Prevotella shahii sp. nov. and *Prevotella salivae* sp. nov., isolated from the human oral cavity

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Two bacterial strains, EHS11^T and EPSA11^T, which were isolated from the human oral cavity, were characterized in terms of phenotypic and biochemical characteristics, cellular fatty acid profiles and phylogenetic position based on 16S rRNA gene sequence analysis. 16S rRNA gene sequence analysis showed that each of the isolates belonged to a novel species of the genus *Prevotella*. Strain EHS11^T was related to *Prevotella loescheii* (about 95 % similarity), whereas strain EPSA11^T was related to *Prevotella oris* (about 94 % similarity). Both strains were obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative rods. The cellular fatty acid composition of strain EPSA11^T was very similar to that of *P. oris* JCM 8540^T. On the other hand, the cellular fatty acid composition of strain EHS11^T was significantly different from those of other *Prevotella* species. The predominant fatty acids in strain EHS11^T are C_{18:1}ω9c, C_{16:0} and C_{16:0} 3-OH, whereas other *Prevotella* species, except for *P. loescheii* JCM 8530^T, possess anteiso-C_{15:0}, iso-C_{17:0} 3-OH and C_{18:1}ω9c. The predominant fatty acids in *P. loescheii* JCM 8530^T are anteiso-C_{15:0}, C_{16:0} and C_{18:1}ω9c. DNA–DNA hybridization experiments revealed a genomic distinction of strains EHS11^T and EPSA11^T from *P. loescheii* JCM 8530^T and *P. oris* JCM 8540^T. On the basis of these data, two novel *Prevotella* species are proposed: *Prevotella shahii* sp. nov. and *Prevotella salivae* sp. nov. The type strains of *P. shahii* and *P. salivae* are EHS11^T (=JCM 12083^T=DSM 15611^T) and EPSA11^T (=JCM 12084^T=DSM 15606^T), respectively.

In the past, because of poor definition of the genus, more than 50 species of *Bacteroides* have been included in *Bergey's Manual of Systematic Bacteriology* (Holdeman *et al.*, 1984) and the Approved Lists of Bacterial Names (Moore *et al.*, 1985). Subsequently, Shah & Collins (1989) formally proposed that the genus *Bacteroides* be restricted to *Bacteroides fragilis* and related taxa, and the description of the genus *Bacteroides* was emended accordingly. Two genera, *Porphyromonas* (Shah & Collins, 1988) for the 'asaccharolytic

pigmented *Bacteroides* species', and *Prevotella* (Shah & Collins, 1990) for the 'moderately saccharolytic, predominantly oral *Bacteroides* species', were thus created. More recently, a novel genus, *Tannerella*, was proposed for the important periodontal pathogen *Bacteroides forsythus* (Sakamoto *et al.*, 2002a). During the study of novel oral phylotypes associated with periodontitis (Sakamoto *et al.*, 2002b), we isolated two strains from the human oral cavity that were obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative rods. These isolates were related phenotypically to the genus *Prevotella*. *Prevotella* species are mainly isolated from the oral cavity and some species are associated with periodontitis. No novel *Prevotella* species have been proposed since Könönen *et al.* (1998) described *Prevotella pallens*. The present study was designed to determine the taxonomic status of these two isolates. On the basis of the results presented here, we propose that these strains should be classified as two novel species of the genus *Prevotella*, namely *Prevotella shahii* sp. nov. and *Prevotella salivae* sp. nov.

Published online ahead of print on 12 December 2003 as DOI 10.1099/ijs.0.02876-0.

Abbreviations: GDH, glutamate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; MDH, malate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences reported in this paper are AB108825 (*Prevotella shahii* EHS11^T) and AB108826 (*Prevotella salivae* EPSA11^T).

Tables of phenotypic characteristics and levels of DNA relatedness, and a phylogenetic tree, are available as supplementary material in IJSEM Online.

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 that contained 100% CO₂. Strain EHS11^T was isolated on EG agar from the saliva of a periodontally healthy subject. Strain EPSA11^T was isolated, also on EG agar, from the saliva of a patient with chronic periodontitis. *Bacteroides bile aesculin* agar (Shah, 1992) was used to check whether growth of the isolates was inhibited on this medium.

Physiological reactions were determined with an API 20A anaerobic test kit in duplicate, as recommended by the manufacturer (bioMérieux). Metabolic end-products were prepared as described previously (Holdeman *et al.*, 1977) and were analysed by GLC (GC-7A; Shimadzu), using a 2.1 m glass column (2.8 mm, FAL-M 25%, Chrommosorb W, 80/100 mesh, AW-DMCS H₃PO₄; Shimadzu). Fatty acid methyl esters were obtained from about 40 mg wet cells by saponification, methylation and extraction, using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). Cellular fatty acid profiles were determined by the MIDI microbial identification system (Microbial ID). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by HPLC with a Cosmosil 5C₁₈ column (4.6 × 150 mm; Nacalai Tesque). Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) activities were determined spectrophotometrically as described previously (Gharbia & Shah, 1991; Bailey & Love, 1995). API ZYM and API An-Ident enzymic substrate tests were performed in duplicate, as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated by using previously described methods (Marmur, 1961; Saito & Miura, 1963), with some modifications. The DNA G+C content was determined by the HPLC method of Tamaoka & Komagata (1984), with some modifications. The DNA homology experiment was carried out in microplate wells as described by Ezaki *et al.* (1989). Hybridization was performed at 44 °C for 16 h. The 16S rRNA gene was analysed as described previously (Sakamoto *et al.*, 2002a). Previously determined 16S rRNA gene sequences used for comparison in this study were retrieved from GenBank/EMBL. Sequence data were aligned with the CLUSTAL W program (Thompson *et al.*, 1994) and 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 by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of tree topologies.

Strain EHS11^T comprised obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. Cells on EG agar were 0.5–0.8 × 8.3–10.0 µm in size and occurred singly. Colonies were 1–2 mm in diameter, tan to light

brown, circular, entire, slightly convex and smooth on EG agar plates. Strain EPSA11^T comprised obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. Cells on EG agar were 0.5–0.8 × 0.8–1.7 µm in size and occurred singly. Colonies were 1–2 mm in diameter, grey to light brown, circular, entire, slightly convex and smooth on EG agar plates. Strain EPSA11^T hydrolysed aesculin on *Bacteroides bile aesculin* agar, whereas strain EHS11^T did not. In addition, *Prevotella loescheii* JCM 8530^T, *Prevotella oralis* JCM 6330, *Prevotella oris* JCM 8540^T and *Prevotella veroralis* JCM 6290^T hydrolysed aesculin on the same agar plates. Growth of the isolates and other *Prevotella* species was inhibited on *Bacteroides bile aesculin* agar. Results of phenotypic tests are given in the species description. In addition, phenotypic characteristics of other *Prevotella* species are available as supplementary material in IJSEM Online. The phenotypic characteristics of strain EHS11^T were similar to those of *P. loescheii* JCM 8530^T and *Prevotella melaninogenica* JCM 6325^T. Strain EHS11^T could not be differentiated from *P. melaninogenica* JCM 6325^T by API 20A tests. On the other hand, strain EHS11^T could be differentiated from *P. loescheii* JCM 8530^T by aesculin hydrolysis. Testing for hydrolysis of aesculin has been reported to be helpful in differentiating *P. loescheii* (formerly *Bacteroides loescheii*) from other related species, such as *P. melaninogenica* (formerly *Bacteroides melaninogenicus*) (Holdeman & Johnson, 1982). Phenotypic characteristics of strain EPSA11^T were similar to those of *P. oris* JCM 8540^T. Strain EPSA11^T could be differentiated from *P. oris* JCM 8540^T by gelatin digestion and D-rhamnose fermentation. However, Holdeman *et al.* (1982) reported that digestion of gelatin and fermentation of D-rhamnose varied between the type strain and 17 other strains of *P. oris* (formerly *Bacteroides oris*).

The cellular fatty acid composition of *Bacteroides* species has been determined (Miyagawa *et al.*, 1979; Shah & Collins, 1980; Mayberry *et al.*, 1982) and reviewed for classification of the genus *Bacteroides* (Shah & Collins, 1983). In this study, the cellular fatty acid composition of strain EPSA11^T was very similar to that of *P. oris* JCM 8540^T (Table 1). Among the strains tested, only these two strains contain C_{17:0} 3-OH (3.4%). On the other hand, the cellular fatty acid composition of strain EHS11^T was significantly different from those of other *Prevotella* species. In strain EHS11^T, the predominant cellular fatty acids are C_{18:1ω9c}, C_{16:0} and C_{16:0} 3-OH, whereas other *Prevotella* species, except for *P. loescheii* JCM 8530^T, possess anteiso-C_{15:0}, iso-C_{17:0} 3-OH and C_{18:1ω9c}. Predominant cellular fatty acids in *P. loescheii* JCM 8530^T are anteiso-C_{15:0}, C_{16:0} and C_{18:1ω9c}. *P. loescheii* has been reported to possess greater relative amounts of C_{16:0} (Wu *et al.*, 1992). It has been reported that anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:0} 3-OH and C_{16:0} are the major cellular fatty acids in the genus *Prevotella* (Moore *et al.*, 1994). According to this report, strain EHS11^T might not be a member of the genus *Prevotella*. However, the predominant cellular fatty acids in *Prevotella dentalis* are C_{16:0} 3-OH, C_{16:0} and iso-C_{14:0}

Table 1. Cellular fatty acid compositions (%) of the two novel *Prevotella* species and related species

Strains: 1, *P. shahii* EHS11^T; 2, *P. salivae* EPSA11^T; 3, *P. bivia* JCM 6331^T; 4, *P. corporis* JCM 8529^T; 5, *P. denticola* JCM 8528; 6, *P. intermedia* JCM 11150^T; 7, *P. loescheii* JCM 8530^T; 8, *P. melaninogenica* JCM 6325^T; 9, *P. nigrescens* JCM 6322^T; 10, *P. oralis* JCM 6330; 11, *P. oris* JCM 8540^T; 12, *P. pallens* JCM 11140^T; 13, *P. veroralis* JCM 6290^T. Values are percentages of total fatty acids. tr, Trace amount (<0.5%). Summed feature 3, iso-C_{15:0} ALDE; summed feature 10, C_{18:1}c11/t9/t6 fatty acid methyl ester.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Saturated straight-chain:													
C _{14:0}	10.9	1.0	tr	0.5	1.0		2.5	1.2			0.6		tr
C _{15:0}	1.0	3.1					0.5	tr			2.3		
C _{16:0}	16.9	7.3	5.9	4.9	6.6	2.9	16.7	9.1	3.4	6.1	8.3	4.7	5.5
C _{17:0}		1.6									1.2		
C _{18:0}	2.8	1.0	1.3	0.7	0.8	1.0	3.4	1.2	0.8	1.2	0.6		0.9
Unsaturated straight-chain:													
C _{13:1} ω12c	0.9	tr					tr	0.5					
C _{16:1} ω9c	2.0	1.2	2.1	2.7	1.6	1.3	2.2	2.0	1.5	1.2	1.5	2.5	1.2
C _{17:1} ω9c		tr	tr	0.6			tr	tr					
C _{18:2} ω9, 12c	8.9	2.0	2.7	1.6	1.1	5.9	4.5	2.3	3.7	1.7	1.0	1.5	1.4
C _{18:1} ω9c	18.7	11.2	18.9	16.4	10.7	10.7	12.3	17.9	11.0	17.6	10.0	14.5	17.2
Hydroxy acids:													
iso-C _{15:0} 3-OH		tr	tr										0.6
C _{15:0} 3-OH		0.9									0.7		
iso-C _{16:0} 3-OH	1.9	0.5	1.6	1.0	1.6	1.6	1.1	1.3	1.5	1.4	0.6		1.3
C _{16:0} 3-OH	16.3	3.0	1.7	1.6	8.3	2.1	8.5	4.8	1.4	3.1	4.2	2.1	2.8
iso-C _{17:0} 3-OH	1.3	12.3	17.9	15.6	11.9	21.0	7.8	9.6	19.9	14.2	11.7	17.7	13.5
anteiso-C _{17:0} 3-OH		1.6	2.0	1.2	1.9	2.2	1.0	1.7	1.5	1.7	1.0	2.9	1.5
C _{17:0} 3-OH		3.4									3.4		
Saturated branched-chain:													
iso-C _{13:0}		tr										1.1	tr
iso-C _{14:0}	4.4	1.4	3.7	6.0	3.5	6.6	2.0	2.8	6.9	3.3	2.5	4.1	3.6
iso-C _{15:0}	3.4	8.3	2.9	8.5	8.6	13.5	8.9	8.2	11.6	6.3	6.7	10.7	6.7
anteiso-C _{15:0}	6.8	25.2	16.9	18.3	30.9	19.6	22.1	25.2	19.4	24.5	26.3	28.8	23.5
iso-C _{16:0}	1.0	1.4	3.8	7.6	2.8	4.2	0.7	1.9	4.9	3.0	3.0	1.5	3.3
iso-C _{17:0}		1.6	5.2	4.6	1.8	1.7		1.2	3.2	2.0	5.5	1.6	2.1
anteiso-C _{17:0}		3.5	4.4	3.5	2.4	1.4	0.6	2.1	2.3	3.5	3.6	1.9	3.4
Summed feature:													
3		5.4	4.0	2.6	2.0	2.5	tr	3.2	5.4	5.2	3.3	2.5	6.9
10	2.0	2.2	3.9	2.0	2.6	1.8	2.3	3.3	1.6	3.9	2.1	2.1	3.7

(Willems & Collins, 1995), which differ from those of other *Prevotella* species, such as strain EHS11^T.

The results of menaquinone analysis are given in the species description. The major menaquinones of clinical isolates and the other *Prevotella* species were MK-10 or MK-11. This result supports findings reported previously (Shah & Collins, 1980, 1983). In our study, the menaquinone composition of strain EPSA11^T was very similar to that of *P. oris* JCM 8540^T. On the other hand, the menaquinone composition of strain EHS11^T was significantly different from that of *P. loescheii* JCM 8530^T or *P. melaninogenica* JCM 6325^T, which are related phenotypically to strain EHS11^T (see supplementary material in IJSEM Online).

The dehydrogenase enzyme pattern is an important criterion that differentiates the genus *Bacteroides sensu*

stricto from other Gram-negative, anaerobic, non-spore-forming rods (Shah & Collins, 1989; Shah, 1992). *B. fragilis* and related species possess enzymes of the hexose monophosphate shunt-pentose phosphate pathway, such as G6PDH, 6PGDH, MDH and GDH. Shah & Collins (1988, 1990) have proposed the novel genera *Porphyromonas* and *Prevotella* because of the lack of enzymes (G6PDH and 6PGDH). In the present study, G6PDH and 6PGDH activities were not detected in extracts of clinical isolates (EHS11^T and EPSA11^T) or other *Prevotella* species. Bailey & Love (1995) have reported that activity of neither enzyme was detected in extracts of *Prevotella bivia*, *Prevotella corporis*, *Prevotella intermedia*, *P. loescheii*, *P. melaninogenica*, *P. oralis*, *P. oris* or *P. veroralis*. Our findings are in good agreement with their report. On the other hand, both MDH and GDH activities were detected in extracts of all strains tested. Among the strains tested, *P. oris* JCM

8540^T and strain EPSA11^T exhibited minor amounts of GDH activity. Furthermore, *P. bivia* JCM 6331^T and *P. corporis* JCM 8529^T exhibited minor amounts of MDH activity.

The API ZYM and API An-Ident systems have been reported to be useful in the identification of oral and non-oral, Gram-negative bacteria (Slots, 1981; Laughon *et al.*, 1982; Tanner *et al.*, 1985). In addition, the RapID-ANA system (Innovative Diagnostics Systems) has been reported to be helpful in the identification of some phenotypically similar, bile-inhibited *Bacteroides* species (Dellinger & Moore, 1986). Biochemical characteristics of clinical isolates and other *Prevotella* species are shown in Table 2. All strains were tested by using API ZYM and API An-Ident. Biochemical characteristics of strain EHS11^T were similar to those of *P. loescheii* JCM 8530^T, *P. melaninogenica* JCM 6325^T and *P. veroralis* JCM 6290^T. However, strain EHS11^T could be differentiated from *P. loescheii* JCM 8530^T, *P. melaninogenica* JCM 6325^T and *P. veroralis* JCM 6290^T by API An-Ident tests. β -Glucosidase, arginine and histidine aminopeptidase activities were helpful in the differentiation of these species. Biochemical characteristics of strain

EPSA11^T were similar to those of *P. oris* JCM 8540^T. However, strain EPSA11^T could be differentiated from *P. oris* JCM 8540^T by α -fucosidase (API ZYM), β -glucosidase (API An-Ident) and β -galactosidase (API An-Ident) activities.

The DNA G+C contents of strains EHS11^T and EPSA11^T were 44.3 and 41.3 mol%, respectively. These values are slightly lower than those for *P. loescheii* JCM 8530^T and *P. oris* JCM 8540^T, which are related phenotypically to strains EHS11^T and EPSA11^T, respectively (Table 3). The relatively low levels of DNA relatedness observed demonstrate the genomic distinction of strains EHS11^T and EPSA11^T from *P. loescheii* JCM 8530^T, *P. melaninogenica* JCM 6325^T and *P. oris* JCM 8540^T ($\leq 21\%$, see supplementary material in IJSEM Online).

Approximately 1500 bp of the 16S rRNA gene sequence was determined for each of the isolates. For the phylogenetic analysis, 1467 bp (positions 34–1483; *Escherichia coli* numbering system) sequence fragments of each species were used. 16S rRNA gene sequence analysis showed that each of the isolates was a species of the genus *Prevotella* (Fig. 1). Strain EHS11^T was related to *P. loescheii* with

Table 2. API ZYM and API An-Ident test results

Strains: 1, *P. shahii* EHS11^T; 2, *P. salivae* EPSA11^T; 3, *P. bivia* JCM 6331^T; 4, *P. corporis* JCM 8529^T; 5, *P. denticola* JCM 8528; 6, *P. intermedia* JCM 11150^T; 7, *P. loescheii* JCM 8530^T; 8, *P. melaninogenica* JCM 6325^T; 9, *Prevotella nigrescens* JCM 6322^T; 10, *P. oralis* JCM 6330; 11, *P. oris* JCM 8540^T; 12, *P. pallens* JCM 11140^T; 13, *P. veroralis* JCM 6290^T. +, Positive reaction (colour range 3–5 on API colour charts); –, negative reaction; w, weak reaction (colour range 1–2 on API colour charts). All strains were positive in API ZYM tests for acid phosphatase and naphthol-AS-BI-phosphohydrolase activities and in API An-Ident tests for α -glucosidase, indoxyl acetate and alanine aminopeptidase activities. All strains were negative in API ZYM tests for esterase (C4), esterase lipase (C4), lipase (C4), leucine, valine and cystine arylamidase, trypsin, chymotrypsin, β -glucuronidase and α -mannosidase activities and in API An-Ident tests for arginine, leucine, proline, tyrosine and phenylalanine aminopeptidase, pyroglutamic acid arylamidase and catalase activities.

Test	1	2	3	4	5	6	7	8	9	10	11	12	13
API ZYM:													
Alkaline phosphatase	w	+	+	+	+	+	+	+	+	+	+	+	+
α -Galactosidase	w	+	–	–	–	–	+	w	–	–	w	–	w
β -Galactosidase	w	+	+	–	w	–	+	+	–	+	w	–	+
α -Glucosidase	w	+	+	w	+	+	+	+	+	+	+	+	+
β -Glucosidase	–	+	–	–	–	–	–	–	–	–	+	–	–
N-Acetyl- β -glucosaminidase	+	+	+	–	+	–	+	+	–	+	+	–	+
α -Fucosidase	w	w	+	–	w	w	w	+	w	–	–	–	w
API An-Ident:													
Indole	–	–	–	–	–	+	–	–	+	–	–	+	–
N-Acetyl- β -D-glucosaminidase	+	+	+	–	+	–	+	+	–	w	+	–	+
α -Arabinofuranosidase	–	+	–	–	–	–	–	–	–	–	+	–	–
β -Glucosidase	–	+	–	–	–	–	+	w	–	w	–	–	w
α -L-Fucosidase	+	+	+	–	+	+	+	+	+	w	w	–	+
Alkaline phosphatase	w	+	+	+	+	+	+	+	+	+	w	+	+
α -Galactosidase	w	w	w	–	w	–	+	+	–	w	w	–	w
β -Galactosidase	w	+	+	–	w	–	+	+	–	w	–	–	+
Arginine aminopeptidase	–	–	–	+	+	+	+	+	+	–	–	+	–
Histidine aminopeptidase	–	–	–	+	–	w	–	+	–	–	–	–	–
Glycine aminopeptidase	–	–	+	–	–	–	–	–	–	–	–	–	–

Table 3. Differential characteristics of *P. shahii* sp. nov., *P. salivae* sp. nov. and some related *Prevotella* species

Strains: 1, *P. shahii* sp. nov.; 2, *P. salivae* sp. nov.; 3, *P. loescheii*; 4, *P. melaninogenica*; 5, *P. oris*. +, Positive; –, negative; W, weak.

Characteristic	1	2	3	4	5
Gelatin digestion	+	–	+	+	+
Aesculin hydrolysis	–	+	+	–	+
Pigment production	–	–	+	–	–
Acid produced from:					
Salicin	–	+	–	–	+
D-Xylose	–	+	–	–	+
L-Arabinose	–	+	–	–	+
D-Cellobiose	–	+	–	–	+
D-Rhamnose	–	–	–	–	+
API ZYM:					
β -Glucosidase	–	+	–	–	+
α -Fucosidase	W	W	W	+	–
API An-Ident:					
α -Arabinofuranosidase	–	+	–	–	+
β -Glucosidase	–	+	+	W	–
β -Galactosidase	W	+	+	+	–
Arginine aminopeptidase	–	–	+	+	–
Histidine aminopeptidase	–	–	–	+	–
Major cellular fatty acids	C _{18:1} ω 9c, C _{16:0} , C _{16:0} 3-OH	ai-C _{15:0} , i-C _{17:0} 3-OH, C _{18:1} ω 9c	ai-C _{15:0} , C _{16:0} , C _{18:1} ω 9c	ai-C _{15:0} , i-C _{17:0} 3-OH, C _{18:1} ω 9c	ai-C _{15:0} , i-C _{17:0} 3-OH, C _{18:1} ω 9c
Predominant menaquinones (MK-)	10, 11, 12	10, 11, 12	10	10, 11	10, 11, 12
DNA G + C content (mol%)	44.3	41.3	46.9	41.1	43.3

about 95 % sequence similarity, whereas strain EPSA11^T was related to *P. oris*, with about 94 % similarity. In addition, strain EHS11^T was related to '*Prevotella* sp. B31FD' (Paster *et al.*, 2001), which was isolated from subgingival dental plaque, with about 98 % similarity, whereas strain EPSA11^T was related to '*Prevotella* sp. oral clone DO033' (Kazor *et al.*, 2003), with about 99 % similarity.

On the basis of the above-mentioned findings and the 16S rRNA gene sequence analysis, we propose two novel

Prevotella species, i.e. *Prevotella shahii* sp. nov. for strain EHS11^T and *Prevotella salivae* sp. nov. for strain EPSA11^T. Differential characteristics of *P. shahii* sp. nov., *P. salivae* sp. nov. and some related *Prevotella* species are shown in Table 3.

Description of *Prevotella shahii* sp. nov.

Prevotella shahii (sha'hi.i. N.L. gen. masc. n. *shahii* of Shah, named after the Trinidadian-born British microbiologist Haroun N. Shah, for his contributions to taxonomy of the genus *Bacteroides* and related taxa).

Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods (0.5–0.8 × 8.3–10.0 µm). Colonies are 1–2 mm in diameter, tan to light brown, circular, entire, slightly convex and smooth on EG agar plates. Growth is inhibited in the presence of 20 % bile. Acid is produced from glucose, lactose, maltose, D-mannose, D-raffinose and sucrose. Acid is not produced from L-arabinose, D-cellobiose, glycerol, D-mannitol, D-melezitose, D-rhamnose, salicin, D-sorbitol, D-trehalose or D-xylose. Aesculin is not hydrolysed. Indole is not produced. Gelatin is digested. Catalase and urease are not produced. Major end products (from 1 % peptone/1 % yeast extract/1 % glucose broth cultures) are succinic and acetic acids. MDH and GDH activities are present, whereas G6PDH and 6PGDH activities are absent. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. Major cellular fatty acids are C_{18:1} ω 9c, C_{16:0}

Fig. 1. Phylogenetic tree showing the relationship between the two novel *Prevotella* species and related species. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Numbers at nodes indicate bootstrap values, out of 100 bootstrap resamplings, for each node. Bar, 0.01 substitutions per nucleotide position.

and C_{16:0} 3-OH. Principal respiratory quinones are menaquinones MK-10 (18 %), MK-11 (58 %) and MK-12 (22 %). Minor menaquinones are MK-8 (1 %) and MK-9 (1 %). DNA G+C content of the type strain is 44.3 mol%.

The type strain of *P. shahii* is EHS11^T (=JCM 12083^T = DSM 15611^T), isolated from the saliva of a periodontally healthy human subject.

Description of *Prevotella salivae* sp. nov.

Prevotella salivae (sa.li'vae. L. fem. gen. n. *salivae* from/of slime, referring to the saliva from which the micro-organism was isolated).

Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods (0.5–0.8 × 0.8–1.7 µm). Colonies are 1–2 mm in diameter, grey to light brown, circular, entire, slightly convex and smooth on EG agar plates. Growth is inhibited in the presence of 20 % bile. Acid is produced from L-arabinose, D-cellobiose, glucose, lactose, maltose, D-mannose, D-raffinose, salicin, sucrose and D-xylose. Acid is not produced from glycerol, D-mannitol, D-melezitose, D-rhamnose, D-sorbitol or D-trehalose. Aesculin is hydrolysed. Indole is not produced. Gelatin is not digested. Catalase and urease are not produced. Major end products (from 1 % peptone/1 % yeast extract/1 % glucose broth cultures) are succinic and acetic acids; small amounts of isovaleric acid are also produced. MDH is present and GDH is present in minor amounts. G6PDH and 6PGDH activities are absent. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. Major cellular fatty acids are anteiso-C_{15:0}, iso-C_{17:0} 3-OH and C_{18:1ω9c}. Predominant respiratory quinones are menaquinones MK-10 (13 %), MK-11 (67 %) and MK-12 (20 %). DNA G+C content of the type strain is 41.3 mol%.

The type strain of *P. salivae* is EPSA11^T (=JCM 12084^T = DSM 15606^T), isolated from the saliva of a patient with chronic periodontitis.

Acknowledgements

We are grateful to Professor H. Trüper, University of Bonn, Germany, for his suggestions regarding nomenclature. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 13672202) from the Japan Society for the Promotion of Science (to M.S.).

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