Peptostreptococcus stomatis sp. nov., isolated from the human oral cavity

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Seven strains of anaerobic Gram-positive cocci isolated from human oral sites were subjected to a comprehensive range of phenotypic and genotypic tests. 16S rRNA gene sequence analysis revealed that the strains constituted a homogeneous group that was distinct from species with validly published names, but related to *Peptostreptococcus anaerobius*. All oral strains tested belonged to this group, whereas all non-oral strains studied were confirmed as *P. anaerobius*. A novel species, *Peptostreptococcus stomatis* sp. nov., is proposed to accommodate these oral strains. *P. stomatis* is weakly saccharolytic and produces acetic, butyric, isobutyric, isovaleric and isocaproic acids as end products of fermentation. The type strain of *P. stomatis* is W2278^T (=DSM 17678^T = CCUG 51858^T); the G + C content of the DNA of this strain is 36 mol%.

The anaerobic Gram-positive cocci comprise a diverse group of organisms. The majority of those associated with humans were formerly included within the genus *Peptostreptococcus*, but polyphasic taxonomic investigations have shown that they vary markedly in fundamental characteristics and a number of new genera, including *Anaerococcus*, *Finegoldia*, *Gallicola*, '*Micromonas*' and *Peptoniphilus*, have been proposed (Ezaki *et al.*, 2001; Murdoch & Shah, 1999).

Members of the type species of the genus *Peptostreptococcus*, *Peptostreptococcus anaerobius*, have been isolated from a wide range of human oral infections including periodontitis, dento-alveolar infections, pericoronitis, dentinal caries and endodontic infections (Moore *et al.*, 1983; Sundqvist, 1992; Tanner *et al.*, 1979). However, a specific PCR assay for the species (Riggio & Lennon, 2002) failed to detect *P. anaerobius* in any of 60 subgingival plaque samples collected from subjects with periodontitis or 43 pus samples from dento-alveolar abscesses.

Molecular ecology studies characterizing the microflora associated with oral infections by 16S rRNA gene sequence analysis have found a number of isolates and cloned 16S rRNA genes with sequences identical to phylotype *Peptostreptococcus* CK035 (GenBank accession no. AF287763) (Munson *et al.*, 2002; Paster *et al.*, 2001), which is closely related to, but distinct from *P. anaerobius*. We hypothesize then, that *P. anaerobius* is not an organism found in the human mouth, but that those oral isolates previously

identified as *P. anaerobius* in fact belong to a closely related, as yet un-named, taxon, corresponding to phylotype *Peptostreptococcus* CK035.

The aim of this study was to characterize oral and non-oral isolates of *P. anaerobius* using a range of phenotypic and genotypic methods, to clarify the taxonomic position of this species and phylogenetically related taxa.

Strains W2175, W2205 and W2278^T were isolated from dento-alveolar abscesses, strains W3855 and W5396 were from endodontic infections, strain W5002 was from a periodontal pocket, strain W1948 was from a pericoronal infection and strain W3412 was from a leg ulcer. Strains AHC 14518 (isolated from a urinary tract infection), AHC 14540 (ankle wound), AHC 15114 (buttock abscess), AHC 50003 (vaginal infection) and AHC 50011 (leg ulcer) were the kind gift of E. Kononen, Anaerobe Reference Laboratory, Helsinki, Finland. The type strain of *P. anaerobius* (NCTC 11460^T) was obtained from the NCTC.

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 on this medium after 5 days incubation were viewed using a dissecting microscope. Cellular morphology was recorded after Gram-staining of smears prepared from 2-day-old FAA cultures.

Biochemical and physiological tests were performed as described previously (Downes *et al.*, 2005). 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 (Columbia agar base; LabM) supplemented with 5 % horse

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Abbreviation: SPS, sodium polyanethol sulfonate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Peptostreptococcus stomatis* W2278^T is DQ160208.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1283 aligned bases showing relationships between strains of *P. anaerobius* and *P. stomatis* sp. nov., *Peptostreptococcus* phylo-type CK035 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 gene sequences are given for each strain. Bar, 0.1 nucleotide substitutions per site.

blood, and were performed in duplicate. Sensitivity to sodium polyanethol sulfonate (SPS) was determined by measuring the diameter of the zone of growth inhibition around an SPS disc (Oxoid) on a 3-day-old FAA plate culture.

The G+C content of the DNA of strains W2278^T and W3855 was estimated by an HPLC method as described previously (Wade *et al.*, 1999). A thermal denaturation method (Huß *et al.*, 1983) was used to determine the extent of DNA–DNA hybridization between strains W2278^T, W3855 and W3412 and the type strain of *P. anaerobius*, NCTC 11460^T. The 16S rRNA genes of the strains were sequenced as described previously (Downes *et al.*, 2005). Sequences were assembled using the program BIOEDIT (Hall, 2004). Phylogenetic analysis was performed using the PHYLIP suite of programs (Felsenstein, 1993). Trees were constructed by three methods: a distance matrix was constructed using the Jukes–Cantor algorithm with DNADIST and NEIGHBOR was used to construct the tree by the neighbour-joining method; the maximum-likelihood method was used by means of DNAML and DNAPARS was used to construct a

tree using a parsimony algorithm. Phylogenetic trees were viewed using TREEVIEW (Page, 1996).

Phylogenetic analysis of 16S rRNA gene sequences showed that the strains fell into two groups (Fig. 1). The three methods of tree construction produced trees with identical topology. The strains isolated from non-oral infections clustered with *P. anaerobius* NCTC 11460^T, whereas the oral strains formed a closely related but distinct cluster, which included oral phylotype *Peptostreptococcus* CK035. Members of the two groups each shared greater than 99.5 % sequence identity with each other but strain W2278^T, which was representative of the oral strains, shared only 97 % sequence identity with strain NCTC 11460^T.

Variable region 1 of the 16S rRNA gene was found to be entirely different in the two groups of strains, i.e. the loop in the secondary structure was 25 bases longer in the oral strains than the non-oral strains (Fig. 2). Interestingly, the specific forward primer described by Riggio & Lennon (2002) was designed to anneal to this region, making it

	70	80	90	100	110
NCTC 11460 ^T	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TNA
AHC 14518	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TGA
AHC 14540	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TGA
AHC 15114	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TNA
AHC 50003	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TAA
AHC 50011	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TGA
W3412	TCGAGCG	-----	CGTCTGATTTGATGCTTGCAT	-----	TGA
CK035	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
W2278 ^T	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
W1948	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
W2205	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
W3855	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
W5002	TCGAGCGAGGGTTTGCTCAGTATTGAGTATTCTAAGACTAGAATGTTCAATTCTGA				
F			CGTCTWATTTNATGCTTGCA		

Fig. 2. 16S rRNA gene sequence alignment of variable region 1 for strains of *P. anaerobius* and *P. stomatis* sp. nov., and the *P. anaerobius*-specific PCR primer (F) described by Riggio & Lennon (2002).

specific for *P. anaerobius*, but not *Peptostreptococcus* CK035. This further supports the hypothesis that *P. anaerobius* is not found in the human mouth.

The seven oral strains were obligately anaerobic, Gram-positive cocci, $0.8 \times 0.8\text{--}0.9\ \mu\text{m}$, and arranged in pairs and short chains. After 5 days incubation on FAA plates, colonies were $0.8\text{--}1.8\ \text{mm}$ in diameter, circular, entire, high convex to pyramidal, opaque, shiny and cream to off-white in colour with a narrow, grey, peripheral outer ring. In contrast, colonies of the seven non-oral *P. anaerobius* strains were $2.2\text{--}4.0\ \text{mm}$ in diameter, circular, entire, convex, opaque, shiny and off-white in colour after 5 days incubation on FAA plates. Growth of all 14 oral and non-oral strains in peptone/yeast extract (PY) broth resulted in a clear broth with a sediment that produced a moderately turbid suspension on shaking (2 to 3+, on a scale of 0 to 4+). Growth was enhanced by the addition of 1% fermentable carbohydrates (3 to 4+). Fructose, glucose and maltose were weakly fermented by all strains, with terminal pH readings of $5.3\text{--}5.8$, whereas arabinose, cellobiose, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose and trehalose were not fermented. Major amounts of acetic and isocaproic acids, minor amounts of isobutyric and isovaleric acids and trace to minor amounts of butyric acid were produced as end products of metabolism in PY and peptone/yeast extract/glucose (PYG) broth, with isobutyric and isovaleric acids more pronounced in PY than PYG broth. All strains produced a characteristic sweet pungent odour. None of the strains hydrolysed aesculin, arginine, gelatin or urea, nitrate was not reduced and catalase and indole were not produced.

The diameter of the zone of growth inhibition around the SPS discs was $15\text{--}17\ \text{mm}$ for the seven non-oral *P. anaerobius* strains and $19\text{--}25\ \text{mm}$ for the seven oral strains. The seven oral strains were positive for α -glucosidase in the Rapid ID 32A identification panel, whereas all other reactions were negative, resulting in a profile of 0400 0000 00. The seven non-oral *P. anaerobius* strains, including the type strain *P. anaerobius* NCTC 11460^T, were positive for proline arylamidase in addition to α -glucosidase resulting in a profile of 0400 0200 00. The G+C content of the DNA of oral strains W2278^T and W3385 was estimated to be 36 mol%.

DNA–DNA hybridization between oral strains W2278^T and W3855 was determined to be 97%. Hybridization between *P. anaerobius* NCTC 11460^T and strains W2278^T and W3855 was 8 and 14%, respectively. Hybridization between *P. anaerobius* NCTC 11460^T and W3412 was 93%.

The oral strains studied here constitute a homogeneous group and are clearly distinct from any species with validly published names. The name *Peptostreptococcus stomatis* sp. nov. is therefore proposed for these strains. Phenotypic characteristics that differentiate *P. stomatis* from *P. anaerobius* are shown in Table 1 and include colony morphology and the production of proline arylamidase by *P. anaerobius*,

but not by *P. stomatis*. Sensitivity to SPS (diameter $\geq 12\ \text{mm}$) has been used to differentiate *P. anaerobius* from other anaerobic Gram-positive cocci that are resistant to SPS (diameter $< 12\ \text{mm}$). *P. stomatis* strains are also sensitive to SPS and the seven strains in this study exhibited larger zones of growth inhibition than the *P. anaerobius* strains tested. Further strains of *P. stomatis* would need to be studied to establish whether the diameter of growth inhibition around an SPS disc is a reliable test to distinguish between *P. stomatis* and *P. anaerobius*.

Description of *Peptostreptococcus stomatis* sp. nov.

Peptostreptococcus stomatis (sto.ma'tis. N.L. gen. n. *stomatis* of the mouth from Gr. n. *stoma* mouth).

The description is based on seven strains isolated from the human oral cavity. Cells are obligately anaerobic, Gram-positive cocci ($0.8 \times 0.8\text{--}0.9\ \mu\text{m}$) occurring in pairs and short chains. After 5 days incubation on FAA plates, colonies are $0.8\text{--}1.8\ \text{mm}$ in diameter, circular, entire, high convex to pyramidal, opaque, shiny and cream to off-white in colour with a narrow, grey, peripheral outer ring. Moderate growth is obtained in broth media and growth is further enhanced by the addition of fermentable carbohydrates. Cells are weakly saccharolytic and ferment fructose, glucose and maltose weakly; arabinose, cellobiose, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose and trehalose are not fermented. Major amounts of acetic and isocaproic acids, minor amounts of isobutyric and isovaleric acids and trace to minor amounts of butyric acid are produced as end products of metabolism in PYG. Aesculin, arginine, gelatin and urea are not hydrolysed. Indole and catalase are not produced and nitrate is not reduced. The G+C content of the DNA of the type strain is 36 mol%.

The type strain is W2278^T (=DSM 17678^T=CCUG 51858^T), isolated from infections of the human oral cavity.

Table 1. Differential characteristics of *P. stomatis* sp. nov. and *P. anaerobius*

SPS diameter is the diameter of growth inhibition around an SPS disc. Colony properties were determined following 5 days incubation on FAA plus 5% horse blood. Proline arylamidase activity was determined with the Rapid ID32A identification panel (bioMérieux).

Characteristic	<i>P. stomatis</i> (n = 7)	<i>P. anaerobius</i> (n = 7)
SPS diameter (mm)	19–25	15–17
Colony morphology	High convex/ pyramidal	Convex
Colony diameter (mm)	0.8–1.8	2.2–4.0
Rapid ID32A profile	0400 0000 00	0400 0200 00
Proline arylamidase	–	+

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