Fatty Acids of *Leptotrichia buccalis*: Taxonomic Implications

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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 Also, analyses of cell wall lipopolysaccharides showed differences amongst the strains examined (Birkeland & Hofstad, unpublished observations).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*

Strain	Fatty acid composition* (% w/w, of total in strain)								
	12:0	14:0	3-OH 14:0	15:0	16:1	16:0	X†	18:1	18:0
ATCC 14201	0.4	5.1	15.1	0.6	2.8	35.2	2.0	35.2	3.7
ATCC 19616	0.7	4.7	15.5	0.4	2.3	41.1	1.0	32.1	2.2
L11	1.6	6.0	12.7	0.4	2.8	42.4	1.4	30.6	2.7
L92	tr	4.1	17.1	0.6	0.9	42.5	1.5	29.5	4.0
L97	0.6	6.5	18.8	0.9	2.9	36.3	1.1	26.8	3.9

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-hydroxyl group.

† A compound which co-chromatographed with octadecadienoate.

equipped with a flame ionization detector, capillary column control (Hewlett-Packard 18740), and a methyl silicone-coated capillary column (25 m × 0.2 mm SE-30 fused silica; Hewlett-Packard) as previously described (Jantzen & Hofstad, 1981). The chromatograms and analytical results were provided by a digital recorder/integrator (Hewlett-Packard 3390A).

The fatty acid methyl esters were identified by comparison of retention data with those of standards. Hydroxy fatty acids were recognized by trifluoroacetylation and rechromatography (Jantzen & Hofstad, 1981).

All structural elucidations were verified by gas chromatography/mass spectrometry [Hewlett-Packard 5992A instrument equipped with a methyl silicone-coated capillary column (25 m × 0.5 mm CP Sil 5 glass capillary column; Chrompack)] (Jantzen & Hofstad, 1981).

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), hexadecenoate (16:1), *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 asaccharoclastic, and, as indicated above, *L. buccalis* is not an obligately anaerobic organism.

Table 2. Main characteristics of *Leptotrichia buccalis* and *Fusobacterium nucleatum*

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

* 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.

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