Prevotella fusca sp. nov. and *Prevotella scopos* sp. nov., isolated from the human oral cavity

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Two 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 belong to two separate taxa. Phylogenetic analysis of full-length 16S rRNA gene sequences showed that the strains were both related to, but distinct from, the type strain of *Prevotella melaninogenica*. Two novel species, *Prevotella fusca* sp. nov. and *Prevotella scopos* sp. nov., are proposed to accommodate these strains. Both strains were saccharolytic and produced acetic and succinic acids, with lesser amounts of lactic and isovaleric acids, as end products of fermentation, and both were sensitive to 20% bile. The principal cellular long-chain fatty acids of both strains were ai-C_{15:0}, 3-OH i-C_{17:0}, 3-OH C_{16:0}, i-C_{15:0} and C_{16:0}. The DNA G + C contents of the type strains of *Prevotella fusca* (W1435^T = DSM 22504^T = CCUG 57946^T) and *Prevotella scopos* (W2052^T = DSM 22613^T = CCUG 57945^T) were 43 and 41 mol%, respectively. The two species could be differentiated by gelatin hydrolysis, cellobiose and ribose fermentation, and production of β -glucosidase.

Members of the genus *Prevotella* are among the species most frequently isolated from the human oral cavity. In this study, two strains provisionally identified as belonging to the genus *Prevotella* by 16S rRNA gene partial sequence analysis, but distinct from current species, were subjected to a range of phenotypic and genetic tests.

Strain W1435^T was isolated from subgingival plaque in an individual with periodontitis and strain W2052^T was from the exudate associated with a failing dental implant. *Prevotella melaninogenica* ATCC 25845^T was obtained from the ATCC. Strains were grown at 37 °C on Fastidious Anaerobe Agar (FAA; LabM) supplemented with 5% horse blood under anaerobic conditions (80% N₂, 10% H₂, 10% CO₂) in an anaerobic workstation (Don Whitley Scientific). Colonial morphologies were determined by using a dissecting microscope after 4 days of incubation on FAA. Cellular morphology was recorded after Gram-staining of smears prepared from 2-day-old FAA plate cultures. Hanging-drop preparations of 18 h cultures in peptone-yeast extract-glucose (PYG) broth were examined for cellular motility by phase-contrast microscopy. The range and optimum temperatures for growth were determined after 48 h of incubation in pre-reduced PYG

broth that had been dispensed into anaerobically sterilized (pre-reduced, anaerobically sterilized; PRAS) tubes in an anaerobic workstation (Don Whitley Scientific). The range and optimum pH for growth were determined in peptone-yeast extract (PY) broth (Holdeman *et al.*, 1977) incubated at 35 °C for 48 h with initial pH values obtained by adding HCl (0.2 M) or Na₂CO₃ (10%, w/v) to the PY broth.

Biochemical and physiological tests were performed using standard methods (Holdeman *et al.*, 1977; Jousimies-Somer *et al.*, 2002). Fermentation tests were performed using PRAS sugars prepared in-house in an anaerobic workstation (Holdeman *et al.*, 1977). Susceptibility to special-potency antibiotic disks of vancomycin (5 µg), kanamycin (1 mg) and colistin (10 µg) was determined on FAA (Jousimies-Somer *et al.*, 2002). Bacterial strains were grown in PY broth (Holdeman *et al.*, 1977) with or without glucose and short-chain volatile and non-volatile fatty acids produced as metabolic end products 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 cells harvested from Columbia agar plates (LabM) supplemented with 5% horse blood. In addition, blood agar plates (Blood Agar Base No. 2; LabM) supplemented with 5% horse blood were also used to prepare the inoculum for strain W1435^T, since this strain grew poorly on Columbia agar. Enzyme tests were performed in triplicate using different batches of the kit. The G + C content of the DNA was determined by HPLC as described previously (Wade *et al.*, 1999).

Abbreviation: PRAS, pre-reduced, anaerobically sterilized.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Prevotella fusca* W1435^T and *Prevotella scopos* W2052^T are FJ545433 and FJ545434, respectively.

Two supplementary tables are available with the online version of this paper.

Analysis of cellular fatty acids was carried out by the DSMZ Identification Service, Braunschweig, Germany. Fatty acid methyl esters were obtained from 50 mg (dry weight) cells by saponification, methylation and extraction using minor modifications of previously described methods (Kuykendall *et al.*, 1988; Miller, 1982). The fatty acid methyl ester mixtures were separated using the Sherlock Microbial Identification System (MIS; MIDI, Microbial ID), which consisted of an Agilent model 6890N GC fitted with a 5% phenyl-methyl silicone capillary column (0.2 mm $\times$ 25 m), a flame-ionization detector, an Agilent model 7683A automatic sampler and an HP computer with MIDI database (Hewlett Packard). Peaks were automatically integrated and fatty acid names and percentages were determined with MIS Standard Software (Microbial ID). The GC parameters were as follows: carrier gas, ultra-high-purity hydrogen; column head pressure, 60 kPa; injection volume, 2 μ l; column split ratio, 100:1; septum purge, 5 ml min⁻¹; column temperature, 170 to 270 °C at 5 °C min⁻¹; injection port temperature, 240 °C; and detector temperature, 300 °C.

The 16S rRNA genes of strains W1435^T and W2052^T were sequenced as described previously (Downes *et al.*, 2005). Sequences were assembled using the program BioEdit (Hall, 2004) and their closest relatives were identified by BLAST interrogation of GenBank (Altschul *et al.*, 1990). Sequences were aligned by using CLUSTAL W within BioEdit. Phylogenetic trees were reconstructed using MEGA4 by the neighbour-joining method from distance matrices prepared using the Jukes–Cantor correction. A thermal denaturation method was used to determine the DNA–DNA hybridization values between strains (Huß *et al.*, 1983).

Results of the phenotypic tests for the two strains are summarized in the species descriptions and can be viewed in table format as supplementary data (Supplementary Table S1) in IJSEM Online. The two strains were obligately anaerobic, non-motile, Gram-negative bacilli and were representatives of two novel taxa.

Cells of strain W1435^T were 0.8 μ m wide by 1.2–6.0 μ m long. After 4 days of incubation on FAA plates, isolated colonies of W1435^T were 1.8–2.2 mm in diameter, circular, entire, high convex to pyramidal, off-white and opaque, with a rough matt surface. On further incubation, colonies developed a deep orange-brown pigment in the centre of the colony (Fig. 1a). Pigmentation was first detectable as a light orange colour in the centre of the colony after 5–6 days of incubation on FAA. The optimum temperature for growth was 35 °C, with good growth at 25 °C and 30 °C, marginal growth at 42 °C and 45 °C, and no growth at 20 °C. The optimum pH for growth was pH 7, with good growth at pH 6, marginal growth at pH 5 and no growth at pH 8. Strain W1435^T was resistant to the special potency disks of kanamycin and vancomycin, but was sensitive to colistin. Growth in PY broth produced a moderately turbid suspension (3+ on a scale of 0 to 4+).

Growth was enhanced by the addition of 1% fermentable carbohydrates (3–4+). The strain was saccharolytic (see species description for sugar reactions) and moderate amounts of acetic acid, major amounts of succinic acid and minor amounts of lactic acid were produced as metabolic end products in PYG. In addition to these acids, moderate amounts of isovaleric acid and trace to minor amounts of isobutyric acid were produced in PY broth. Strain W1435^T gave positive reactions in the Rapid ID 32A panel for α -galactosidase, β -galactosidase, β -galactosidase 6-phosphate, α -glucosidase, β -glucosidase, β -N-acetylglucosaminidase, α -fucosidase, mannose fermentation, raffinose fermentation, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Reactions to arginine arylamidase were weak or variable depending on the batch of the kit used. Negative reactions were obtained for the remaining 15 enzymes resulting in a profile of 4717 4⁴/502 22.

Cells of strain W2052^T were 0.7 μ m wide by 0.9–3.0 μ m long. After 4 days of incubation on FAA plates, colonies were 2–3 mm in diameter, circular, entire, convex, smooth, opaque and off-white, with a light orange pigment in the centre of the colonies. On further incubation, colonies developed a sharp, distinct area of dark brown pigment in the centre of the colony to form a 'bullseye' colony (Fig. 1b). The optimum temperature for growth was 35 °C, with good growth at 30 °C and 42 °C, marginal growth at 25 °C and no growth at 45 °C. The optimum pH for growth was pH 7, with good growth at pH 6 and marginal growth at pH 5 and pH 8. Strain W2052^T was resistant to the special potency disks colistin, kanamycin and vancomycin. Growth in PY broth produced a moderately turbid suspension (3+) and growth was enhanced by the addition of 1% fermentable carbohydrates (3–4+). The strain was saccharolytic (see species description for sugar reactions) and moderate amounts of acetic acid, major amounts of succinic acid, and trace amounts of isovaleric and lactic acids were produced as metabolic end products in PYG. In addition to these acids, trace to minor amounts of isobutyric acid and moderate rather than trace amounts of isovaleric acid were produced in PY broth. Gelatin hydrolysis was positive, but other biochemical tests were negative (see description of species). Strain W2052^T gave

Fig. 1. Morphology of colonies of strain W1435^T (a) and strain W2052^T (b) after 10 days of incubation on FAA. Bars, 1 mm.

positive reactions in the Rapid ID 32A panel for α -galactosidase, β -galactosidase, β -galactosidase 6-phosphate, α -glucosidase, β -N-acetylglucosaminidase, α -fucosidase, mannose fermentation, raffinose fermentation, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Reactions to leucine arylamidase and histidine arylamidase were weak or variable depending on the batch of the kit used. Negative reactions were obtained for the remaining 14 enzymes resulting in a profile of 4707 45⁰/22²/3.

The cellular fatty acid methyl ester results for strains W1435^T and W2052^T are shown in Supplementary Table S2. The principal cellular long-chain fatty acids identified in W1435^T were ai-C_{15:0} (28.0 %), 3-OH i-C_{17:0} (29.2 %), 3-OH C_{16:0} (11.0 %), i-C_{15:0} (7.8 %) and C_{16:0} (5.2 %); those for W2052^T were also ai-C_{15:0} (39.0 %), 3-OH i-C_{17:0} (9.5 %), 3-OH C_{16:0} (8.0 %), i-C_{15:0} (11.8 %) and C_{16:0} (7.2 %). These profiles are comparable to those of other species of the genus *Prevotella* previously analysed (Downes *et al.*, 2007).

Phylogenetic analysis of the 16S rRNA gene sequences of strains W1435^T and W2052^T revealed that both strains belonged to the genus *Prevotella* and were most closely related to the type strain of *P. melaninogenica* (Fig. 2). Strains W1435^T and W2052^T had sequence similarities of 95.9 and 98.1 %, respectively, with *P. melaninogenica* ATCC 25845^T over 1452 unambiguously aligned bases. As W2052^T had >97 % sequence similarity with *P. melaninogenica* ATCC 25845^T, the extent of DNA–DNA hybridization was estimated, as recently recommended (Stackebrandt & Ebers, 2006), and found to be 26 %, which is consistent with W2052^T representing a novel species.

The strains studied here represent two novel taxa that are clearly distinct from any species with validly published names; the names *Prevotella fusca* sp. nov. (type strain W1435^T) and *Prevotella scopos* sp. nov. (type strain W2052^T) are therefore proposed. Phenotypic characteristics that distinguish *P. fusca* and *P. scopos* from related species of the genus *Prevotella* are shown in Table 1. *P. scopos* and *Prevotella shahii* cannot be differentiated on the basis of these tests; they are easily identified, however, by 16S rRNA gene sequence analysis.

Description of *Prevotella fusca* sp. nov.

Prevotella fusca (fus'ca. L. fem. adj. *fusca* dark-coloured, relating to the dark colour of colonies of the type strain).

The description is based on one strain. Cells are obligately anaerobic, non-motile, Gram-negative bacilli (0.8 × 1.2–6.0 µm). After 4 days of incubation on FAA plates, isolated colonies are 1.8–2.2 mm in diameter, circular, entire, high convex to pyramidal, off-white and opaque, with a rough matt surface. On further incubation, colonies develop a deep orange-brown pigment in the centre of the colony. Growth in broth media produces moderate turbidity that is

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1361 aligned bases showing the relationship between *Prevotella fusca* sp. nov., *Prevotella scopos* sp. nov. and related species of the genus *Prevotella*. The tree was reconstructed using the neighbour-joining method from a distance matrix constructed from aligned sequences using the Jukes–Cantor correction. Numbers represent bootstrap values for each branch based on data for 500 trees. Accession numbers for 16S rRNA gene sequences are given for each strain. Bar, 0.02 nucleotide substitutions per site.

enhanced by the addition of fermentable carbohydrates. Cells are saccharolytic and ferment cellobiose, fructose, glucose, lactose, maltose, mannose, raffinose, ribose and sucrose; arabinose, mannitol, melezitose, rhamnose, sorbitol, trehalose and xylose are not fermented, whereas salicin is weakly fermented. Moderate amounts of acetic acid, major amounts succinic acid and minor amounts of lactic acid are produced as end products of metabolism in PYG broth. In addition to these acids, moderate amounts of isovaleric acid and trace to minor amounts of isobutyric acid are produced as metabolic by-products in PY broth. Gelatin, arginine, aesculin 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 4717 4⁴/502 22. The principal cellular long-chain fatty acids are ai-C_{15:0}, 3-OH i-C_{17:0}, 3-OH C_{16:0}, i-C_{15:0} and C_{16:0}. *P. fusca* is Human Oral Taxon 782 in the Human Oral Microbiome Database (www.homd.org).

The type strain is W1435^T (=DSM 22504^T =CCUG 57946^T), isolated from the human oral cavity from subgingival plaque. The DNA G+C content of the type strain is 43 mol%.

Description of *Prevotella scopos* sp. nov.

Prevotella scopos [sco'pos. L. masc. n. *scopos* (nominative in apposition) a mark, aim at which one shoots, referring to the 'bullseye' appearance of older colonies of the type strain].

The description is based on one strain. Cells are obligately anaerobic, non-motile, pigmented Gram-negative bacilli (0.7 × 0.9–3.0 µm). After 4 days of incubation on FAA

Table 1. Phenotypic characteristics that differentiate *Prevotella fusca* sp. nov. and *Prevotella scopos* sp. nov. from related species of the genus *Prevotella*

Species: 1, *P. fusca* sp. nov.; 2, *P. scopos* sp. nov.; 3, *P. buccae* (data from Holdeman *et al.*, 1982); 4, *P. dentalis* (Haapasalo *et al.*, 1986); 5, *P. oris* (Holdeman *et al.*, 1982); 6, *P. salivae* (Sakamoto *et al.*, 2004); 7, *P. maculosa* (Downes *et al.*, 2007); 8, *P. multisaccharivorax* (Sakamoto *et al.*, 2005b); 9, *P. oralis* (Loesche *et al.*, 1964; Watabe *et al.*, 1983); 10, *P. baroniae* (Downes *et al.*, 2005); 11, *P. micans* (Downes *et al.*, 2009); 12, *P. buccalis* (Shah & Collins, 1981); 13, *P. loeschei* (Holdeman and Johnson, 1982); 14, *P. veroralis* (Watabe *et al.*, 1983); 15, *P. multiformis* (Sakamoto *et al.*, 2005a); 16, *P. nanceiensis* (Alauzet *et al.*, 2007); 17, *P. denticola* (Shah & Collins, 1982); 18, *P. melaninogenica* (Holdeman and Johnson, 1982); 19, *P. histicola* (Downes *et al.*, 2008); 20, *P. shahii* (Sakamoto *et al.*, 2004); 21, *P. oulorum* (Shah *et al.*, 1985); 22, *P. enoea* (Moore *et al.*, 1994); 23, *P. pleuritidis* (Sakamoto *et al.*, 2007); 24, *P. tanneri* (Moore *et al.*, 1994); 25, *P. intermedia* (Johnson and Holdeman, 1983); 26, *P. nigrescens* (Shah & Gharbia, 1992); 27, *P. marshii* (Downes *et al.*, 2005); 28, *P. pallens* (Könönen *et al.*, 1998). +, Positive; –, negative; w, weakly positive; v, variable.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Pigment*	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	–	+	+	v	+	–	–	–	+	+	+	–	+
Fermentation of:																												
Arabinose	–	–	+	+	+	+	+	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	v	–	–	–	–	–	–	–	–	–	–	–	–
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	–	–	–	–
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	v	v	+	v	–
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	v	v	+	–	+
Salicin	w	–	+	–	+	+	+	v	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sucrose	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	v	+	+	–	+
Indole production	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+
Hydrolysis of:																												
Aesculin	–	–	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	v	–	–	+	v	–	–	–	–	–	–
Gelatin	–	+	+	–	v	–	–	+	v	w	+	–	+	v	+	–	+	+	+	+	–	+	+	+	+	+	+	+

*Pigmentation on blood agar may take up to 14 days and varies from tan to brown to black depending on the species.

plates, colonies are 2–3 mm in diameter, circular, entire, convex, smooth, opaque and off-white, with a light orange pigment in the centre of the colonies. On further incubation, colonies develop a distinct area of dark brown pigment in the centre of the colony to form a 'bullseye' colony. Growth in broth media produces moderate turbidity that is enhanced by the addition of fermentable carbohydrates. Cells are saccharolytic and ferment fructose, glucose, lactose, maltose, mannose, raffinose and sucrose; arabinose, cellobiose, mannitol, melezitose, rhamnose, ribose, salicin, sorbitol, trehalose and xylose are not fermented. Moderate amounts of acetic acid, major amounts of succinic acid, and trace amounts of isovaleric and lactic acids are produced as end products of metabolism in PYG broth. In addition to these acids, trace to minor amounts of isobutyric acid and moderate rather than trace amounts of isovaleric acid are produced as metabolic by-products in PY broth. Gelatin is hydrolysed; arginine, aesculin 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 4707 45⁰/22 2²/3. The principal cellular long-chain fatty acids are ai-C_{15:0}, 3-OH i-C_{17:0}, 3-OH C_{16:0}, i-C_{15:0} and C_{16:0}.

The type strain is W2052^T (=DSM 22613^T =CCUG 57945^T), isolated from an implant in the human oral cavity. The DNA G + C content of the type strain is 41 mol%.

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