Multivariate analyses of fatty acid data from whole-cell methanolysates of *Prevotella*, *Bacteroides* and *Porphyromonas* spp.

ILIA BRONDZ,^{1*}† INGAR OLSEN,² MARKUS HAAPASALO³ and ARIE JAN VAN WINKELHOFF⁴

¹ Research Department, National Institute of Public Health, Umeå, Sweden

² Department of Microbiology, Dental Faculty, University of Oslo, Oslo, Norway

³ Department of Cariology, University of Helsinki, Helsinki, Finland

⁴ Department of Oral Microbiology, Schools of Dentistry and Medicine, Free University, Amsterdam, The Netherlands

(Received 2 August 1990; revised 19 November 1990; accepted 13 February 1991)

The genus *Bacteroides* contains a number of biochemically and physiologically heterogeneous groups of organisms and needs taxonomic revision. In this study cellular fatty acids from a number of *Bacteroides* spp. were identified and quantified using gas chromatography and gas chromatography–mass spectrometry. The chemical data were then subjected to principal components analysis. In *B. fragilis*, which is the type species of the genus *Bacteroides*, C_{3-OH-iso17} was the predominant fatty acid (38.0%) and C_{ante15} was present in higher amounts (32.7%) than C_{iso15} (14.6%). *B. fragilis* thus differed from all the other species examined: *Prevotella* (*Bacteroides*) *buccae*, *P. (B.) oralis*, *P. (B.) oris*, *P. (B.) disiens*, *P. (B.) veroralis*, *P. (B.) heparinolytica* and *Porphyromonas* (*Bacteroides*) *endodontalis*. Principal components analysis also enabled the closely related *P. buccae*, *P. oralis* and *P. oris* to be differentiated.

Introduction

Bacteroides species are obligately anaerobic, Gram-negative, non-motile and motile rods commonly encountered in polymicrobial infections at various sites in humans and animals, including the oral cavity, the urinary, gastrointestinal and respiratory tracts, and post-surgical wounds (Finegold & George, 1989). Due to their major role in anaerobic infections of these sites, *Bacteroides* species have been under intensive study in recent years.

According to the *Approved Lists of Bacterial Names* (Moore *et al.*, 1985), the genus *Bacteroides* comprises more than 50 species and has been used continuously as a repository for a varied collection of obligately anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria (Shah & Collins, 1989). Therefore, this genus now contains biochemically and physiologically heterogeneous groups of organisms and is in need of taxonomic revision. Some efforts have been made already to achieve this. Several *Bacteroides* species have been proposed to have separate generic status, e.g. *Anaerorhabdus* for *B. furcosus* (Shah &

Collins, 1986); *Megamonas* for *B. hypermegas* (Shah & Collins, 1982a); *Mitsuokella* for *B. multiacidus* (Shah & Collins, 1982b); *Porphyromonas* for *B. asaccharolyticus*, *B. endodontalis* and *B. gingivalis* (Shah & Collins, 1988); *Rikenella* for *B. microfus* (Collins *et al.*, 1985); *Sebaldella* for *B. termiditis* (Collins & Shah, 1986a); *Tissierella* for *B. praeacutus* (Collins & Shah, 1986b); and *Prevotella* for *B. melaninogenicus*, *B. oralis* and related species (Shah & Collins, 1990).

There is biochemical, chemical and molecular biological evidence that the genus *Bacteroides* should be restricted to the type species *B. fragilis* and closely related organisms such as *B. caccae*, *B. distasonis*, *B. eggerthii*, *B. merdae*, *B. ovatus*, *B. stercoris*, *B. thetaiotaomicron*, *B. uniformis* and *B. vulgatus* (Collins & Shah, 1987; Shah & Collins, 1989). The *B. fragilis* group has a narrow G + C range and is biochemically and taxonomically homogeneous (Shah & Collins, 1983; Collins & Shah, 1987). Also, rRNA homology studies have shown that *B. fragilis* and related species are coherent and distinct from other *Bacteroides* species (Johnson & Harich, 1986).

In the past, the genus *Bacteroides* was frequently speciated according to the production of pigmented colonies on blood agar, its sugar fermentative ability, and the sensitivity to bile. However, the patterns of fatty acids, polar lipids and isoprenoid quinones may also

† Present address: Norwegian Plant Protection Institute, Box 70, N-1432 Ås-NLH, Norway.

Abbreviation: PCA, principal components analysis.

Table 1. List of organisms investigated

Species	Strain*	Sample no.	Isolated from:
Non-pigmented			
<i>Prevotella (Bacteroides) buccae</i>	ES9-1	1	Dental root canal
	ES12-B	2	Dental root canal
	ES17-1	3	Dental root canal
	ATCC 33574 ^T	6	Gingival sulcus
<i>Prevotella (Bacteroides) oris</i>	ES14B-3A	4	Dental root canal
	ES9-3	5	Dental root canal
	ES7-B0	7	Dental root canal
	ATCC 33573 ^T	8	Gingival sulcus
<i>Prevotella (Bacteroides) oralis</i>	ESB4-B	9	Dental root canal
	ES14B-1B	10	Dental root canal
	ES15-2	11	Dental root canal
<i>Prevotella (Bacteroides) distiens</i>	DSM 20516 ^T	12	Bartholin abscess
<i>Prevotella (Bacteroides) veroralis</i>	ATCC 33779 ^T	13	Oral cavity
<i>Prevotella (Bacteroides) heparinolytica</i>	ATCC 35895 ^T	14	Periodontal pocket
<i>Bacteroides fragilis</i>	ATCC 25285 ^T	15	Appendix abscess
Pigmented			
<i>Porphyromonas (Bacteroides) endodontalis</i>	HG 412	16	Dental root canal
	HG 370 ^T (ATCC 35406 ^T)	17	Dental root canal
	HG 182 (BN 11a-f)	18	Dental root canal
	HG 181 (H 11a-e)	19	Dental root canal

* ES, phenotypically characterized by M. Haapasalo; ATCC, American Type Culture Collection, Rockville, Md., USA; DSM, German Collection of Micro-organisms and Tissue Cultures, Braunschweig, FRG; HG, characterized by Van Winkelhoff *et al.* (1985); T, type strain.

assist in rationalizing the systematics of these organisms (for a review, see Wilkinson, 1988).

The present study, applying principal components analysis of fatty acid data from whole cells, was undertaken in an attempt to clarify further the inter- and intrageneric relationships of *Bacteroides*. Gas chromatographic (GC) and gas chromatographic-mass spectrometric (GC-MS) analyses of the cellular fatty acid composition are generally accepted as suitable and reliable methods in microbial chemotaxonomy (Brondz & Olsen, 1986; Lechevalier & Lechevalier, 1988). We have previously used multivariate analyses of cellular fatty acid data, obtained through GC and GC-MS analyses, to define genera of bacteria and yeasts such as *Porphyromonas* (Brondz *et al.*, 1989a), *Actinobacillus*, *Haemophilus* and *Pasteurella* (Brondz *et al.*, 1990), *Treponema* (Brondz *et al.*, 1991), and *Candida*, *Torulopsis* and *Saccharomyces* (Brondz *et al.*, 1989b; Brondz & Olsen, 1990).

Methods

Organisms and growth conditions. Bacterial strains used in this study are listed in Table 1. The reference strains were obtained directly from the American Type Culture Collection (ATCC) or the German Collection of Micro-organisms and Tissue Cultures (DSM), except *Prevotella (Bacteroides) heparinolytica* which was received from Dr K. Okuda, Tokyo Dental College, Tokyo, Japan, *P. (B.) veroralis*, which was sent to us by Dr H. N. Shah, London Hospital Medical College, London, UK, and *Porphyromonas (Bacteroides) endodontalis* strains HG 181 and 182, which were received from Dr G. Sundquist, University of Umeå, Umeå, Sweden. All clinical isolates were from necrotic root canals (Sundquist, 1976; Van Steenberg *et al.*, 1984; Van Winkelhoff *et al.*, 1985; Haapasalo, 1986). For fatty acid analyses, the organisms were grown for 2–5 d at 37 °C under anaerobic conditions in Mycoplasma broth (BBL) supplemented with menadione (0.5 mg l⁻¹), haemin (5 mg l⁻¹) and cysteine (0.5 g l⁻¹), harvested by centrifugation, washed in distilled water, and lyophilized.

Methanolysis and derivatization. Lyophilized whole cells were methanolysed with 2 M-HCl in anhydrous methanol for 24 h at 95 °C (Brondz & Olsen, 1984b). Excess methanol/HCl was evaporated with a stream of N₂ in an ice-bath and the methanolysate was then extracted

with n-hexane. HCl, methanol and n-hexane were obtained from E. Merck. The above procedure was used for routine examination of fatty acid contents. For assessment of hydroxy fatty acids, the hydroxy groups were derivatized with trifluoroacetic acid anhydride. This was done in a 7:3 (v/v) mixture of acetonitrile (Rathburn Chemicals) and trifluoroacetic acid anhydride (Fluka Chemie) at 90 °C for 3 min. Before GC the solution was diluted with acetonitrile so that it contained 10% trifluoroacetic acid anhydride.

GC and GC-MS. A model 8700 gas chromatograph (Perkin Elmer) was used for fatty acid analyses. The column was an HP Ultra 1, 25 m × 0.20 mm i.d. Helium was the carrier gas, at a flow rate of 1.5 ml min⁻¹. The temperature of the injector was 200 °C, and the temperature of the flame-ionization detector was 275 °C. The programme was as follows: the temperature was held for 1 min at 90 °C and then rose from 90 °C to 275 °C at a rate of 6 °C min⁻¹. The attenuator was set at 16. Paper speed was 5 mm min⁻¹. The sample (0.2 µl) was delivered as a splitless injection. The identities of the methanolysed and derivatized fatty acids were established by direct co-chromatography with authentic standards and by GC-MS (Brondz & Olsen, 1984b; 1985). From each duplicate sample two independent derivatizations were made, and three injections were made from each of the latter. The quantity of the substances, expressed as the relative percentage, was calculated from the area under each peak and corrected with the molar response factor (Brondz & Olsen, 1984b). The sum of the identified substances was considered to be 100%. For identification of fatty acids, methyl ester standards of decanoic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic and octadecanoic acids were obtained from Sigma. Bacterial acid methyl esters mixture CP, gas-liquid chromatography standard mixture GLC 70, American Oil Chemists' Society oil reference mixture, RM-1 rapeseed, National Institutes of Health reference mixtures and FA-FAME kit 14 were obtained from Supelco. 13-Methyltetradecanoic acid methyl ester, methyl-12-methyltetradecanoic acid ester, methyl-14-methylhexadecanoic acid ester, methyl-14-methylpentadecanoic acid ester, methyl-15-methylhexadecanoic acid ester and methyl-11-methyltridecanoic acid ester were from Larodan Fine Chemicals. Phenol/water extracted lipopolysaccharides from *Porphyromonas (Bacteroides) gingivalis* ATCC 33277^T (Brondz *et al.*, 1989a) and *Actinobacillus (Haemophilus) actinomycetemcomitans* ATCC 33384^T (Brondz & Olsen, 1984a) were also used as standards for identification of fatty acids.

Statistical analyses. Principal components analysis (PCA) (Jolliffe, 1986; Wold *et al.*, 1987) was used for quantitative analysis of fatty acid data. With this method the original space for variable measurements was projected down onto two low-dimensional subspaces. One of these was sample-related, the other was variable-related. By plotting the sample-oriented parameters t_1 versus t_2 , information on the dominating behaviour of the samples was gained, and by plotting the variable loadings p_1 versus p_2 , information was obtained on which variables contributed to the corresponding sample-oriented projection. The complexity and stability of both models was determined by cross-validation (Wold, 1976, 1978).

Results

GC and GC-MS analyses

The distribution of fatty acids in whole-cell methanolysates from the non-pigmented and pigmented species tested is shown in Table 2. Thirteen different fatty acids including normal fatty acids, saturated fatty acids, iso-

and anteisobranched fatty acids and hydroxy fatty acids were detected. Unsaturated fatty acids were not found. It was noteworthy that *B. fragilis*, the type species of the genus *Bacteroides*, differed from all other species examined by having 3-hydroxy-15-methylhexadecanoic acid (C_{3-OH-iso17}) as the predominant acid. Further, the 12-methyltetradecanoic acid (C_{ante15}) content was greater than the 13-methyltetradecanoic acid (C_{iso15}) content in this organism. Other characteristic findings were as follows: in most saccharoclastic, non-pigmented organisms [*Prevotella (Bacteroides) buccae*, *P. (B.) oris*, *P. (B.) oralis*, *P. (B.) veroralis* and *P. (B.) disiens*] 12-methyltetradecanoic acid was the predominant fatty acid, whereas in asaccharoclastic, black-pigmented organisms [*Porphyromonas (Bacteroides) endodontalis*] and in the saccharoclastic, non-pigmented *Prevotella (Bacteroides) heparinolytica*, 13-methyltetradecanoic acid predominated. *Porphyromonas (Bacteroides) endodontalis*, *Prevotella (Bacteroides) heparinolytica*, and, as mentioned, *B. fragilis*, contained large amounts of 3-hydroxy-15-methylhexadecanoic acid. Whereas 13-methyltetradecanoic acid constituted a significant portion of the cellular fatty acids in *Prevotella (Bacteroides) oris*, the content was much lower in *P. (B.) buccae* and *P. (B.) oralis*. *P. oralis* seemed to have a higher content of hexadecanoic (C_{16:0}) acid than the other organisms.

Statistical analyses

In the PCA, the two principal score vectors, t_1 and t_2 were plotted against each other (Fig. 1). This sample-oriented (i.e. bacterial-strain-oriented) projection described the two largest variants of the data matrix in Table 2. The two principal components, t_1 and t_2 , accounted for 33.1% and 26.4%, respectively, of the variance. In this projection, *Prevotella (Bacteroides) buccae* (samples 1, 2, 3 and 6) and *P. (B.) oralis* (samples 9, 10 and 11) constituted two overlapping groups of organisms. *P. (B.) oris* (samples 4, 5, 7 and 8), *P. (B.) heparinolytica* (sample 14), *P. (B.) disiens* (sample 12) and *P. (B.) veroralis* (sample 13) were distinct from *P. (B.) buccae* and *P. (B.) oralis*. *Porphyromonas (Bacteroides) endodontalis* (samples 16, 17, 18 and 19) formed a homogeneous cluster distinct from the remaining species examined.

The first and the third principal score vectors, t_1 and t_3 , were also plotted against each other (Fig. 2). This sample-oriented projection described the largest and the third largest variant of the data matrix, constituting 33.1% and 11.0%, respectively, of the variance. In this projection there was a possible separation of *Prevotella (Bacteroides) buccae* (samples 1, 2, 3 and 6) and *P. (B.) oralis* (samples 9, 10 and 11), but no definite conclusion

Organism	Sample no.	Relative amount (%) of:													
		C _{iso13}	C _{antel13}	C _{iso14}	C ₁₄₊₀	C _{iso15}	C _{antel15}	C _{iso16}	C ₁₆₊₀	C _{3OH-15}	C _{iso17}	C _{antel17}	C _{3OH-16}	C _{3OH-iso17}	
<i>Prevotella (Bacteroides) buccae</i>															
ES9-1	1	0.7	0.4	2.4	0.9	6.5	51.9	2.7	8.7	2.1	6.8	4.5	3.3	9.1	
ES12-B	2	0.1	0.1	2.5	1.2	5.9	50.9	2.6	12.4	2.4	5.6	4.5	3.4	8.4	
ES17-1	3	1.2	0.8	2.2	2.5	5.7	50.1	1.7	9.7	2.0	5.7	4.0	5.7	8.9	
ATCC 33574 ^T	6	0.7	0.4	2.4	1.5	6.0	51.0	2.3	12.2	2.2	5.4	4.0	4.1	7.8	
<i>Prevotella (Bacteroides) oris</i>															
ES14B-3A	4	2.1	0.4	4.7	3.3	20.0	32.7	0.7	15.6	0.7	2.8	0.05	7.8	9.2	
ES9-3	5	1.2	0.4	4.1	3.4	20.7	44.2	3.1	10.6	0.8	4.4	0.05	3.5	6.6	
ES7-B0	7	1.8	0.6	4.5	3.2	22.4	37.6	1.9	12.4	0.5	4.1	0.05	3.9	7.1	
ATCC 33573 ^T	8	2.1	0.9	4.7	2.9	26.6	35.9	2.0	11.0	0.05	5.1	0.05	3.3	5.6	
<i>Prevotella (Bacteroides) oralis</i>															
ES4-B	9	0.2	0.05	3.9	2.2	11.2	40.2	3.2	22.6	2.0	2.3	1.4	4.8	5.9	
ES14B-1B	10	0.05	0.05	4.7	2.6	11.3	35.6	3.0	20.4	2.7	8.7	3.3	3.7	4.0	
ES15-2	11	0.05	0.05	3.5	2.2	7.9	46.4	3.6	19.4	2.1	2.6	3.1	3.5	5.7	
<i>Prevotella (Bacteroides) disiens</i>															
DSM 20516 ^T	12	5.8	2.2	6.8	6.7	16.0	34.0	4.2	4.4	1.3	2.6	2.8	3.2	10.0	
<i>Prevotella (Bacteroides) veroralis</i>															
ATCC 33779 ^T	13	5.7	1.7	7.9	9.7	26.5	35.2	2.6	6.6	0.05	0.05	0.05	2.7	1.7	
<i>Prevotella (Bacteroides) heparinolytica</i>															
ATCC 35895 ^T	14	1.7	0.5	0.05	2.4	40.1	21.9	0.05	4.0	2.9	5.6	0.05	4.1	17.1	
<i>Bacteroides fragilis</i>															
ATCC 25285 ^T	15	0	0	0	0.2	14.6	32.7	0	5.8	0	0	0	8.2	38.0	
<i>Porphyromonas (Bacteroides) endodontalis</i>															
HG 412	16	1.8	0.05	0.05	4.0	61.1	3.2	0.05	9.8	0.6	0.05	0.05	2.6	16.9	
HG 370 ^T (ATCC 35406 ^T)	17	1.8	0.05	0.05	3.6	60.6	4.2	0.05	7.5	0.6	0.05	0.05	0.6	16.9	
HG 182	18	4.8	0.05	0.05	4.8	68.5	4.4	0.05	6.6	0.05	0.05	0.05	0.1	10.8	
HG 181	19	1.8	0.05	0.05	3.5	67.0	4.4	0.05	8.4	1.2	0.05	0.05	0.1	13.8

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Sample-oriented (i.e. bacterial-strain-oriented) principal components projection (t_1 versus t_2). These two principal components describe 33.1% and 26.4%, respectively, of the variance of the data in Table 2. Samples include: *Prevotella* (*Bacteroides*) *buccae* (samples 1, 2, 3 and 6), *P. (B.) oris* (samples 4, 5, 7 and 8), *P. (B.) oralis* (samples 9, 10 and 11), *P. (B.) disiens* (sample 12), *P. (B.) veroralis* (sample 13), *P. (B.) heparinolytica* (sample 14), *B. fragilis* (sample 15) and *Porphyromonas* (*Bacteroides*) *endodontalis* (samples 16, 17, 18 and 19). A complete line (—) surrounds samples representing the same species; closely related species are surrounded by a dashed line (---).

Fig. 2. Sample-oriented principal components projection (t_1 versus t_3). These two principal components describe 33.1% and 11.1%, respectively, of the variance of the data in Table 2. Sample numbers are as in Fig. 1. A complete line (—) surrounds samples representing the same species; closely related species are surrounded by a dashed line (---).

Fig. 3. Sample-oriented principal components projection (t_1 versus t_2). These two principal components describe 38.0% and 24.3%, respectively, of the variance in the data (Table 2) related exclusively to *P. (B.) buccae*, *P. (B.) oris* and *P. (B.) oralis*. Sample numbers representing these species are as in Fig. 1.

Fig. 4. Variable-oriented (i.e. bacterial-character-oriented) principal components projection (p_1 versus p_2). The numbers refer to the following variables (Table 2): 1, 11-methyldodecanoic acid ($C_{10:13}$); 2, 10-methyldodecanoic acid (C_{ante13}); 3, 12-methyltridecanoic acid (C_{iso14}); 4, tetradecanoic acid ($C_{14:0}$); 5, 13-methyltetradecanoic acid (C_{iso15}); 6, 12-methyltetradecanoic acid (C_{ante15}); 7, 14-methylpentadecanoic acid (C_{iso16}); 8, hexadecanoic acid ($C_{16:0}$); 9, 3-hydroxypentadecanoic acid ($C_{3-OH-15}$); 10, 15-methylhexadecanoic acid (C_{iso17}); 11, 14-methylhexadecanoic acid (C_{ante17}); 12, 3-hydroxyhexadecanoic acid ($C_{3-OH-16}$); and 13, 3-hydroxy-15-methylhexadecanoic acid ($C_{3-OH-iso17}$). It can be seen from the plot that the variables that contribute most to the first score vector (t_1) are variables 13 and 8, and those variables that contribute most to the second score vector (t_2) are variables 1, 3, 5, 4, 9 and 6. (See text for description of method.)

could be made. Separation of the other species examined, however, remained distinct. *B. fragilis* was distant from species proposed to belong to *Porphyromonas* (Shah & Collins, 1988) and *Prevotella* (Shah & Collins, 1990).

To determine whether *P. (B.) buccae*, *P. (B.) oris* and *P. (B.) oralis* really differed in their fatty acid contents, a PCA was done exclusively for these species. In the

sample-oriented projection shown in Fig. 3 the two largest variants, t_1 and t_2 , described 38.0% and 24.3%, respectively, of the variance in the fatty acid data (Table 2) from these species. This projection provided a clear separation between *P. (B.) buccae* (samples 1, 2, 3 and 6), *P. (B.) oris* (samples 4, 5, 7 and 8) and *P. (B.) oralis* (samples 9, 10 and 11).

The variables (i.e. bacterial characters, Table 2) that contributed most to the first score vector (t_1) were those with high absolute loading values, namely variable 13 (3-hydroxy-15-methylhexadecanoic acid) and variable 8 (hexadecanoic acid) (Fig. 4). Those variables that contributed most to the second score vector (t_2) were variables 1 (11-methyldodecanoic acid), 3 (12-methyltridecanoic acid), 5 (13-methyltetradecanoic acid), 4 (tetradecanoic acid), 11 (14-methylhexadecanoic acid), 9 (3-hydroxypentadecanoic acid) and 6 (12-methyltetradecanoic acid).

Discussion

The present study, which used PCA of cellular fatty acid data, has provided new information on the intrageneric and intergeneric relationships of *Bacteroides*. The fatty acid content of the bile-resistant, non-pigmented *B. fragilis* was similar to that reported previously (Brondz *et al.*, 1989a). *B. fragilis* was the only species where the $C_{3-OH-iso17}$ acid dominated and the C_{ante15} acid content was higher than that of the C_{iso15} acid and is thus entirely different from the other species examined. The present findings support the proposal that *B. fragilis* and closely related species, should form the genus *Bacteroides sensu stricto* (Collins & Shah, 1987; Shah & Collins, 1989).

Whereas asaccharoclastic, black-pigmented organisms, as represented by *Porphyromonas* (*Bacteroides*) *endodontalis*, contained large amounts of 13-methyltetradecanoic acid (C_{iso15}), most saccharoclastic, bile-sensitive, non-pigmented species [*Prevotella* (*Bacteroides*) *buccae*, *P. (B.) oris*, *P. (B.) oralis*, *P. (B.) disiens* and *P. (B.) veroralis*] had 12-methyltetradecanoic acid (C_{ante15}) as the predominant fatty acid. Asaccharoclastic, black-pigmented organisms also tended to contain more 3-hydroxy-15-methylhexadecanoic acid ($C_{3-OH-iso17}$) than did saccharoclastic, non-pigmented species with the exception of *P. (B.) heparinolytica* and *B. fragilis*. This supported the proposed distinction between the *Bacteroides*-derived genera *Porphyromonas* (Shah & Collins, 1988) and *Prevotella* (Shah & Collins, 1990). The fatty acid profile of *Porphyromonas* (*Bacteroides*) *endodontalis* was quite similar to that reported previously for *P. (B.) endodontalis*, *P. (B.) gingivalis* and *P. (B.) asaccharolytica* (Brondz *et al.*, 1989a). Multivariate fatty acid analyses have also distinguished *Porphyromonas* species from other black-pigmented species such as *Prevotella* (*Bacteroides*) *melaninogenica*, *P. (B.) intermedia* and *B. levii* (Brondz *et al.*, 1989a).

Prevotella (*Bacteroides*) *heparinolytica* differed from the other non-pigmented, bile-sensitive, saccharoclastic species by having 13-methyltetradecanoic acid as its major fatty acid. It also contained large amounts of 3-

hydroxy-15-methylhexadecanoic acid, as did black-pigmented species. However, PCA of the fatty acid content confirmed that *P. (B.) heparinolytica* is distinct from asaccharolytic species. It has been established previously (Okuda *et al.*, 1985) that there are no antigenic or serological relationships between *P. (B.) heparinolytica* and other *Bacteroides* species. The sole characteristic that reliably differentiated this species from other non-pigmented saccharoclastic *Bacteroides* species was its heparin-degrading activity. The present study suggested that multivariate cellular fatty acid analyses could also be useful for this distinction.

Very few criteria are available for taxonomic distinction between *Prevotella* (*Bacteroides*) *buccae*, *P. (B.) oris* and *P. (B.) oralis* (Holdeman *et al.*, 1982, 1984). Enzyme patterns have been useful in differentiating these species (Holdeman *et al.*, 1984; Haapasalo, 1986). Much confusion has existed as to the identification of *P. (B.) oralis*, and some strains previously referred to as *P. (B.) oralis* in the literature were, in fact, *P. (B.) oris* or *P. (B.) buccae* (Holdeman *et al.*, 1984; Johnson & Holdeman, 1985). *B. pentosaceus* and *B. capillus* appear to be synonyms of *P. (B.) buccae* (Holdeman *et al.*, 1984; Johnson & Holdeman, 1985). Further, it has been suggested that *P. (B.) oralis* is a non-pigmented variant of *B. melaninogenica* subsp. *melaninogenica* (Harding *et al.*, 1976; Holbrook *et al.*, 1977), *P. (B.) loescheii* has been identified as *P. (B.) oralis* or *P. (B.) melaninogenica* (Holdeman & Johnson, 1982). The similarity of *P. (B.) oralis* strains to *P. (B.) ruminicola* has also been noted (Sundquist, 1976). *P. (B.) veroralis* is phenotypically quite similar to *P. (B.) oralis* (Watabe *et al.*, 1983; Holdeman *et al.*, 1984). Helpful phenotypic characteristics to differentiate these species have been fermentation of aesculin and salicin by *P. (B.) oralis* but not by *P. (B.) veroralis* (Holdeman *et al.*, 1984). In the present study *P. (B.) oralis* and *P. (B.) veroralis* appeared to be clearly distinct. Our findings suggested that PCA of cellular fatty acids can assist in the differentiation of *P. (B.) oralis* from closely related species.

Identifying *P. (B.) disiens* may occasionally be problematic since this species has a sugar fermentation pattern quite similar to that of the black-pigmented *P. (B.) corporis* (Holdeman & Johnson, 1977; Holdeman *et al.*, 1984). Non-hydroxy straight-chain and branched-chain fatty acids in *P. (B.) disiens* were found by Mayberry (1980) to be similar to those in *P. (B.) oralis*. Multivariate fatty acid analyses (this study) show that *P. (B.) disiens* is clearly different from all the other *Prevotella* species examined.

We thank Norsk Dental Depot's Fond for Odontologisk Forskning, Tannlegeundervisnings Fond, and Nordisk Ministerråd (Nordisk forskarstipendium no. 035/90), for financial support.

References

- BRONDZ, I. & OLSEN, I. (1984a). Differentiation between *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus* based on carbohydrates in lipopolysaccharide. *Journal of Chromatography* **310**, 261–272.
- BRONDZ, I. & OLSEN, I. (1984b). Whole-cell methanolysis as a rapid method for differentiation between *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*. *Journal of Chromatography* **311**, 347–353.
- BRONDZ, I. & OLSEN, I. (1985). Differentiation between major species of the *Actinobacillus-Haemophilus-Pasteurella* group by gas chromatography of trifluoroacetic acid anhydride derivatives from whole-cell methanolysates. *Journal of Chromatography* **342**, 13–23.
- BRONDZ, I. & OLSEN, I. (1986). Microbial chemotaxonomy. Chromatography, electrophoresis and relevant profiling techniques. *Journal of Chromatography* **379**, 367–411.
- BRONDZ, I. & OLSEN, I. (1990). Multivariate analyses of cellular carbohydrates and fatty acids in *Candida albicans*, *Torulopsis glabrata* and *Saccharomyces cerevisiae*. *Journal of Clinical Microbiology* **28**, 1854–1857.
- BRONDZ, I., CARLSSON, J., SJÖSTRÖM, M. & SUNDQUIST, G. (1989a). Significance of cellular fatty acids and sugars in defining the genus *Porphyromonas*. *International Journal of Systematic Bacteriology* **39**, 314–318.
- BRONDZ, I., OLSEN, I. & SJÖSTRÖM, M. (1989b). Gas chromatographic assessment of alcoholized fatty acids from yeasts: a new chemotaxonomic method. *Journal of Clinical Microbiology* **27**, 2815–2819.
- BRONDZ, I., OLSEN, I. & SJÖSTRÖM, M. (1990). Multivariate analysis of quantitative chemical and enzymic characterization data in classification of *Actinobacillus*, *Haemophilus* and *Pasteurella* spp. *Journal of General Microbiology* **136**, 507–513.
- BRONDZ, I., FICHN, N.-E., OLSEN, I. & SJÖSTRÖM, M. (1991). Multivariate analyses of cellular fatty acids and carbohydrates of 1:2:1 and 2:4:2 spirochetes. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **99**, (in the Press).
- COLLINS, M. D. & SHAH, H. N. (1986a). Reclassification of *Bacteroides termiditis* Sebald (Holdeman and Moore) in a new genus *Sebaldella*, as *Sebaldella termiditis* comb. nov. *International Journal of Systematic Bacteriology* **36**, 349–350.
- COLLINS, M. D. & SHAH, H. N. (1986b). Reclassification of *Bacteroides praeacutus* Tissier (Holdeman and Moore) in a new genus, *Tissierella*, as *Tissierella praeacuta* comb. nov. *International Journal of Systematic Bacteriology* **36**, 461–463.
- COLLINS, M. D. & SHAH, H. N. (1987). Recent advances in the taxonomy of the genus *Bacteroides*. In *Recent Advances in Anaerobic Bacteriology*, pp. 249–258. Edited by S. P. Borrellio and others. Dordrecht, The Netherlands: Martinus Nijhoff.
- COLLINS, M. D., SHAH, H. N. & MITSUOKA, T. (1985). Reclassification of *Bacteroides microfus* (Kaneuchi and Mitsuoka) in a new genus *Rikenella*, as *Rikenella microfus* comb. nov. *Systematic and Applied Microbiology* **6**, 79–81.
- FINEGOLD, S. M. & GEORGE, W. L. (1989). *Anaerobic Infections in Humans*. San Diego: Academic Press.
- HAAPASALO, M. (1986). *Bacteroides buccae* and related taxa in necrotic root canal infections. *Infection and Immunity* **53**, 149–153.
- HARDING, G. K. M., SUTTER, V. L., FINEGOLD, S. M. & BRICKNELL, K. S. (1976). Characterization of *Bacteroides melaninogenicus*. *Journal of Clinical Microbiology* **4**, 354–359.
- HOLBROOK, W. P., DUERDEN, B. I. & DEACON, A. G. (1977). The classification of *Bacteroides melaninogenicus* and related species. *Journal of Applied Bacteriology* **42**, 259–273.
- HOLDEMAN, L. V. & JOHNSON, J. L. (1977). *Bacteroides disiens* sp. nov. and *Bacteroides bivius* sp. nov. from human clinical infections. *International Journal of Systematic Bacteriology* **27**, 337–345.
- HOLDEMAN, L. V. & JOHNSON, J. L. (1982). Description of *Bacteroides loeschii* sp. nov. and emendation of the descriptions of *Bacteroides melaninogenicus* (Oliver and Wherry) Roy and Kelly 1939 and *Bacteroides denticola* Shah and Collins 1981. *International Journal of Systematic Bacteriology* **32**, 399–409.
- HOLDEMAN, L. V., MOORE, W. E. C., CHURN, P. J. & JOHNSON, J. L. (1982). *Bacteroides oris* and *Bacteroides buccae*, new species from human periodontitis and other human infections. *International Journal of Systematic Bacteriology* **32**, 125–131.
- HOLDEMAN, L. V., KELLEY, R. W. & MOORE, W. E. C. (1984). Genus I. *Bacteroides* Castellani and Chalmers 1919. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 604–631. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- JOHNSON, J. L. & HARICH, B. (1986). Ribosomal ribonucleic acid homology among species of the genus *Bacteroides*. *International Journal of Systematic Bacteriology* **36**, 71–79.
- JOHNSON, J. L. & HOLDEMAN, L. V. (1985). *Bacteroides capillus* Kornman and Holt and *Bacteroides pentosaceus* Shah and Collins, later synonyms of *Bacteroides buccae* Holdeman et al. *International Journal of Systematic Bacteriology* **35**, 114.
- JOLIFFE, I. T. (1986). *Principal Component Analysis*. New York: Springer-Verlag.
- LECHEVALIER, H. & LECHEVALIER, M. P. (1988). Chemotaxonomic use of lipids – an overview. In *Microbial Lipids*, vol. 1, pp. 869–902. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press.
- MAYBERRY, W. R. (1980). Hydroxy fatty acids in *Bacteroides* species: D-(–)-3-hydroxy-15-methylhexadecanoate and its homologs. *Journal of Bacteriology* **143**, 582–587.
- MOORE, W. E. C., CATO, E. P. & MOORE, L. V. (1985). Index of the bacterial and yeast nomenclatural changes published in the *International Journal of Systematic Bacteriology* since the 1980 Approved Lists of Bacterial Names (1 January 1980 to January 1985). *International Journal of Systematic Bacteriology* **35**, 382–407.
- OKUDA, K., KATO, T., SHIOZU, J., TAKAZOE, I. & NAKAMURA, T. (1985). *Bacteroides heparinolyticus* sp. nov. isolated from humans with periodontitis. *International Journal of Systematic Bacteriology* **35**, 438–442.
- SHAH, H. N. & COLLINS, M. D. (1982a). Reclassification of *Bacteroides hypermegas* (Harrison and Hansen) in a new genus *Megamonas*, as *Megamonas hypermegas* comb. nov. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. (Abteilung I, Originale Reihe C)* **3**, 394–398.
- SHAH, H. N. & COLLINS, M. D. (1982b). Reclassification of *Bacteroides multiacidus* (Mitsuoka, Terada, Watanabe and Uchida) in a new genus *Mitsuokella*, as *Mitsuokella multiacidus* comb. nov. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I, Originale Reihe C)* **3**, 491–494.
- SHAH, H. N. & COLLINS, M. D. (1983). Genus *Bacteroides*: a chemotaxonomic perspective. *Journal of Applied Bacteriology* **55**, 403–416.
- SHAH, H. N. & COLLINS, M. D. (1986). Reclassification of *Bacteroides furcosus* Veillon and Zuber (Hauduroy, Ehringer, Urbain, Guillot and Magron) in a new genus *Anaerorhabdus*, as *Anaerorhabdus furcosus* comb. nov. *Systematic and Applied Bacteriology* **8**, 86–88.
- SHAH, H. N. & COLLINS, M. D. (1988). Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus, *Porphyromonas*. *International Journal of Systematic Bacteriology* **38**, 128–131.
- SHAH, H. N. & COLLINS, M. D. (1989). Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species. *International Journal of Systematic Bacteriology* **39**, 85–87.
- SHAH, H. N. & COLLINS, M. D. (1990). *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *International Journal of Systematic Bacteriology* **40**, 205–208.
- SUNDQUIST, G. (1976). *Bacteriologic studies of necrotic dental pulps*. Umeå University Odontological Dissertation no. 7, University of Umeå, Umeå, Sweden.
- VAN STEENBERGEN, T. J. M., VAN WINKELHOFF, A. J., MAYRAND, D., GRENIER, D. & DE GRAAFF, J. (1984). *Bacteroides endodontalis* sp. nov., an asaccharolytic black-pigmented *Bacteroides* species from infected dental root canals. *International Journal of Systematic Bacteriology* **34**, 118–120.
- VAN WINKELHOFF, A. J., VAN STEENBERGEN, T. J. M., KIPPUW, N. & DE GRAAFF, J. (1985). Further characterization of *Bacteroides endodontalis*, an asaccharolytic black-pigmented *Bacteroides* species from the oral cavity. *Journal of Clinical Microbiology* **22**, 75–79.

- WATABE, J., BENNO, Y. & MITSUOKA, T. (1983). Taxonomic study of *Bacteroides oralis* and related organisms and proposal of *Bacteroides veroralis* sp. nov. *International Journal of Systematic Bacteriology* **33**, 57–64.
- WILKINSON, S. G. (1988). Gram-negative bacteria. In *Microbial Lipids*, vol. 1, pp. 299–488. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press.
- WOLD, S. (1976). Pattern recognition by means of disjoint principal components models. *Pattern Recognition* **8**, 127–139.
- WOLD, S. (1978). Cross-validatory estimation of the number of components in factor and principal components models. *Technometrics* **20**, 397–405.
- WOLD, S., ESBENDSEN, K. & GELADI, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems* **2**, 37–52.