Prevotella marshii sp. nov. and *Prevotella baroniae* sp. nov., isolated from the human oral cavity

Julia Downes,¹ Iain Sutcliffe,² Anne C. R. Tanner³ and William G. Wade^{1,3}

Correspondence

William G. Wade
william.wade@kcl.ac.uk

¹Infection Research Group, Dental Institute, King's College London, London SE1 9RT, UK

²Division of Biomedical Sciences, School of Applied Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, UK

³Department of Molecular Genetics, The Forsyth Institute, Boston, MA 02115, USA

Eleven strains of anaerobic Gram-negative bacilli isolated from the human oral cavity were subjected to a comprehensive range of phenotypic and genotypic tests and were found to comprise two homogeneous groups, designated E2 and E4. 16S rRNA gene sequence analysis revealed that members of both groups belonged to the genus *Prevotella* but were distinct from any species with validly published names. This distinction was confirmed by DNA–DNA hybridization and phenotypic tests. Two novel species are therefore proposed: *Prevotella marshii* sp. nov. (group E2) and *Prevotella baroniae* (group E4). Both species are saccharolytic; the end-products of fermentation for *P. marshii* are acetic, propionic and succinic acids, while *P. baroniae* produces acetic and succinic acids with minor amounts of isovaleric and isobutyric acids. The G+C content of the DNA of the type strain of *Prevotella marshii* is 51 mol% and that of *Prevotella baroniae* is 52 mol%. The type strain for *P. marshii* is E9.34^T (=DSM 16973^T = CCUG 50419^T) and that for *P. baroniae* is E9.33^T (=DSM 16972^T = CCUG 50418^T).

The genus *Prevotella* includes moderately saccharolytic, bile-sensitive species formerly belonging to the genus *Bacteroides* (Shah & Collins, 1990). Seventeen of the *Prevotella* species with validly published names are found in the oral cavity. The use of molecular methods, particularly 16S rRNA gene sequence analysis, for the identification of oral bacterial isolates has revealed that a significant proportion of strains do not correspond to species with validly published names (Kroes *et al.*, 1999; Munson *et al.*, 2002, 2004). In this study, 11 strains of anaerobic Gram-negative bacilli, isolated from human oral infections and dental plaque in healthy subjects, provisionally identified as belonging to the genus *Prevotella*, were subjected to a range of phenotypic and genotypic tests.

Strains E9.33^T, E9.34^T and W7346 were isolated from endodontic lesions, W5520 and W5712 were isolated from dental

abscesses, W1833 was isolated from a pericoronitis lesion, D016C-10 and D031A-28 were isolated from supragingival plaque from individuals with periodontitis and strains D190A-02, D153G-12 and W6967 were isolated from subgingival plaque in periodontally healthy individuals. Strains D016C-10, D031A-28, D190A-02 and D153G-12 were obtained from the collection of W. E. C. Moore and L. V. Holdeman Moore, formerly of the Virginia Polytechnic Institute. *Prevotella denticola* ATCC 35308^T, *Prevotella melaninogenica* ATCC 25845^T, *Prevotella veroralis* ATCC 33779^T, *Prevotella loescheii* NCTC 11321^T, *Prevotella oralis* NCTC 11459^T, *Prevotella shahii* DSM 15611^T and *Prevotella buccae* CCUG 15401^T were obtained from the American Type Culture Collection (Manassas, VA, USA), the National Collection of Type Cultures, Central Public Health Laboratory (London, UK), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) or the Culture Collection of the University of Göteborg (Gothenburg, Sweden), respectively.

Strains were grown at 37 °C on fastidious anaerobe agar (FAA; LabM), supplemented with 5 % horse blood, under anaerobic conditions (in N₂/H₂/CO₂: 80/10/10, by vol.) in an MAC anaerobic chamber (Don Whitley). Colonial morphologies were determined using a plate microscope after 5 days incubation. Cellular morphology was recorded after Gram staining of 2-day-old plate cultures. Hanging-drop preparations of 18 h cultures of peptone/yeast extract/glucose (PYG; Holdeman *et al.*, 1977) broth were examined

Published online ahead of print on 1 March 2005 as DOI 10.1099/ijs.0.63634-0.

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *P. marshii* E9.34^T, *P. baroniae* E9.33^T, *P. loescheii* NCTC 11321^T, *P. oralis* NCTC 11459^T, *P. denticola* ATCC 35308^T, *P. melaninogenica* ATCC 25845^T and *P. veroralis* ATCC 33779^T are AF481227, AY840553, AY836508, AY323522, AY323524, AY323525 and AY836507, respectively.

Transmission electron micrographs of *P. baroniae* E9.33^T and *P. marshii* E9.34^T, an extended phylogenetic tree and details of FAME profiles are available as supplementary material in IJSEM Online.

under phase-contrast microscopy (Axioskop; Zeiss) for cellular motility. Transmission electron microscopy (H7600; Hitachi) was used to examine the cell-wall ultrastructure of strains E9.34^T and E9.33^T as described previously (Downes *et al.*, 2003).

Fermentation tests were performed using pre-reduced, anaerobically sterilized sugars prepared in an anaerobic workstation (Holdeman *et al.*, 1977). Other biochemical tests were performed using standard methods (Holdeman *et al.*, 1977; Jousimies-Somer *et al.*, 2002). Bacterial strains were grown in peptone/yeast extract (PY) broth (Holdeman *et al.*, 1977) with and without glucose and short-chain volatile and non-volatile fatty acids were extracted by standard methods and analysed by GC (Holdeman *et al.*, 1977). Enzyme profiles were generated with the Rapid ID 32A anaerobe identification kit (bioMérieux), according to the manufacturer's instructions, using bacteria harvested from blood agar plates (blood agar base no. 2; LabM) supplemented with 5% horse blood, and performed in triplicate.

The cellular fatty acid composition was determined for 10 strains as described previously (Sutcliffe, 2000). In brief, fatty acids from lyophilized whole cells were derivatized to produce fatty acid methyl esters (FAMES) by acid-catalysed methanolysis. FAMES were recovered by hexane extraction and concentration under nitrogen, followed by GC analysis (Sutcliffe, 2000). FAMES were identified by comparison with authentic FAME standards (Sigma).

The G+C content of the DNA was estimated by using an HPLC method for E2 strains E9.34^T and D153G-12 and E4 strains E9.33^T and W1833 as described previously (Wade *et al.*, 1999). A thermal denaturation method (Huß *et al.*, 1983) was used to determine the DNA–DNA relatedness between strains.

DNA was isolated from the bacteria by using a standard method. Briefly, cells were suspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8; Sigma) and treated with lysozyme (500 µg ml⁻¹) for 30 min at 37 °C followed by proteinase K (500 µg ml⁻¹) and 20% *N*-lauroyl-sarcosine (Sarkosyl; Sigma) (2%) for 2 h at 37 °C. After two phenol/chloroform extractions and one chloroform extraction, DNA was precipitated by the addition of two volumes of ethanol (stored at -20 °C) and washed in 70% ethanol. The 16S rRNA gene was amplified by a PCR with primers 27F and 1492R (Lane, 1991) and a Uno II Thermocycler (Biomtra) using Ready to Go PCR beads (Pharmacia/Amersham). PCR products were sequenced directly using a dye terminator cycle sequencing kit (CEQ DTCS; Beckman Coulter) and 60 ng template DNA, according to the manufacturer's instructions. Sequencing was performed using an automated sequencer (CEQ2000; Beckman Coulter) with primers 27f, 342r, 357f, 519r, 907r, 926f, 1100r, 1114f and 1492r (Lane, 1991); primers 1100r and 1114f had minor modifications (1100Ar, 5'-GGGTTGCGCTCGTTA-3'; 1114ATf, 5'-ATAACGAGCGCAACCC-3'). Sequences

were connected using the BioEdit program (Hall, 2004) and identified by BLAST interrogation of the GenBank database (Altschul *et al.*, 1990). Related sequences were aligned by means of CLUSTAL X (Thompson *et al.*, 1997). Further analysis was performed using the PHYLIP suite of programs (Felsenstein, 1993). Specifically, a distance matrix was constructed using the Jukes–Cantor algorithm with DNADIST, and NEIGHBOR was used to construct phylogenetic trees, which were viewed using TREEVIEW (Page, 1996).

The 11 strains studied were found to fall into two groups. Five strains, E9.34^T, D190A-02, D153G-12, D016C-10 and D031A-28, designated group E2, had cells that were 0.4 µm wide by 0.9–3 µm long, with the occasional cell reaching 6 µm in length, and occurred singly and in pairs. After 5 days incubation on FAA plates, E2 colonies were 1.8–3.5 mm in diameter, circular and entire, convex, watery, opaque and grey to off-white/grey to greenish grey. Areas of heavier growth at the end of the streak lines on FAA were cream in colour. Viewed under a plate microscope, the colonies had a striated appearance with concentric rings of varying opacity and iridescent hues of pink, green and yellow.

The remaining six strains, designated group E4, consisted of coccoid and short bacilli, the majority of cells being 0.6 × 0.6–2.0 µm, with occasional cells ranging from 3 to 8 µm in length. After 5 days incubation on FAA plates, E4 colonies were 1.2–3.8 mm in diameter, circular, high convex and opaque with an off-white centre, which appeared matt in some strains and had a shiny grey periphery. Colony morphology varied from entire to undulate; this variation appeared to be strain-related but in some instances both forms were present in the same strain.

Transmission electron microscopic examination of ultra-thin sections through the cells of E9.34^T (group E2) and E9.33^T (group E4) showed the presence of a typical Gram-negative cell wall composed of a thin peptidoglycan layer surrounded by an outer membrane (micrographs are available as Supplementary Fig. S1 in IJSEM Online).

Growth of all E2 strains in PY broth was good and produced a moderately turbid suspension (3+ on a scale of 0 to 4+) with a deposit of cells on standing. Growth was markedly enhanced by the addition of either glucose, fructose or maltose (1%, w/v, in each case), resulting in a heavily turbid suspension of 4+. Strains were saccharolytic and fermented glucose and maltose strongly and fructose and mannose weakly and variably. Other sugars tested, listed in the species description, were not fermented. The major end-products of fermentation were acetic acid and succinic acid; minor to moderate amounts of propionic acid were also produced. Hydrolysis of gelatin was strongly positive, with pre-reduced, anaerobically sterilized gelatin tests not solidifying at 4 °C. All E2 strains were positive for α-glucosidase, alkaline phosphatase, leucyl glycine arylamidase, pyroglutamic acid arylamidase and alanine arylamidase in the Rapid ID 32A identification panel, while

reactions for glutamyl glutamic acid arylamidase were weak and variable. All strains gave negative results in the remaining 23 tests, resulting in a Rapid ID 32A profile of 0400 4442 0⁰/₂. The G+C content of the DNA of strains E9.34^T and D153G-12 was 51 mol%.

Growth of E4 strains in PY broth was good (3+) and produced a deposit of cells with a slightly turbid suspension (1+) in PY broth and a clear supernatant in PYG broth on standing. Growth was somewhat enhanced by the addition of 1% (w/v) fermentable carbohydrates. The major end-products of fermentation in PYG broth were acetic acid and succinic acid; isovaleric acid was a minor end-product. In addition to these acids, minor amounts of isobutyric acid were produced in PY broth, and larger amounts of isovaleric acid were produced in PY broth than in PYG broth. All E4 strains were positive for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and α -fucosidase in the Rapid ID 32A identification panel, while reactions for mannose and raffinose fermentation and for glutamyl glutamic acid arylamidase were variable. All strains were negative for the remaining 17 tests, resulting in a Rapid ID 32A profile of 451¹/₅ 4402 2⁰/₂. The G+C content of the DNA of strains E9.33^T and W1833 was 52 mol%.

A phylogenetic tree showing the relationship between representative strains of groups E2 and E4 and other closely related species of the genus *Prevotella* is shown in Fig. 1. The 16S rRNA genes of the type strains of *P. denticola*, *P. loescheii*, *P. melaninogenica*, *P. oralis* and *P. veroralis* were sequenced and deposited with GenBank to improve the quality of the phylogenetic analysis, in view of the relatively

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1411 aligned bases showing the relationship between *P. marshii*, *P. baroniae* and related species. The tree was constructed using the neighbour-joining method following distance analysis of aligned sequences. Numbers represent bootstrap values for each branch, based on data for 100 trees. Accession numbers for 16S rRNA sequences are given for each strain. Bar, 0.1 nucleotide substitutions per site. A fuller phylogenetic tree is available as Supplementary Fig. S2 in IJSEM Online.

large number of ambiguous bases present in the sequences then available for these strains in the database. Group E2 strain E9.34^T was found to be most closely related to *P. shahii* (92.2% 16S rRNA gene sequence identity over 1455 unambiguously aligned bases). In addition, the 16S rRNA gene was sequenced in E2 strains D190A-02, D153G-12, D016C-10 and D031A-28: over 1415 bases, each strain had >99.7% sequence similarity to the other three strains and to strain E9.34^T. E4 strain E9.33^T was most closely related to *P. buccae* (91.3% sequence identity over 1440 bases). E4 strains W1833, W6967 and W7346 each exhibited >99.5% 16S rRNA gene sequence identity to the other two strains and to strain E9.33^T over 1404 bases.

The DNA–DNA relatedness between group E2 strains E9.34^T and D153G-12 was determined to be 80%. The relatedness values between strain E9.34^T and *P. loescheii* NCTC 11321^T, *P. oralis* NCTC 11459^T and *P. shahii* DSM 15611^T were estimated to be 23, 41 and 42%, respectively. The DNA–DNA relatedness between group E4 strains E9.33^T and W1833 was 78% and that between each of these strains and *P. buccae* CCUG 15401^T was 28 and 20%, respectively.

Prevotella species are reported to synthesize both non-hydroxylated and 3-hydroxylated fatty acids (Shah & Collins, 1990; Willems & Collins, 1995). More than 90% of the FAMES recovered from the strains studied were identifiable by GC as being non-hydroxylated (FAME data are available as Supplementary Table S1 in IJSEM Online). Of the non-hydroxylated fatty acids in *Prevotella*, methyl-branched fatty acids (notably anteiso-C_{15:0}) are reported to predominate, along with C_{16:0} (Logar *et al.*, 2001; Moore *et al.*, 1994; Sakamoto *et al.*, 2004). Consistent with the assignment of E2 and E4 to the genus *Prevotella*, the main FAMES detected in the present study were iso-C_{15:0} and anteiso-C_{15:0}, whilst C_{16:0} was abundant in strains from group E4. However, strains from group E4 were also distinguished by a high content of anteiso-C_{17:0} FAMES. Strains from group E2 were distinguished by a reduced content of C_{16:0} and markedly increased C_{15:0} content. Intriguingly, Sakamoto *et al.* (2004) identified significant quantities of unsaturated fatty acids in representative *Prevotella* species, including *P. shahii* and *P. loescheii*, which are phylogenetically closely related to group E2. However, unsaturated fatty acids have not been noted as major components by others using GC (Debelian *et al.*, 1997; Logar *et al.*, 2001; Moore *et al.*, 1994) and were not observed in strains of groups E2 and E4 (see Supplementary Table S1). It is noted that mass spectrometry studies have also identified unsaturated fatty acids in *Prevotella* species, including mono-, di- and tri-unsaturated forms (Radcliffe *et al.*, 2001; Tavana *et al.*, 1998). It is clear that a comprehensive, integrated re-examination of the hydroxylated and non-hydroxylated fatty acid composition of members of the genus *Prevotella* is warranted.

The phenotypic and genotypic tests described here show that the strains tested fall into two groups that are distinct

from any *Prevotella* species with validly published names. We propose the name *Prevotella marshii* sp. nov. for the strains in the E2 group and *Prevotella baroniae* sp. nov. for the E4 group, with E9.34^T (=DSM 16973^T=CCUG 50419^T) and E9.33^T (=DSM 16972^T=CCUG 50418^T) as the respective type strains.

Phenotypic characteristics that distinguish *P. marshii* and *P. baroniae* from other *Prevotella* species are shown in Table 1. *P. marshii* is unusual in that it produces minor to moderate amounts of propionic acid as a by-product of fermentation. Although the characteristics shown will help in the identification of clinical isolates, unequivocal identification will not be possible in all cases. 16S rRNA gene sequence analysis is recommended for the identification of clinical isolates in this genus, not least because the many additional unnamed *Prevotella* taxa will be recognized by this method.

Description of *Prevotella marshii* sp. nov.

Prevotella marshii (mar'shi.i. N.L. gen. n. *marshii* of Marsh, named in honour of British microbiologist Philip Marsh, for his contributions to oral microbiology).

The description is based on five strains isolated from the human oral cavity. Cells are obligately anaerobic, non-motile, Gram-negative bacilli (0.4 × 0.9–3 µm) occurring singly and in pairs with an occasional elongated cell up to 6 µm long. After 5 days incubation on FAA plates, colonies are 1.8–3.5 mm in diameter, circular and entire, convex, watery, opaque and grey to off-white/grey to greenish grey. Areas of heavier growth at the end of the streak lines on FAA are cream in colour. Viewed under a plate microscope the colonies have a striated appearance with concentric rings of varying opacity and iridescent hues of pink, green

and yellow. Growth in broth media produces moderate turbidity that is markedly enhanced by the addition of glucose, fructose or maltose. Cells are saccharolytic and ferment glucose and maltose strongly and fructose and mannose weakly and variably. Arabinose, cellobiose, lactose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose and trehalose are not fermented. Major amounts of acetic acid and succinic acid and moderate amounts of propionic acid are produced as end-products of metabolism in PYG broth. Gelatin is hydrolysed; aesculin, arginine and urea are not hydrolysed. Indole and catalase are not produced and nitrate is not reduced. There is no growth in 20 % bile. The Rapid ID 32A profile is 0400 4442 0⁰/2. The fatty acid profile predominantly comprises anteiso-C_{15:0}, iso-C_{15:0}, C_{15:0} and iso-C_{14:0}. The G + C content of the DNA of the type strain is 51 mol%.

Isolated from the human oral cavity in subjects with endodontic and periodontal infections and from subgingival dental plaque in healthy subjects. The type strain is E9.34^T (=DSM 16973^T=CCUG 50419^T).

Description of *Prevotella baroniae* sp. nov.

Prevotella baroniae (bar'on.i.ae. N.L. gen. n. *baroniae* of Baron, named in honour of Ellen Jo Baron, the American microbiologist, for her contributions to clinical microbiology).

The description is based on six strains isolated from the human oral cavity. Cells are obligately anaerobic, non-motile, Gram-negative coccoid and short bacilli (0.6 µm × 0.6–2.0 µm) with occasional cells ranging from 3 to 8 µm long. After 5 days incubation on FAA plates, colonies are 1.2–3.8 mm in diameter, circular, high convex and opaque with a shiny grey periphery and an off-white

Table 1. Characteristics that distinguish *P. baroniae* sp. nov. and *P. marshii* sp. nov. from related *Prevotella* species

1, *P. buccae*; 2, *P. dentalis*; 3, *P. oris*; 4, *P. salivae*; 5, *P. buccalis*; 6, *P. loescheii*; 7, *P. oralis*; 8, *P. veroralis*; 9, *P. baroniae* sp. nov.; 10, *P. denticola*; 11, *P. enoea*; 12, *P. melaninogenica*; 13, *P. shahii*; 14, *P. oulorum*; 15, *P. intermedia*; 16, *P. nigrescens*; 17, *P. pallens*; 18, *P. tanneriae*; 19, *P. marshii* sp. nov. +, Positive; –, negative; v, variable; w, weak.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Production of pigment on blood agar	–	–	–	–	–	+	–	–	–	+	–	+	+	–	+	+	+	+	–
Fermentation of:																			
Arabinose	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	v	–
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	–	v	v
Raffinose	+	+	+	+	+	+	+	+	+	+	–	+	+	+	v	+	+	v	–
Salicin	+	–	+	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–
Sucrose	+	w	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	v	–
Xylose	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Indole production	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	v	v	–	+	–	–	–	–	–
Gelatin liquefaction	+	–	v	–	–	+	v	v	w	+	+	+	+	–	+	+	+	+	+

centre, which appears matt in some strains. Colony morphology varies from entire to undulate. Growth in PY broth is good and is somewhat enhanced by the addition of 1% (w/v) fermentable carbohydrates. Cells are saccharolytic and ferment cellobiose, fructose, glucose, lactose, maltose, mannose, melibiose, raffinose, salicin and sucrose. Arabinose, mannitol, melezitose, rhamnose, ribose, sorbitol and trehalose are not fermented. End-products of fermentation in PYG broth are major acetic and succinic acids and minor isovaleric acid. Minor amounts of isobutyric acid are also produced in PY broth. Aesculin is hydrolysed, gelatin is weakly hydrolysed and arginine and urea are not hydrolysed. Indole and catalase are not produced and nitrate is not reduced. There is no growth in 20% bile. The Rapid ID 32A profile is 451¹/₅/7 4402 2⁰/₂. The non-hydroxylated fatty acid profile predominantly comprises iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0} and anteiso-C_{17:0}. The G + C content of the DNA of the type strain is 52 mol%.

Isolated from the human oral cavity in patients with endodontic and periodontal infections or dentoalveolar abscesses and from the dental plaque of healthy subjects. The type strain is E9.33^T (= DSM 16972^T = CCUG 50418^T).

Acknowledgements

This work was supported by grants from the Guy's and St Thomas' Charitable Foundation (ref. R020725) and National Institute of Dental and Craniofacial Research (ref. DE 09513).

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Debelian, G. J., Olsen, I. & Tronstad, L. (1997). Distinction of *Prevotella intermedia* and *Prevotella nigrescens* from endodontic bacteremia through their fatty acid contents. *Anaerobe* **3**, 61–68.
- Downes, J., Munson, M. & Wade, W. G. (2003). *Dialister invisus* sp. nov., isolated from the human oral cavity. *Int J Syst Evol Microbiol* **53**, 1937–1940.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Hall, T. (2004). BioEdit. Biological sequence alignment editor for Win95/98/NT/2K/XP. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- Holdeman, L. V. H., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.
- Huß, V. A. R., Festl, H. & Schleifer, K.-H. (1983). Studies on the spectrophotometric determination of DNA hybridisation from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jousimies-Somer, H., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. & Finegold, S. M. (2002). *Wadsworth Anaerobic Bacteriology Manual*, 6th edn. Belmont, CA: Star Publishing.
- Kroes, I., Lepp, P. W. & Relman, D. A. (1999). Bacterial diversity within the human subgingival crevice. *Proc Natl Acad Sci U S A* **96**, 14547–14552.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester, UK: Wiley.
- Logar, R. M., Zorec, M. & Kopečny, J. (2001). Reliable identification of *Prevotella* and *Butyrivibrio* spp. from rumen by fatty acid methyl ester profiles. *Folia Microbiol* **46**, 57–59.
- Moore, L. V., Bourne, D. M. & Moore, W. E. (1994). Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic Gram-negative bacilli. *Int J Syst Bacteriol* **44**, 338–347.
- Munson, M. A., Pitt-Ford, T., Chong, B., Weightman, A. & Wade, W. G. (2002). Molecular and cultural analysis of the microflora associated with endodontic infections. *J Dent Res* **81**, 761–766.
- Munson, M. A., Banerjee, A., Watson, T. F. & Wade, W. G. (2004). Molecular analysis of the microflora associated with dental caries. *J Clin Microbiol* **42**, 3023–3029.
- Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Radcliffe, C. E., Korachi, M. A. O., Könönen, E., Boote, V. & Drucker, D. B. (2001). Phospholipid analogue distribution of *Prevotella pallens* and related species analysed by fast atom bombardment mass spectrometry. *Anaerobe* **7**, 87–91.
- Sakamoto, M., Suzuki, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y. (2004). *Prevotella shahii* sp. nov. and *Prevotella salivae* sp. nov., isolated from the human oral cavity. *Int J Syst Evol Microbiol* **54**, 877–883.
- Shah, H. N. & Collins, D. M. (1990). *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* **40**, 205–208.
- Sutcliffe, I. C. (2000). Characterisation of a lipomannan lipoglycan from the mycolic acid containing actinomycete *Dietzia maris*. *Antonie van Leeuwenhoek* **78**, 195–201.
- Tavana, A. M., Drucker, D. B., Hull, P. S. & Boote, V. (1998). Phospholipid molecular species distribution of oral *Prevotella corporis* clinical isolates. *FEMS Immunol Med Microbiol* **21**, 57–64.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wade, W. G., Downes, J., Dymock, B., Hiom, S. J., Weightman, A. J., Dewhirst, F. E., Paster, B. J., Tzellas, N. & Coleman, B. (1999). The family *Coriobacteriaceae*: reclassification of *Eubacterium exiguum* (Poco *et al.* 1996) and *Peptostreptococcus heliotrinireducens* (Lanigan 1976) as *Slackia exigua* gen. nov., comb. nov. and *Slackia heliotrinireducens* gen. nov., comb. nov., and *Eubacterium lentum* (Prevot 1938) as *Eggerthella lenta* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 595–600.
- Willems, A. & Collins, M. D. (1995). 16S rRNA gene similarities indicate that *Hallella seregens* (Moore and Moore) and *Mitsuokella dentalis* (Haapsalo *et al.*) are genealogically highly related and are members of the genus *Prevotella*: emended description of the genus *Prevotella* (Shah and Collins) and description of *Prevotella dentalis* comb. nov. *Int J Syst Bacteriol* **45**, 832–836.