition characterized by relatively large amounts of n-hexadecanoate (16:0), octadecenoate (18:1) and 3-hydroxytetradecanoate (3-OH-14:0) (Table 1). Present in all the strains, but in smaller amounts, were n-dodecanoate (12:0), n-tetradecanoate (14:0), n-pentadecanoate (15:0), hexadecanoate (16:0), n-octadecanoate (18:0), and a constituent which co-chromatographed with octadecadienoate (18:2) (Jantzen & Hofstad, 1981). Cyclopropane-substituted acids and methyl-branched isomers could not be detected. This general fatty acid profile for L. buccalis was similar to the profiles for Fusobacterium species (Jantzen & Hofstad, 1981).

DISCUSSION

Leptotrichia buccalis has a fatty acid composition characteristic of a Gram-negative bacterium. The close similarity in fatty acid patterns amongst the five strains examined substantiates the designation of L. buccalis as a species.

There are distinct similarities between L. buccalis and Fusobacterium nucleatum (Table 2). Both organisms appear in Gram-stained smears as Gram-negative fusiform rods. The guanine plus cytosine (G + C) contents of the DNA are similar in both organisms (25–27%) and unusually low (Page & Krywolap, 1976). With the exception of 3-hydroxyhexadecanoic acid, which is a taxonomic marker for F. nucleatum (Jantzen & Hofstad, 1981), the two organisms have a grossly similar fatty acid pattern. The relative amount of octadecenoic acid is higher in L. buccalis than in F. nucleatum, whereas F. nucleatum contains relatively more n-tetradecanoic acid. The principle phenotypic differences between the two organisms are that L. buccalis metabolizes a range of different sugars whereas F. nucleatum is asaccharoelastic, and, as indicated above, L. buccalis is not an obligately anaerobic organism.
Fatty acids of Leptotrichia

Table 2. Main characteristics of Leptotrichia buccalis and Fusobacterium nucleatum

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Leptotrichia buccalis</th>
<th>Fusobacterium nucleatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Fusiform</td>
<td>Fusiform</td>
</tr>
<tr>
<td>Habitat</td>
<td>Human oral cavity</td>
<td>Human oral cavity</td>
</tr>
<tr>
<td>Diamino acid of the peptidoglycan*</td>
<td>Diaminopimelic acid</td>
<td>Lanthionine</td>
</tr>
<tr>
<td>Main fatty acids</td>
<td>16:0, 18:1, 3-OH-14:0</td>
<td>14:0, 16:0, 3-OH-14:0 (3-OH-16:0†)</td>
</tr>
<tr>
<td>Fermentation of carbohydrates</td>
<td>Saccharoclastic</td>
<td>Asaccharoclastic</td>
</tr>
<tr>
<td>Main metabolic end-product</td>
<td>Lactic acid</td>
<td>Butyric acid</td>
</tr>
<tr>
<td>Relation to oxygen</td>
<td>Faculative or anaerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Mol % G + C ‡</td>
<td>25</td>
<td>25–27</td>
</tr>
</tbody>
</table>

* From Davis & Baird-Parker (1959) and Vasstrand et al. (1979).
† Specific to F. nucleatum, not found in other species of Fusobacterium (Jantzen & Hofstad, 1981).
‡ From Page & Krywolap (1976).

The taxonomic position of L. buccalis is still uncertain, but awaiting further data, the present position in the family Bacteroidaceae should be maintained.

The study was supported in part by the Norwegian Research Council for Science and the Humanities.

REFERENCES