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Note

Cellular fatty acids of *Bacteroides forsythus*

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Bacteroides forsythus is a Gram-negative anaerobic rod isolated from advanced periodontal lesions in human oral cavities [1]. It was originally described as an fastidious organism growing slowly on blood agar and giving inconsistent growth in broth media [1]. Recently, it was detected that this organism had a unique nutritional requirement for N-acetylmuramic acid [2]. Addition of N-acetylmuramic acid to conventional bateriological media now allows routine cultivation of this organism [2]. The taxonomic position of *B. forsythus* is currently under reconsideration [3].

Cellular fatty acid profiles obtained by gas chromatography (GC) and mass spectrometry (MS) after methylation and derivatization of whole cells have proved to be useful in the taxonomy of the genus *Bacteroides* [4,5]. The present paper describes the cellular fatty acids of *B. forsythus*.

EXPERIMENTAL

Bacteria

The type strain of *B. forsythus* (American Type Culture Collection 43037) and two other strains of this organism (BF331 and BF472) were obtained from C. Wyss, Dental Institute of the University of Zürich. They were grown on the surface of blood agar plates at 37°C in an anaerobic chamber with an atmosphere containing 5% dioxide and 10% hydrogen in nitrogen. After five days the colonies on the plates were scraped off and lyophilized. The blood agar medium was prepared by the method of Holdeman *et al.* [6]. Defibrinated horse blood (50 ml)

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was haemolysed by freeze–thawing before it was added to 1 l of the medium, together with 10 ml of a filter-sterilized solution containing 100 mg of N-acetylmuramic acid.

Methanolysis and derivatization

Lyophilized whole cells were methanolyzed with 2 *M* hydrochloric acid in anhydrous methanol for 24 h at 95°C [7]. The methanolysate was kept in an ice-bath, evaporated with nitrogen and then extracted with *n*-hexane.

The above procedure was used for routine examination of the fatty acid content. For assessment of hydroxy fatty acids, the hydroxy groups were derivatized with a mixture of acetonitrile (Rathburn Chemicals, Walkenburn, U.K.) and 10% (v/v) trifluoroacetic acid anhydride (Fluka, Buchs, Switzerland) at 90°C for 3 min. Thereafter, the solution was diluted with acetonitrile so that it contained 1% trichloroacetic acid anhydride [7].

Gas chromatography and gas chromatography–mass spectrometry

A Model 8700 gas chromatograph (Perkin Elmer, Norwalk, CT, U.S.A.) was used. The HP Ultra Performance Column Ultra 1 (Hewlett-Packard, Avondale, PA, U.S.A) was used (25 m × 0.20 mm I.D.)

Helium served as carrier gas at a flow-rate of 2.0 ml/min. The temperature of the injector was 200°C and that of the flame ionization detector 290°C. The programme was as follows: the temperature was held for 1 min at 90°C and then it was increased at 6°C/min to 275°C. The attenuator was set at 16, and the paper speed was 5 mm/min. The sample (0.2 μl) was delivered by splitless injection.

The identities of the methanolysed and derivatized fatty acids were confirmed by co-chromatography with authentic standards and by gas chromatography–mass spectrometry (GC–MS). The instrument used for GC–MS was a Model 4200 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a Model 7072F mass spectrometer (Vg Micromass, Cheshire, U.K.) and a Type 2200 data system (Vg Micromass). From each sample two independent derivatizations were prepared. Three injections were made from each derivative. The amounts of the substances, expressed in relative percentages, were calculated from the area under each peak and corrected with the molar response factor [7]. The sum of identified substances was considered to be 100%.

For identification of fatty acids, methyl esters of decanoic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic and octadecanoic acid were obtained from Sigma (St. Louis, MO, U.S.A.). From Supelco (Bellefonte, PA, U.S A.) were received bacterial acid methyl esters mixture Cp (No. 4-7080), GC standard mixture GLC 70 (No. 4-7044), American Oil Chemists' Society oil reference mixture, PM-1 rapeseed oil (No. 4-7019), National Institutes of Health reference mixtures (Nos. A-NHI-C 4-7010, A-NHI-D and A-NHI-F 4-7013) and FA-FAME kit 14 (No. 1039). From Larodan Fine Chemicals (Malmö, Sweden) were obtained 13-methyltetradecanoic acid methyl ester, 12-methyltetradecanoic acid

methyl ester, 14-methylhexadecanoic methyl ester, 14-methylpentadecanoic methyl ester, 15-methylhexadecanoic acid methyl ester and 11-methyltridecanoic methyl ester. Lipopolysaccharides extracted from *Porphyromonas gingivalis* ATCC 33277 and *Actinobacillus actinomycetemcomitans* ATCC 33384 [7] in phenol–water were methanolysed and derivatized for use as standards for identification of fatty acids.

RESULTS

The distribution of fatty acids in whole-cell methanolysates of three strains of *B. forsythus* is shown in Table I. Characteristics of this profile are the high level of 12-methyltetradecanoic acid and low levels of hydroxy acids (Fig. 1).

DISCUSSION

The low levels of hydroxy fatty acids in whole-cell methanolysates of *B. forsythus* is remarkable. It may mean that this species does not have the conventional cell-wall lipopolysaccharide of Gram-negative bacteria.

The taxonomic position of *B. forsythus* is uncertain [3]. This is the first report on the whole-cell fatty acid profile of the organism. No other species of *Bacteroides* exhibit either such a very high ratio of 12-methyltetradodecanoic acid to 13-methyltetradodecanoic acid or the absence of 3-hydroxy-15-methylhexadeda-

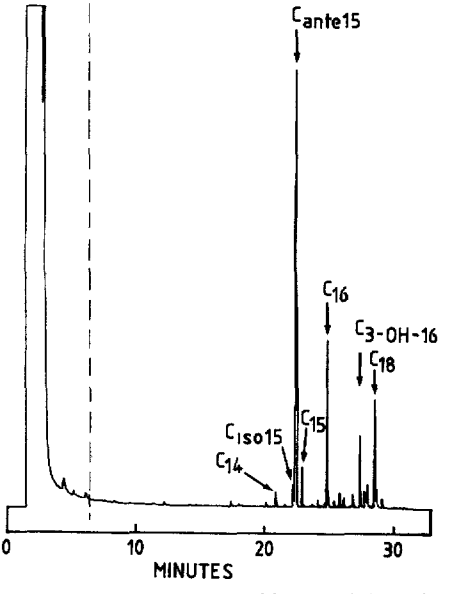

Fig. 1 Gas chromatogram of fatty acids in *B forsythus* strain BF472 Chromatographic details are given in the text

TABLE I

FATTY ACIDS IN THREE STRAINS OF *B FORSYTHUS* AS REVEALED BY GC–MS AFTER METHYLATION OF WHOLE CELLS

Bacterial strain	Relative amounts[a] (%)						
	$C_{14\,0}$	C_{iso15}	C_{ante15}	$C_{15\,0}$	$C_{16\,0}$	$C_{3\text{-}OH\text{-}16}$	$C_{18\,0}$
ATCC 43037	1 0	3.8	62 8	2 1	7 0	3 4	7 5
BF331	4.3	4.3	52.8	1 4	10 0	8 6	5 6
BF472	1.4	2 6	60.4	2 5	12 6	3.6	5 5

[a] $n = 6$, S D = 3%.

noic acid [4,5,8]. This finding supports the suggestion that *B. forsythus* should be removed from the genus *Bacteroides* [3]. Knowledge of the whole-cell fatty acid composition will facilitate the definition of the proper taxonomic position of the organism.

CONCLUSIONS

B. forsythus has a unique requirement of N-acetylmuramic acid for growth, and its taxonomic position is under reconsideration. The whole-cell fatty acid profile of the organism demonstrated in the present study indicates that the organism should not be a member of the genus *Bacteroides*.

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