Characterisation of non-pigmented species of the genus *Prevotella* by polyacrylamide gel electrophoresis

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Summary. Gram-negative anaerobic bacilli previously known as the melaninogenicus-oralis group of *Bacteroides* have been assigned to a new genus, *Prevotella*. The non-pigmented members of this genus share several general characteristics and cannot be readily distinguished by routine tests. A polyacrylamide slab gel electrophoresis procedure, with visual analysis of protein patterns, was used to compare cellular protein patterns from clinical isolates with those from collection (reference) strains. Reference strains of *P. oralis*, *P. veroralis*, *P. buccalis*, *P. oris*, *P. buccae*, *P. zooglyphiformans*, *P. bivia*, *P. disiens*, *P. oulora*, *B. (P.) capillus* and *B. (P.) pentosaceus*, and 91 non-pigmented isolates from patients with adult periodontal disease were examined by conventional biochemical tests, gas-liquid chromatography (GLC) and enzyme tests, and whole-cell protein profiles were obtained by SDS-PAGE. There was close correlation between patterns of results in biochemical and GLC tests and the SDS-PAGE profiles, and the species were readily distinguished in SDS-PAGE. The periodontal isolates were assigned to 10 groups by conventional test reaction patterns and nine groups by SDS-PAGE; the profiles of 79 isolates corresponded to those of seven species reference strains. By SDS-PAGE, clinical isolates of *P. buccae* (42 isolates) and *P. oralis* (eight isolates) showed good similarity with reference strains. However, for *P. veroralis* (15), *P. oris* (7), *P. bivia* (4), *P. zooglyphiformans* (2) and *P. buccalis* (1), clinical isolates showed some minor variations from reference strains. Twelve isolates remained undesignated in SDS-PAGE analysis. Variant SDS-PAGE profiles divided clinical isolates of *P. buccae* into two subgroups and those of *P. veroralis* into five subgroups.

Introduction

Small gram-negative obligately anaerobic bacilli of the genus *Prevotella* (*Bacteroides* of the melaninogenicus-oralis group) are important members of the normal flora of the mouth and significant causes of periodontal diseases^{1–4} and other infections, particularly of the urogenital tract, the head and neck, and the lungs.^{4–7}

In recent years, the genus *Bacteroides* has undergone major taxonomic revision and several species have been given separate genus status.^{2,8} It has been proposed that the genus *Bacteroides* be restricted to *B. fragilis* (the type species) and closely related organisms.⁸ However, the position of the moderately saccharolytic, predominantly oral *Bacteroides* spp. (*B. melaninogenicus*, *B. oralis* and related species) remained unresolved.² These species share several

general characteristics and form a phenotypically and phylogenetically coherent group that differs significantly from the emended description of the genus *Bacteroides*.² These species have now been assigned to a new genus *Prevotella*.² The black-pigmented members of *Prevotella* are well known, but the non-pigmented species have received less attention. Most of these non-pigmented species have been described only recently and are still generally reported as either *B. (P.) oralis* or *B. (P.) ruminicola*. So far, little information is available on their characteristics and their role in human disease.

Several authors have reported the use of SDS-PAGE of soluble cellular proteins to distinguish species or to determine the similarity between isolates of bacteroides.^{9,10}

In the present study, collection (reference) strains of *Prevotella*, and non-pigmented clinical isolates from patients with periodontal disease were examined. They had been characterised by biochemical, gas-liquid chromatography (GLC) and enzyme tests and the results of these tests were compared with the patterns obtained by SDS-PAGE of whole-cell proteins.

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Table I. Collection strains studied

Species	Designation	Source
<i>P. oralis</i>	NCTC 11459	Periodontal pocket
<i>P. veroralis</i>	ATCC 33779	Oral cavity
<i>P. veroralis</i>	83-457F*	
<i>P. buccalis</i>	ATCC 35310	Dental plaque
<i>P. bivia</i>	VPI 6622	Endometrium
<i>P. bivia</i>	VPI 5540†	
<i>P. disiens</i>	NCTC 11157	Bartholin abscess
<i>P. oulora</i>	NCTC 11871	Subgingival plaque
<i>P. buccae</i>	ATCC 33574	Gingival crevice
<i>P. buccae</i>	83-617*	
<i>P. oris</i>	ATCC 33573	Gingival crevice
<i>P. oris</i>	86-201DD*	
<i>P. zoogloformans</i>	ATCC 33285	Oral cavity
<i>B. (P.) capillus</i> ‡	ATCC 33690	Periodontal pocket
<i>B. (P.) pentosaceus</i> ‡	NP 333	Dental plaque
<i>B. (P.) pentosaceus</i> ‡	WPH 61	Dental plaque

* Donated by Dr S. M. Finegold, V.A. Wadsworth Medical Centre, California.

† Donated by Dr E. M. Barnes, A.F.R.C. Food Research Institute, Colney Lane, Norwich.

‡ These species have been suggested to be synonymous with *P. buccae*.²⁴

Materials and methods

Bacterial strains

The strains studied comprised 16 reference strains from collections (table I) and 91 clinical isolates from 21 adult patients with periodontal disease. All were checked for purity on BM agar,¹¹ incubated anaerobically (H₂ 10%, CO₂ 10%, N₂ 80%) at 37°C for 72 h and on blood agar incubated aerobically with CO₂ 10%; growth from anaerobic cultures was then suspended in a freezing medium of Nutrient Broth No. 2 (Oxoid) with inactivated horse serum (Wellcome) 10% and glucose 1% and held at -70°C in liquid nitrogen.

Characterisation tests

The strains were characterised and identified by a combination of biochemical reactions, antibiotic disk susceptibility and tolerance tests, fluorescence under UV light, GLC analysis and tests for enzyme activity as previously described¹² and summarised briefly below.

Biochemical tests. Strains were tested for production of indole; hydrolysis of aesculin and starch; production of acid from glucose, lactose, sucrose, rhamnose, trehalose, xylose, maltose, arabinose, cellobiose, salicin, inulin and xylan;^{12,13} hydrolysis of hippurate with diagnostic tablets;¹⁴ and hydrolysis of indoxyl acetate by the method of Popovic-Uroic *et al.*¹⁵

GLC analysis. Volatile (VFA) and non-volatile (NVFA) fatty acid end-products of metabolism were determined after growth in Fastidious Anaerobe Broth (FAB) (Lab M, Bury, Lancs) for 4–5 days by a modification of the method of Holdeman *et al.*¹⁶

Enzyme activities. The production of β -xylosidase, β -glucuronidase, α - and β -glucosidase, α - and β -galactosidase, α -fucosidase, β -N-acetylglucosaminidase, and α -mannosidase was tested with Rosco Diagnostic tablets (Lab M).¹⁴

SDS-PAGE

SDS-PAGE was performed by a modification of the method of Laemmli.¹⁷

Preparation of protein samples. Strains were grown on BM agar¹¹ for 2–3 days in an anaerobic cabinet (Don Whitley Scientific Ltd, Shipley, W. Yorkshire) in an atmosphere of H₂ 10%, CO₂ 10%, N₂ 80%. Growth was harvested and the weight of the wet pellets was standardised to between 100 and 150 mg. The pellets were washed three times in 1 ml of sterile distilled water and suspended in 150 μ l of double strength lysis buffer containing SDS 6% w/v, glycerol 20% v/v, 2-mercaptophenol 10% v/v, 0.125 M Tris-HCl (pH 6.8) and bromophenol blue 0.005% w/v. The suspensions were boiled in a water bath at 100°C for 5 min; 150 μ l of sterile distilled water was added and the samples were boiled again for 5 min. Lysates were centrifuged and the supernates were tested.

Loading and running the gels. The separating gel contained (w/v) polyacrylamide 10%, SDS 0.1%, ammonium persulphate (APS) 0.06%, TEMED 0.1% and 0.375 M Tris-HCl (pH 8.8). The stacking gel contained (w/v) polyacrylamide 5%, SDS 0.1%, APS 0.05%, TEMED 0.1% and 0.125 M Tris-HCl (pH 6.8). The apparatus was a vertical electrophoresis tank (model V16; BRL, Bethesda, MD, USA). The gels were 1.5 mm thick with 17 wells in each; 10 μ l of sample was loaded into each well with protein mol. wt markers (29–205 kDa; Sigma) in one well of each gel. Electrophoresis was at constant 60 V until the tracking dye reached the bottom of the separating gel (approx. 12 h).

Staining. Gels were stained for 3 h in Coomassie Brilliant Blue (CBB) R-250 (CBB 2.5 g, methanol 500 ml, trichloroacetic acid 100 g, distilled water 400 ml), destained with several changes of methanol 20% v/v and glacial acetic acid 7.5% v/v in water, and photographed through an orange filter (23 A).

Results

The gels and photographs were examined with the naked eye and strains were separated into similar groups, based on the number and position of protein bands (figs. 1–8); at least one reference strain of another *Prevotella* sp. was included as a control in each gel that was used primarily to show similarities between strains of a single species or group.

Protein profiles of 91 oral non-pigmented clinical isolates previously assigned to 10 distinct groups by the results of biochemical, GLC and enzyme tests¹² (table II) were compared with profiles of the reference

Table II. Summary of the biochemical, GLC and enzyme results of clinical isolates

Test	Patterns of results									
Dye tolerance										
Victoria blue 4R	+	I	I	+/I	+	+/I	+	I	+	+
Indole production	—	—	—	—	—	—	—	—	—	—
Hydrolysis of:										
aesculin	+	+	+	+	+	+	+	w	—	—
hippurate	—	—	—	—	—	—	+	—	+	—
starch	+	+	+	+	+	+/-	w	—	+	v
indoxyl acetate	+	+	+	+	+	v	v	+	+	w
Fluorescence	—	—	—	+ ^p	—	+ ^r	—	—	+ ^r	+ ^r
Pigmentation	—	—	—	—	—	v	—	—	—	—
Acid produced from:										
lactose	+	+	+	+	+	+	+	+	+	w/+
sucrose	+	+	+	+	+	+	+	+	—	w/+
rhamnose	+/-	—	—	—	—	—	—	—	—	—
trehalose	—	—	—	—	—	—	—	—	—	—
xylose	+	+	+	+	—	—	—	—	—	—
arabinose	+	+	+	+	—	—	—	—	—	—
cellobiose	+	+	+	+	+	+/-	+	—	—	—
salicin	+	+	+	+	+/-	—	—	—	—	—
inulin	+/-	+	+	—	w	+/-	w	—	—	—
xylan	+/-	—	—	—	—	v	—	—	—	—
End products (GLC):										
acetic	++	+	++	++	++	++	++	++	++	++
propionic	tr	++	—	+	—	—	—	+	—	—
iso-butyric	tr	++	++	—	—	+/-	+	—	+	+/-tr
butyric	—	—	—	tr	tr	—	—	tr	—	—
iso-valeric	++	++	++	tr	tr	+/-	++	+	++	++
pyruvic	—	—	—	—	—	tr/-	tr	—	—	—
lactic	tr	tr	+	tr	+	+	+	+	+	+
succinic	++	++	++	++	++	++	++	++	++	++
phenylacetic	—	+	—	—	tr	—	—	—	—	—
Enzyme production:										
β -xylosidase	+	+	+	-/+	+/-	—	—	—	—	—
β -glucuronidase	—	+	—	+	—	—	—	—	—	—
β -glucosidase	+	+	+	+	+	+	+	—	—	—
α -galactosidase	+	+	+	+	+	+	+	—	+/-	+
β -galactosidase	+	+	+	+	+	+	+	—	—	+/-
α -fucosidase	—	+	+	+	+	+	+	+	+	+
β -NAGase	—	+	+	+	+	+	+	+	+	+
α -mannosidase	—	+	+	+/-	-/+	+	+	w	+/-	+/-
Numbers of isolates	42	2	4	7	8	15	1	1	4	7
Groups	A	B	C	D	E	F	G	H	I	J

I, inhibition; +, growth; +, positive reaction; —, negative reaction; +/-, most reactions positive; -/+, most reactions negative; w, weak reaction; v, variable reaction; p, pale fluorescence; r, red fluorescence; ++, major production; +, minor; tr, trace; —, none produced; β -NAGase, β -N-acetyl-glucosaminidase.

strains whose test results are shown in table III. Protein profiles for the majority of isolates were similar to those of seven reference species and were allocated to nine groups:

Group I—42 isolates (group A, table II) biochemically identified as *P. buccae* (column 1, table III) gave very similar SDS-PAGE profiles closely corresponding to that of the reference strain *P. buccae* ATCC 33574; one collection strain (*P. buccae* 83-617) and six clinical isolates lacked a major protein band of c. 116 kDa, and formed the minor subgroup Ib (fig. 1). *P. oris* ATCC 33573 (track 2) was included for comparative purposes and differed from *P. buccae* ATCC 33574 and 83-617 in the regions of c. 25–30 kDa and other minor bands (fig. 1).

Group II—six isolates (group B and C, table II) biochemically identified as *P. zoogloiformans* (column 2, table III) gave two distinct subgroups by SDS-

PAGE profiles; subgroup IIa (two isolates; group B, table II) differed from the *P. zoogloiformans* reference strain ATCC 33285 only by the presence of two protein bands in the mol. wt regions c. 27 and 37 kDa, but subgroup IIb (four isolates; group C, table II) differed in bands at 48, 66, 97.4 and 116 kDa; thus, subgroup IIb remained in an undesignated SDS-PAGE group (fig. 2). *P. oris* ATCC 33573 (track 2). *B. (P.) pentosaceus* NP 333 and WPH 61 (tracks 3 and 4) and *P. buccae* ATCC 33574 (track 5) were applied as reference controls and differed from *P. zoogloiformans* in several major and minor bands (fig. 2). These strains were very similar in conventional biochemical tests.

Group III—seven isolates (group D, table II) biochemically identified as *P. oris* (column 4, table III) had similar GLC patterns in spite of their variation in biochemical tests. They differed from the *P. oris* reference strain ATCC 33573 by SDS-PAGE profile in

Table III. Summary of the biochemical, GLC and enzyme results of the reference strains

Test	Results obtained with strains									
	1	2	3	4	5	6	7	8	9	10
Dye tolerance										
Victoria blue 4R	+	I	+	I	+	+	+	I	+	+
Indole production	—	—	—	—	—	—	—	—	—	—
Hydrolysis of:										
aesculin	+	+	+	+	+	+	+	—	—	—
hippurate	—	—	—	—	—	—	+	—	+	+
starch	+	—	+	+	w	v	—	—	+	w
indoxyl acetate	+	+	+	+	w	w	v	+	+	+
Fluorescence	—	—	—	—	—	+ ^p	—	—	+ ^r	+ ^r
Pigmentation	—	—	—	—	—	—	—	—	—	—
Acid produced from:										
lactose	+	+	+	+	+	+	+	+	+	—
sucrose	+	+	+	+	+	+	+	+	—	—
rhamnose	+/-	—	—	—	—	—	—	—	—	—
trehalose	—	—	—	—	—	—	—	—	—	—
xylose	+	+	+	+	—	—	—	—	—	—
arabinose	+	+	+	+	—	—	—	—	—	—
cellobiose	+	+	+	+	+	+	+	—	—	—
salicin	+	+	+	+	+	—	—	—	—	—
inulin	+	+	+	+	w	+	v	—	—	—
xylan	+	—	—	—	—	w	—	—	—	—
End products (GLC):										
acetic	++	+	+	++	++	++	++	++	++	++
propionic	tr	++	++	+	—	—	—	++	—	—
iso-butyric	tr	++	++	—	—	tr	+	—	++	tr
butyric	—	tr	—	—	tr	—	—	tr	tr	tr
iso-valeric	+	++	++	+	tr	++	++	tr	++	+
pyruvic	—	—	—	—	—	tr/-	tr	—	—	—
lactic	tr	tr	tr	tr	+	+	+	+	+	+
succinic	++	++	++	++	++	++	++	++	++	++
phenylacetic	—	+	+	+/-	tr	—	—	—	—	—
Enzyme production:										
β -xylosidase	+	—	—	—	w	—	—	—	—	—
β -glucuronidase	—	+	—	+	—	—	—	—	—	—
β -glucosidase	+	+	+	+	+	+	+	—	—	—
α -galactosidase	+	+	+	+	+	+	+	+	—	+
β -galactosidase	+	+	+	+	+	+	+	+	v	—
α -fucosidase	—	+	+	+	+	+	+	—	+	—
β -NAGase	—	+	+	+	+	+	+	w	w	—
α -mannosidase	—	v	—	+	—	+	+	+	+	v

See footnote to table II; 1, *P. buccae* ATCC 33574, 83-617 and *B. (P.) capillus* ATCC 33690; 2, *P. zoogloeiformans* ATCC 33285; 3, *B. (P.) pentosaceus* NP 333 and WPH 61; 4, *P. oris* ATCC 33573 and 86-20IDD; 5, *P. oralis* NCTC 11459; 6, *P. veroralis* ATCC 33779 and 83-457F; 7, *P. buccalis* ATCC 35310; 8, *P. oulora* NCTC 11871; 9, *P. bivia* VPI 5540 and VPI 6622; 10, *P. disiens* NCTC 11157.

protein bands in the mol. wt regions *c.* 18, 25, 29 and 63 kDa (fig. 3). *B. (P.) pentosaceus* NP 333 and *P. buccae* ATCC 33574 (tracks 2 and 3) were applied for comparative purposes and differed from *P. oris* 86-20IDD and ATCC 33573 (tracks 3 and 4) in several proteins bands, particularly in the regions 29–66 kDa (fig. 3).

Group IV—eight isolates (group E, table II) identified as *P. oralis* (column 5, table III), but with some variation in conventional tests, gave SDS-PAGE profiles identical to that of the *P. oralis* reference strain NCTC 11459; one isolate showed slight differences with a single protein band in the mol. wt region *c.* 87 kDa (fig. 4). *P. veroralis* ATCC 33779 and *P. buccalis* ATCC 35310 (tracks 2 and 3) were used as reference controls and differed from *P. oralis* NCTC 11459 (track 4) in a few protein bands, particularly in the regions *c.* 25 and 116 kDa (fig. 4).

Group V—strains in this biochemically hetero-

geneous group of 15 isolates (group F, table II) gave an identical pattern in GLC and enzyme tests and were similar to *P. veroralis* (column 6, table III). They were separated into five subgroups (a, b, c, d and e) in SDS-PAGE profiles, which differed from that of the *P. veroralis* reference strain ATCC 33779 in protein bands of *c.* 25, 27, 31, 65, 67 and 116 kDa (fig. 5).

Groups VI and VII—each comprised a single isolate with distinct characteristics (groups G and H, table II). Group VI showed some variation from *P. buccalis* ATCC 35310 (column 7, table III) in conventional test reaction patterns and SDS-PAGE profiles (fig. 6). Group VII was biochemically similar to *P. oulora* NCTC 11871 (column 8, table III), but there were more differences in their SDS-PAGE profiles than in other tests; therefore, this single clinical isolate remained in an undesignated SDS-PAGE group (fig. 6).

Group VIII—four isolates (group I, table II) identified as *P. bivia* (column 9, table III) gave similar

Fig. 1. SDS-PAGE profiles of clinical isolates identified as *P. buccae* (group I). Track 1, mol. wt markers; 2, *P. oris* ATCC 33573; 3 and 4, *P. buccae* 83-617 and ATCC 33574; 5-11 and 13-15, examples of clinical isolates subgroup a; 12, example of subgroup b.

Fig. 3. SDS-PAGE protein profiles of clinical isolates identified as *P. oris* (group III). Track 1, mol. wt markers; 2, *B. (P.) pentosaceus* NP 333; 3, *P. buccae* ATCC 33574; 4, *P. oris* 86-201DD; 5, *P. oris* ATCC 33573; 6-12, clinical isolates.

SDS-PAGE profiles except for the absence of a few bands at *c.* 26, 37 and 17 kDa (fig. 7). *P. disiens* NCTC 11157 (track 2) was applied as a reference control and differed from *P. bivia* VPI 6622 and VPI 5540 (tracks 3 and 4) in several protein bands, particularly in the regions *c.* 29-66 and 116 kDa (fig. 7).

Group IX—these seven isolates (group J, table II) did not correspond with any of the reference strains. They were divided into two subgroups in SDS-PAGE. The five isolates of subgroup IXa had similar patterns of results in biochemical, GLC and enzyme tests and similar SDS-PAGE profiles. The two isolates of IXb showed minor variations in biochemical and SDS-PAGE profiles (fig. 8).

Fig. 2. SDS-PAGE profiles of clinical isolates identified as *P. zoogloeiformans* (group II). Track 1, mol. wt markers; 2, *P. oris* ATCC 33573; 3-4, *B. (P.) pentosaceus* WPH 61, NP 333; 5, *P. buccae* ATCC 33574; 6, *P. zoogloeiformans* ATCC 33285; 7 and 8, clinical isolates subgroup a; 9-12, clinical isolates subgroup b (undesignated SDS-PAGE group).

Fig. 4. SDS-PAGE profiles of clinical isolates identified as *P. oralis* (group IV). Track 1, mol. wt markers; 2, *P. buccalis* ATCC 35310; 3, *P. veroralis* ATCC 33779; 4, *P. oralis* NCTC 11459; 5-12, clinical isolates.

Fig. 5. SDS-PAGE profiles of clinical isolates identified as *P. veroralis* (group V). Track 1, mol. wt markers; 2, *P. veroralis* ATCC 33779; 3–8 clinical isolates of subgroup a; 9, subgroup b; 10–13, subgroup c; 14 and 15, subgroup d; 16 and 17, subgroup e.

Fig. 6. SDS-PAGE profiles of clinical isolates of groups VI and VII. Tracks 3 and 6, mol. wt markers; 1, clinical isolate (group VI); 2, *P. buccalis* ATCC 35310; 4, clinical isolate (undesignated SDS-PAGE group); 5, *P. oulora* NCTC 11871.

Fig. 7. SDS-PAGE profiles of clinical isolates identified as *P. bivia*. Track 1, mol. wt markers; 2, *P. disiens* NCTC 11157; 3 and 4, *P. bivia* VPI 6622 and VPI 5540; 5–8, clinical isolates.

Discussion

Among the recognised species of the genus *Prevotella*, the black-pigmented species have been well studied and classified. However, the non-pigmented species have not been so closely studied. Most of the non-pigmented species (*P. veroralis*, *P. oulora*, *P. buccalis* and *P. zooglyphiformans*) have been described

only recently,^{18–21} are generally still reported as either “*B. (P.) oralis*” or “*B. (P.) ruminicola*”, and few data are available on their general characteristics and role in human disease.

In the previous study,¹² each of the three analytical approaches (biochemical, GLC and enzyme tests) contributed to the identification of the species within this group. The technique of polyacrylamide slab gel

Fig. 8. SDS-PAGE profiles of group IX (undesigned spp.) 1-5, clinical isolates of subgroup a; 6 and 7, subgroup b; 8, mol. wt markers.

electrophoresis also appeared to be useful in characterisation and classification of strains within the genus *Prevotella*. The use of slab gels enabled resolution of proteins in a mol. wt range from c. 15-205 kDa in a gel 14 cm long, and the protein profiles of the majority of the species produced discrete bands allowing good differentiation. For each strain, the profiles were reproducible in successive extractions and gels. The strains of each species gave similar overall profiles, but the profiles could differ in specific bands. Species discrimination was mainly obtained by well stained, major protein bands. The degree of resemblance of strains was assessed visually. This was adequate for strains adjacent to each other on a gel, particularly if they demonstrated identical protein patterns. The age of the culture or number of subcultures did not appear to result in a significant variation in the protein profiles. Re-analysis of the protein extracts after storage at -20°C for several weeks caused the omission of some minor protein bands. The most critical factor affecting reproducibility of the gel patterns appeared to be sensitivity of the acrylamide solutions to oxygen, and it was necessary to de-gas the acrylamide solutions thoroughly before the ammonium persulphate was added.

Some groups of strains (*P. buccae*, *B. (P.) capillus*, *P. disiens*, *P. bivia*, *P. zoogloformans* and *B. (P.) pentosaceus*) were easily lysed during the process of protein extraction (the EL group), whereas others (*P. oralis*, *P. veroralis*, *P. buccalis*, *P. oulora* and *P. oris*) were difficult to lyse (the DL group). Although *P. zoogloformans* and *B. (P.) pentosaceus* were readily lysed, they produced a viscous and sticky protein extract. In these species the high concentration of soluble protein in the extracts caused problems with

band smearing and over-staining, making the SDS-PAGE patterns difficult to interpret.

In this study, the SDS-PAGE profiles of most (79) clinical isolates were similar to those of seven reference strains, and enabled species differentiation. The isolates that were provisionally classified as *P. buccae* and *P. oralis* by conventional tests,¹² gave protein profiles identical with reference strains. However, isolates identified as *P. veroralis*, *P. oris*, *P. bivia*, *P. zoogloformans* and *P. buccalis* differed from reference strains by some protein bands; 12 clinical isolates remained in three undesigned SDS-PAGE groups (IIb, VII and IXa) which may represent previously undesigned species.

P. buccae (group I) was the most commonly isolated of the non-pigmented *Prevotella* spp. from periodontal pockets, in agreement with findings of Haapasalo^{4,22} and Johnston *et al.*²³ SDS-PAGE confirmed that these strains (group I) were correctly identified as *P. buccae* in the previous conventional study.¹² *P. buccae* ATCC 33574, *B. (P.) capillus* ATCC 33690 and *B. (P.) pentosaceus* NP 333 and WPH 61 had similar biochemical properties and have recently been combined in the single species *P. buccae*.²⁴ In a previous study,¹² we found that *P. buccae* ATCC 33574 and *B. (P.) capillus* ATCC 33690 were similar in all three approaches, but *B. (P.) pentosaceus* NP 333 and WPH 61 gave different results in GLC and enzyme tests from *P. buccae* ATCC 33574. Results obtained by SDS-PAGE confirmed this finding; *P. buccae* and *B. (P.) capillus* gave identical protein profiles, but *B. (P.) pentosaceus* and *P. buccae* showed many profile differences (fig. 9). *B. (P.) pentosaceus* NP 333 and *B. (P.) pentosaceus* WPH 61 gave similar results in conventional tests and GLC,¹² but they gave slightly different SDS-PAGE

Fig. 9. SDS-PAGE protein profiles of collection strains. Track 1, high mol. wt markers; 2 and 3, *P. oris* ATCC 33573 and 86-201DD; 4 and 5, *P. buccae* 83-617 and ATCC 33574; 6, *B. (P.) capillus* ATCC 33690; 7 and 8, *B. (P.) pentosaceus* NP 333 and WPH 61; 9, *P. oralis* NCTC 11459; 10 and 11, *P. veroralis* ATCC 33779 and 83-457F; 12, *P. zoogloformans* ATCC 33285; 13, *P. buccalis* ATCC 35310; 14, *P. oulora* NCTC 11871; 15 and 16, *P. bivia* VPI 6822 and VPI 5540; 17, *P. disiens* NCTC 11157.

profiles (fig. 9). Protein profiles of *P. buccae* (subgroup Ia) ATCC 33574 and *P. buccae* (subgroup Ib) 83-617 appeared to be identical except for the absence of a major protein band in the region c. 116 kDa.

Of the six isolates (group II) initially identified as *P. zoogloeiformans* in conventional biochemical tests,¹² only two gave GLC patterns identical with those of the reference strain (*P. zoogloeiformans*). The four remaining isolates showed GLC and enzyme patterns different from *P. zoogloeiformans*. SDS-PAGE confirmed this division; two isolates (subgroup IIa) gave electrophoretic patterns similar to *P. zoogloeiformans*, but four isolates (subgroup IIb) showed many differences in their profiles. Therefore, these four isolates remained in an undesignated SDS-PAGE group (fig. 2).

Clinical isolates that were identified as *P. oris* (group III) differed slightly from the reference strain (*P. oris* ATCC 33573); common bands were seen, but there were also easily recognisable differences. Although clinical isolates of *P. oralis* (group IV) showed some variations in biochemical tests, they displayed homogeneous electrophoretic patterns. The strains of *P.*

veroralis (group V), formed an electrophoretically and biochemically heterogeneous collection of strains (subgroups Va-e) despite their similarity in GLC and enzyme patterns. *P. oulora* (group VII) gave a unique protein profile and differed from other non-pigmented species by several major protein bands. The single strain that was biochemically similar to *P. oulora* showed many differences in protein profile from the reference strain.

For the majority of isolates, SDS-PAGE protein profiles enabled good differentiation and characterisation of non-pigmented species of the genus *Prevotella*. SDS-PAGE revealed two previously unrecognised centres of variation within the group, and indicated some heterogeneity of isolates identified as *P. veroralis* and *P. buccae*. Previous DNA hybridisation work indicating that *B. (P.) capillus* and *P. buccae* were synonymous²⁴ was supported by the SDS-PAGE results in this study. By contrast the contention that *B. (P.) pentosaceus* and *P. buccae* are synonymous, made in the same paper,²⁴ was not supported; these two species showed many differences in SDS-PAGE profiles and in conventional test reaction patterns.

References

1. Duerden BI. The isolation and identification of *Bacteroides* spp. from the normal human gingival flora. *J Med Microbiol* 1980; **13**: 89-101.
2. Shah HN, Collins MD. *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* 1990; **40**: 205-208.
3. Duerden BI, Goodwin L, O'Neil TCA. Identification of *Bacteroides* species from adult periodontal disease. *J Med Microbiol* 1987; **24**: 133-137.
4. Haapasalo M. The genus *Bacteroides* in human dental root canal infections. Taxonomic, ultrastructural and clinical studies. *Proc Finn Dent Soc* 1986; **82** Suppl XI-XIII: 1-87.
5. Holdeman LV, Johnson JL. *Bacteroides disiens* sp. nov. and *Bacteroides bivius* sp. nov. from human clinical infections. *Int J Syst Bacteriol* 1977; **27**: 337-345.
6. Brusis T, Luckhaupt H. Anaerobier infektionen bei exulzierten Tumoren im Kopf-Hals-Bereich. Ein Beitrag zum Foetorproblem. *Laryngol Rhinol Otol* 1986; **65**: 65-68.
7. Brook I. Direct and indirect pathogenicity of anaerobic bacteria in respiratory tract infections in children. *Adv Pediatr* 1987; **34**: 357-377.
8. Shah HN, Collins MD. Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species. *Int J Syst Bacteriol* 1989; **39**: 85-87.
9. Storm A, Dyer JK, Marsh C, Tribble JK. Identification and characterisation of species of the family Bacteroidaceae by polyacrylamide gel electrophoresis. *J Dent Res* 1976; **55**: 252-256.
10. Moore WEC, Hash DE, Holdeman LV, Cato EP. Polyacrylamide slab gel electrophoresis of soluble proteins for studies of bacterial floras. *Appl Environ Microbiol* 1980; **39**: 900-907.
11. Holbrook WP, Ogston SA, Ross PW. A method for the isolation of *Bacteroides melaninogenicus* from the human mouth. *J Med Microbiol* 1978; **11**: 203-207.
12. Yousefi-Mashouf R, Duerden BI. An identification scheme for oral non-pigmented *Prevotella* (*Bacteroides*) species. *Microbial Ecol Health Dis* (in press).
13. Rotimi VO, Faulkner J, Duerden BI. Rapid methods for identification of clinical isolates of gram-negative anaerobic bacilli. *Med Lab Sci* 1980; **37**: 331-339.
14. Casals JB, Pringle N. Diagnostic tablets for bacterial identification. Rosco Diagnostica, Taastrop, Denmark, 1987.
15. Popovic-Uroic T, Patton CM, Nicholson MA, Kiehlbauch JA. Evaluation of the indoxyl acetate hydrolysis test for rapid differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species. *J Clin Microbiol* 1990; **28**: 2335-2339.
16. Holdeman LV, Cato EP, Moore WEC (eds). Anaerobe laboratory manual, 4th edn. Blacksburg, VA, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, 1977.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685.
18. Watabe J, Benno Y, Mitsuoka T. Taxonomic study of *Bacteroides oralis* and related organisms and proposal of *Bacteroides veroralis* sp. nov. *Int J Syst Bacteriol* 1985; **33**: 57-64.
19. Shah HN, Collins MD, Watabe J, Mitsuoka T. *Bacteroides oulorum* sp. nov., a nonpigmented saccharolytic species from the oral cavity. *Int J Syst Bacteriol* 1985; **35**: 193-197.
20. Shah HN, Collins MD. *Bacteroides buccalis*, sp. nov., *Bacteroides denticola*, sp. nov., *Bacteroides pentosaceus*, sp. nov., new species of the genus *Bacteroides* from the oral cavity. *Zentralbl Bakteriell Hyg Abt 1 Orig C* 1981; **2**: 235-241.
21. Cato EP, Kelley RW, Moore WEC, Holdeman LV. *Bacteroides zoogloeiformans* (Weinberg, Nativelle, and Prevot 1983) corrig., comb. nov.: emended description. *Int J Syst Bacteriol* 1982; **32**: 271-274.
22. Haapasalo M. *Bacteroides buccae* and related taxa in necrotic root canal infections. *J Clin Microbiol* 1986; **24**: 940-944.
23. Johnston BL, Edelstein MAC, Holloway EY, Finegold SM. Bacteriologic and clinical study of *Bacteroides oris* and *Bacteroides buccae*. *J Clin Microbiol* 1987; **25**: 491-493.
24. Johnson JL, Holdeman LV. *Bacteroides capillus* Korman and Holt and *Bacteroides pentosaceus* Shah and Collins, later synonyms of *Bacteroides buccae* Holdeman et al. *Int J Syst Bacteriol* 1985; **35**: 114.