Prevotella falsenii sp. nov., a *Prevotella intermedia*-like organism isolated from monkey dental plaque

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Eight anaerobic, pigmented, non-spore-forming, Gram-negative, rod-shaped strains isolated from monkey oral cavities were characterized phenotypically and chemotaxonomically and their phylogenetic positions were determined using 16S rRNA gene sequence analysis. The 16S rRNA gene sequence analysis showed that these isolates represent a single species of the genus *Prevotella*. These strains were most closely related to *Prevotella intermedia* ATCC 25611^T, with 95.0% 16S rRNA gene sequence similarity. The next most closely related species were *Prevotella pallens* and *Prevotella nigrescens* (92.7 and 92.1% similarity to the respective type strains). The phenotypic and biochemical characteristics of the isolates were the same as those of *P. intermedia* JCM 12248^T and *P. nigrescens* JCM 12250^T. The isolates could be differentiated from *P. pallens* JCM 11140^T on the basis of mannose fermentation and α -fucosidase activity. The isolates could not be distinguished from *P. intermedia* or *P. nigrescens* using conventional biochemical tests. DNA–DNA hybridization experiments revealed the genomic distinctiveness of these eight strains with respect to *P. pallens* JCM 11140^T, *P. intermedia* JCM 12248^T and *P. nigrescens* JCM 12250^T. On the basis of these data, strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 represent a novel *Prevotella* species, for which the name *Prevotella falsenii* sp. nov. is proposed. The type strain is 04052^T (=JCM 15124^T =CCUG 56137^T).

During studies on the oral microbiota of long-tailed macaques (*Macaca fascicularis*), dental plaque was the source of a number of strains comprising obligately anaerobic, pigmented, non-spore-forming, non-motile, Gram-negative rods. These isolates were phenotypically most closely related to *Prevotella intermedia* and *Prevotella nigrescens*. In addition, the isolates were tentatively identified, using a PCR technique (Baumgartner *et al.*, 1999), as belonging to the species *P. intermedia*. The present study was designed to determine the taxonomic status of the isolates. On the basis of the results presented

here, we propose that these strains represent a novel species of the genus *Prevotella*.

The strains used in the present study were maintained on Eggerth Gagnon (EG) agar (Merck), supplemented with 5% (v/v) horse blood, for 2 days at 37 °C in an atmosphere containing 100% CO₂. Strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 were isolated from dental plaque collected from 12 male long-tailed macaques that were 5–6 years old. Bacteroides bile aesculin agar (Shah, 1992) was used to check whether the growth of the isolates was inhibited on this medium.

Physiological reactions were determined with an API 20A anaerobe test kit, in duplicate, as recommended by the manufacturer (bioMérieux). Metabolic end products were prepared (Holdeman *et al.*, 1977) and analysed (Sakamoto *et al.*, 2005a) as described previously. Fatty acid methyl esters were obtained from about 40 mg wet cells by saponification, methylation and extraction using the method of Miller (1982), with minor modifications

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 are AB429501–AB429508, respectively.

The sequence of the primer pair for *P. intermedia* and alignment of the 16S rRNA gene sequences of related species are presented in a supplementary figure available with the online version of this paper.

(Kuykendall *et al.*, 1988). Cellular fatty acid profiles were determined using the MIDI microbial identification system (Microbial ID). Biochemical reactions were determined with the API Rapid ID 32A anaerobe identification kit, in duplicate, as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated by previously described methods (Marmur, 1961; Saito & Miura, 1963), with some modifications. DNA G + C contents were determined using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M $\text{NH}_4\text{H}_2\text{PO}_4$ and acetonitrile (20:1, v/v). The DNA–DNA hybridization experiment was carried out in microplate wells, as described by Ezaki *et al.* (1989). Hybridization was performed at 43 °C for 16 h. 16S rRNA gene sequences were analysed as described previously (Sakamoto *et al.*, 2002). Related sequences were aligned using the CLUSTAL X 2.0 program (Larkin *et al.*, 2007) and the alignment was corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980) after gaps and unknown bases had been eliminated. The phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of tree topologies.

Strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 were obligately anaerobic, pigmented (black), non-spore-forming, non-motile, Gram-negative rods or coccobacilli. Cells on EG agar were 0.5–0.8 by 1.6 µm or 0.8 by 0.9–1.0 µm in size and occurred singly. Colonies were 1–2 mm in diameter, brown to black, circular, entire, slightly convex and smooth on EG agar plates. Colonies of strain 04021 were slightly smaller than those of the other isolates. In addition, this strain formed aggregates in GAM broth (Nissui Pharmaceutical) culture. On Bacteroides bile aesculin agar, growth of the isolates was inhibited and aesculin hydrolysis was absent. Phenotypic and biochemical characteristics are given in the species description. The phenotypic and biochemical characteristics of the eight strains were the same as those of *P. intermedia* JCM 12248^T and *P. nigrescens* JCM 12250^T. The strains could be differentiated from *Prevotella pallens* JCM 11140^T on the basis of mannose fermentation and α -fucosidase activity.

The cellular fatty acid compositions of *Prevotella* species have been determined previously (Sakamoto *et al.*, 2004, 2005a, b). In this study, the cellular fatty acid compositions of the eight isolates were found to be similar to those of *P. pallens* JCM 11140^T, *P. intermedia* JCM 12248^T and *P. nigrescens* JCM 12250^T (Table 1). The amounts of anteiso-C_{15:0} and summed feature 3 were slightly higher than in *P. intermedia* JCM 12248^T, while the amount of iso-C_{17:0} 3-OH was slightly low relative to that in *P. intermedia* JCM 12248^T.

Approximately 1500 bases of the 16S rRNA gene sequence were determined for each of the new isolates. For the phylogenetic analysis, 1359 bp (positions 56–1391 in the *Escherichia coli* numbering system) sequences of each species were used. 16S rRNA gene sequence analysis

Table 1. Cellular fatty acid compositions of the novel strains and type strains of related species

Strains: 1, strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 (means $\pm$ SD); 2, *P. intermedia* JCM 12248^T; 3, *P. nigrescens* JCM 12250^T; 4, *P. pallens* JCM 11140^T. Values are percentages of total fatty acids. tr, Trace amount (<0.5%); –, not detected.

Fatty acid	1	2	3	4
Saturated straight-chain				
C _{14:0}	0.6 $\pm$ 0.1	0.6	–	1.1
C _{16:0}	6.2 $\pm$ 0.9	5.1	4.1	5.1
C _{18:0}	1.4 $\pm$ 0.4	1.1	0.5	0.8
Unsaturated straight-chain				
C _{13:1} ω1c	1.6 $\pm$ 0.3	–	1.0	–
C _{14:1} ω5c	tr	tr	–	0.6
C _{16:1} ω5c	–	tr	–	–
C _{16:1} ω7c	1.9 $\pm$ 0.2	2.1	2.0	2.6
C _{17:1} ω8c	–	0.5	0.5	–
C _{18:2} ω6,9c	5.2 $\pm$ 0.7	5.3	3.3	1.4
C _{18:1} ω9c	18.0 $\pm$ 2.7	16.3	12.7	19.8
Hydroxy				
iso-C _{16:0} 3-OH	1.1 $\pm$ 0.3	1.0	1.0	–
C _{16:0} 3-OH	1.2 $\pm$ 0.5	2.5	tr	2.2
iso-C _{17:0} 3-OH	6.3 $\pm$ 2.7	16.1	3.3	15.7
anteiso-C _{17:0} 3-OH	1.1 $\pm$ 0.1	1.3	–	1.7
Saturated branched-chain				
iso-C _{10:0}	tr	tr	–	tr
iso-C _{13:0}	0.6 $\pm$ 0.1	0.7	0.8	1.1
iso-C _{14:0}	5.5 $\pm$ 1.6	5.5	9.0	4.3
iso-C _{15:0}	7.7 $\pm$ 1.8	10.8	12.5	12.1
anteiso-C _{15:0}	21.6 $\pm$ 2.4	14.0	22.4	24.5
iso-C _{16:0}	3.0 $\pm$ 1.1	6.2	5.3	1.5
iso-C _{17:0}	2.2 $\pm$ 0.5	3.8	3.2	1.4
anteiso-C _{17:0}	2.8 $\pm$ 0.5	2.5	2.2	1.5
iso-C _{18:0}	–	tr	–	–
iso-C _{19:0}	–	0.5	–	–
Summed features*				
3	12.5 $\pm$ 4.7	1.2	14.3	–
10	2.1 $\pm$ 0.3	1.7	1.3	2.2

*Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3 contained an unknown fatty acid with an equivalent chain-length of 13.570 and/or iso-C_{15:0} ALDE. Summed feature 10 contained one or more of an unknown fatty acid with an equivalent chain-length of 17.834 and/or C_{18:1}ω11c/ω9t/ω6t.

showed that the isolates represented a single species of the genus *Prevotella* (Fig. 1). The levels of sequence similarity among the eight strains were above 99.0%. These eight strains were most closely related to *P. intermedia* ATCC 25611^T, with 95.0% 16S rRNA gene sequence similarity; the next most closely related species were *P. pallens* and *P. nigrescens* (92.7 and 92.1% similarity to the respective type strains). In a preliminary examination using the species-specific primer for *P. intermedia* (Baumgartner *et al.*, 1999), these isolates were identified as

The DNA G + C contents of strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 were 44.9, 47.7, 46.0, 45.2, 44.7, 45.9, 44.7 and 44.6 mol%, respectively. These values are slightly higher than those of *P. pallens* JCM 11140^T (40.4 mol%), *P. intermedia* JCM 12248^T (43.1 mol%) and *P. nigrescens* (40–44 mol%; Shah & Gharbia, 1992). The levels of DNA relatedness between the isolates and *P. pallens* JCM 11140^T, *P. intermedia* JCM 12248^T and *P. nigrescens* JCM 12250^T were 3.6–18.2, 2.0–3.8 and 11.8–31.0%, respectively. The levels of DNA relatedness observed demonstrate the genomic distinctiveness of these eight strains with respect to the type strains of *P. pallens*, *P. intermedia* and *P. nigrescens*.

On the basis of the above-mentioned findings, strains 04013, 04021, 04043, 04052^T, 0406, 04113, 04111 and 04161 represent a novel species of the genus *Prevotella*, for

Description of *Prevotella falsenii* sp. nov.

Cells are obligately anaerobic, pigmented, non-spore-forming, non-motile, Gram-negative rods or coccobacilli ($0.5\text{--}0.8 \times 1.6\text{ }\mu\text{m}$ or $0.8 \times 0.9\text{--}1.0\text{ }\mu\text{m}$). Colonies are 1–2 mm in diameter, brown to black, circular, entire, slightly convex and smooth on EG agar plates. Growth is inhibited in the presence of 20 % (w/v) bile. Acid is produced from glucose, maltose, D-mannose, raffinose and sucrose, but not from L-arabinose, cellobiose, glycerol, lactose, D-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol, trehalose or D-xylose. Aesculin is not hydrolysed. Indole is produced. Gelatin is digested. Catalase and urease are not

produced. With API Rapid ID 32A, positive reactions are obtained for α -glucosidase, α -fucosidase, indole production, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Mannose and raffinose are fermented. All of the other tests performed (for urease, arginine dihydrolase, α -galactosidase, β -galactosidase, 6-phospho- β -galactosidase, β -glucosidase, α -arabinosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, glutamic acid decarboxylase, nitrate reduction, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase) give negative results. The major end products (from 1% peptone/1% yeast extract/1% glucose broth cultures) are succinic and acetic acids. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The major cellular fatty acids are anteiso- $C_{15:0}$ and $C_{18:1\omega 9c}$. The DNA G + C content of the type strain is 45.2 mol%.

The type strain, 04052^T (=JCM 15124^T =CCUG 56137^T), was isolated from monkey dental plaque. Seven additional strains [04013 (=JCM 15121), 04021 (=JCM 15122), 04043 (=JCM 15123), 0406 (=JCM 15125), 04113 (=JCM 15126), 04111 (=JCM 15127) and 04161 (=JCM 15128)], from the same source, are included in this species.

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