***Streptococcus peroris* sp. nov. and *Streptococcus infantis* sp. nov., new members of the *Streptococcus mitis* group, isolated from human clinical specimens**

Yoshiaki Kawamura,¹ Xiao-Gang Hou,¹ Yuko Todome,²
Ferdousi Sultana,¹ Kenji Hirose,¹ Sin-Ei Shu,¹ Takayuki Ezaki¹
and Hisashi Ohkuni²

Author for correspondence: Yoshiaki Kawamura. Tel: +81 58 267 2240. Fax: +81 58 267 0156.
e-mail: kawamura@cc.gifu-u.ac.jp

¹ Department of Microbiology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan

² Department of Immunology and Infectious Diseases, Institute of Gerontology, Nippon Medical School, 1-396, Kosugi-cho, Nakahara-ku, Kawasaki, Kanagawa 211, Japan

Taxonomic studies were performed on eight strains of α -haemolytic streptococci that showed very low DNA–DNA hybridization similarity values with all established members of the mitis group of the genus *Streptococcus*. These strains were isolated from the tooth surface and pharynx of humans. 16S rRNA gene sequence analysis showed that these strains belonged to the mitis group, but that they fell into two new branches. DNA–DNA hybridization demonstrated two new similarity groups. From the results of the present study, the names *Streptococcus peroris* sp. nov. and *Streptococcus infantis* sp. nov. are proposed for these new groups. The type strains are O-66^T (= GTC 848^T = JCM 10158^T) and O-122^T (= GTC 849^T = JCM 10157^T), respectively.

Keywords: α -haemolytic streptococci, the mitis group, *S. peroris*, *S. infantis*, 16S rRNA, DNA–DNA hybridization

INTRODUCTION

In the past decade, some species or species groups of the genus *Streptococcus*, such as the enterococci, lactococci, anaerobic streptococci and nutritionally variant streptococci, have been transferred to other genera (Collins & Wallbanks, 1992; Ezaki *et al.*, 1994; Kawamura *et al.*, 1995a; Kilpper-Balz & Schleifer, 1988; Schleifer & Kilpper-Balz, 1984; Schleifer *et al.*, 1985). At the same time, several new members of the genus *Streptococcus* have been proposed (Devriese *et al.*, 1988, 1997; Eldar *et al.*, 1994; Kilian *et al.*, 1989; Osawa *et al.*, 1995; Robinson *et al.*, 1988; Skaar *et al.*, 1994; Vandamme *et al.*, 1996; Whaley *et al.*, 1988, 1990; Whaley & Hardie, 1988; Williams & Collins, 1990). Presently, 42 species and four subspecies are recognized as members of the genus *Streptococcus*. α -Haemolytic streptococci, especially the members of the mitis group (Bentley *et al.*, 1991; Kawamura *et al.*, 1995b) have been considered difficult to classify and identify. In a previous DNA–DNA hybridization study, we confirmed the difficulty in identifying

members of the mitis group by biochemical methods; many strains identified biochemically as *Streptococcus mitis*, *Streptococcus oralis* or *Streptococcus sanguinis* (formerly *Streptococcus sanguis*) (Trüper & de' Clari, 1997) remained unconfirmed by DNA–DNA hybridization (Ezaki *et al.*, 1988). This difficulty in identification was due to a lack of reliable, accumulated biochemical traits. Furthermore, we believe that some previously undescribed taxa that should be classified as new species or subspecies were present among the clinical isolates that had been identified as members of the mitis group. Unfortunately, members of the mitis group are genetically closely related to each other, and it is very difficult to differentiate between them even by genetic methods; some members of the mitis group share more than 99% 16S rRNA gene sequence similarity and show 40–60% DNA–DNA homology (Kawamura *et al.*, 1995b).

During the present taxonomic study of the mitis group, we have found that some clinical strains showing very low DNA–DNA homology to all members of the mitis group formed two separate groups. We have determined the phylogenetic position and biochemical traits of these strains, and we propose two new species, *Streptococcus peroris* and *Streptococcus infantis*.

The DDBJ accession numbers for the 16S rRNA sequences of *S. peroris*, *S. infantis* and *S. cristatus* are AB008314, AB008315 and AB008313, respectively.

Table 1. DNA–DNA hybridization similarity values

Each value represents the mean of two experiments. We used 31 and 41 °C for optimal and stringent hybridization conditions, respectively. The hybridization solution contained 50 % formamide, which decreased the hybridization temperature by approximately 30 °C (Meinkoth & Wahl, 1984).

Species/group	Strain	Percentage DNA similarities with biotin-labelled DNA from:							
		O-66 ^T		O-105		O-122 ^T		O-134	
		Optimal	Stringent	Optimal	Stringent	Optimal	Stringent	Optimal	Stringent
Similarity group I									
	O-66 ^T	100.0	100.0	83.4	75.1	39.5	31.4	50.5	32.0
	O-91	96.1	94.5	98.8	99.3	35.2	24.4	61.6	23.0
	O-105	83.7	75.3	100.0	100.0	38.8	37.8	49.5	28.2
Similarity group II									
	O-92	39.8	30.3	53.7	30.4	77.6	75.4	76.8	81.7
	O-101	47.9	41.0	50.6	29.6	81.1	75.3	90.4	88.7
	O-103	57.8	52.0	61.9	40.8	73.6	76.9	73.8	71.1
	O-122 ^T	43.7	37.7	42.3	41.2	100.0	100.0	76.3	73.3
	O-134	55.2	35.2	53.6	34.4	80.5	75.8	100.0	100.0
<i>S. mitis</i>	GTC 495 ^T (NCTC 12261 ^T)	20.4	8.6	28.6	11.7	18.8	10.6	13.8	14.5
<i>S. oralis</i>	GTC 276 ^T (NCTC 11427 ^T)	19.8	11.5	32.3	14.8	23.9	11.6	17.9	18.8
<i>S. pneumoniae</i>	GTC 261 ^T (NCTC 7465 ^T)	21.5	13.6	27.9	22.2	26.5	15.2	21.7	17.1
<i>S. gordonii</i>	GTC 497 ^T (ATCC 10558 ^T)	7.3	5.2	13.1	9.2	7.5	6.4	8.3	6.0
<i>S. cristatus</i>	GTC 631 ^T (ATCC 12479 ^T)	16.6	13.5	17.4	12.8	13.5	7.4	17.0	7.8
<i>S. sanguinis</i>	GTC 217 ^T (ATCC 10556 ^T)	8.7	4.9	13.7	7.5	8.5	4.3	7.6	5.5
<i>S. parasanguinis</i>	GTC 498 ^T (ATCC 15912 ^T)	11.0	8.4	21.9	12.8	16.2	11.2	12.4	13.0
<i>S. salivarius</i>	GTC 215 ^T (ATCC 7073 ^T)	15.0	11.7	16.5	10.6	10.5	6.1	14.7	7.8
<i>S. bovis</i>	GTC 235 ^T (NCFB 597 ^T)	6.6	5.4	8.0	5.8	4.9	3.6	7.7	3.3

METHODS

Strains and cultivation. Strains used in this study were virtually all isolated from human (3-month- to 3-year-old children from Tokyo) clinical sources between 1990 and 1991: O-66^T [GTC 848^T = JCM 10158^T], pharynx, Kawasaki disease (KD); O-91, pharynx, KD; O-105, tooth surface, acute pharyngitis (group I); O-92, pharynx, KD; O-101, tooth surface, KD; O-103, pharynx, bronchiostenosis (group II). Strains O-122^T [GTC 849^T = JCM 10157^T] and O-134 (group II) were isolated from the pharynx of a healthy Japanese child in 1990 (location unknown) and from the human pharynx (KD status, date and location unknown), respectively (Ohkuni *et al.*, 1993). These strains were grown on Columbia blood (5% defibrinated sheep blood) agar plates (CBA; bioMérieux) at 37 °C under aerobic conditions.

Microplate DNA–DNA hybridization. Quantitative microplate DNA–DNA hybridization was carried out as described previously (Ezaki *et al.*, 1989). Hybridization experiments were carried out at 31 °C (optimal conditions) and 41 °C (stringent conditions) using 2 × SSC and 50 % formamide. The optimal temperature was 55 °C lower than the thermal denaturation temperature; because formamide lowered the hybridization temperature (Meinkoth & Wahl, 1984).

The type strains used for DNA–DNA hybridization were as follows: *Streptococcus mitis* GTC 495^T (= NCTC 12261^T), *Streptococcus oralis* GTC 276^T (= NCTC 11427^T), *Streptococcus pneumoniae* GTC 261^T (= NCTC 7465^T), *Streptococcus gordonii* GTC 497^T (= ATCC 10558^T), *Streptococcus cristatus* [formerly *Streptococcus crista* (Trüper & de' Clari, 1997)] GTC 631^T (= NCTC 12479^T), *Streptococcus sanguinis*

GTC 217^T (= ATCC 10556^T), *Streptococcus parasanguinis* [formerly *Streptococcus parasanguis* (Trüper & de' Clari, 1997)] GTC 498^T (= ATCC 15912^T), *Streptococcus bovis* GTC 235^T (= NCFB 597^T), and *Streptococcus salivarius* GTC 215^T (= ATCC 7073^T).

Mol% G + C content of DNA. The mol% G + C content of the DNA was determined by HPLC as described previously (Ezaki *et al.*, 1990). Briefly, 10 µl purified DNA (1 mg ml⁻¹) was heat-denatured, and after cooling the DNA solution, 10 µl Nuclease P1 solution (2 U ml⁻¹) was added and incubated at 50 °C for 1 h. Then 10 µl alkaline phosphatase solution (2.4 U ml⁻¹) was added and allowed to incubate at 37 °C for 30 min. The digested DNA solution was analysed by HPLC using a packed column (Wakosil 5C18; Wako). The G + C content was calculated based on the *Escherichia coli* K-12 strain DNA as a standard (51.12 mol% G + C).

16S rRNA gene sequence and analysis. The 16S rRNA genes were amplified by PCR as previously described (Ezaki *et al.*, 1994). The sequence was determined using an automatic sequencer (ALF-express) with an auto-load sequencing kit (both from Pharmacia Biotech). The sequence from positions 45–1380 (*E. coli* numbering) of each 16S rRNA was determined.

The sequences of the other members of the genus *Streptococcus* used for alignment and for calculating the homology levels were obtained from the DDBJ, GenBank and EMBL databases. The CLUSTAL W software originally described by Thompson *et al.* (1994) was used to align the sequences, and the phylogenetic distance was calculated using the neighbour-joining method. The phylogenetic tree was drawn using TREEVIEW software.

Biochemical traits. The biochemical traits were determined with Rapid ID32 Strep (bioMérieux) and STREPTOGRAM (Wako) (Kawamura *et al.*, 1995c) according to the manufacturers' recommendations. We confirmed the hydrolytic ability of arginine in Moeller-decarboxylase medium containing 1% D-arginine. We also determined aesculin hydrolysis in a heart infusion broth containing 0.1% aesculin and 0.05% ferric citrate. After inoculation into these media, cultures were observed continuously for 1 week.

RESULTS

Colony morphology

All strains of group I formed pin-point colonies (less than 0.1 mm in diameter) on CBA-plates at 37 °C under aerobic conditions after 1 d incubation. After 3 d incubation, they made 'middle size' colonies (approximately 0.3–0.8 mm in diameter). The strains of group II also formed small colonies after 1 d incubation. However, they grew more rapidly than the strains of group I, and they made colonies 0.3–0.8 mm in diameter after 2 d incubation. All strains of groups I and II showed α -haemolysis on CBA plates.

DNA–DNA hybridization

The DNA–DNA hybridization similarity values obtained under optimal (31 °C) and stringent (41 °C) conditions are shown in Table 1. Three strains of group I showed more than 83 and 75% similarity under optimal and stringent conditions, respectively. Similarly, five strains of group II showed more than 71% similarity under both optimal and stringent conditions. These two new groups showed less than 33% similarity to all established members of the mitis group as well as to other α -haemolytic streptococci (*S. salivarius* and *S. bovis*). These data clearly demonstrated that these two new groups were genetically independent taxa.

16S rRNA analysis

Before we performed the phylogenetic analysis of groups I and II, we determined the 16S rRNA sequence and phylogenetic position of the type strain of *S. cristatus*, as this species had previously been designated as *S. sanguinis* and was strongly suspected of belonging to the mitis group. As expected, the branching root of *S. cristatus* was located within the mitis group cluster, and the highest level of sequence homology to *S. cristatus* was exhibited by *S. sanguinis* (97.1%).

The sequences from positions 45 to 1380 (*E. coli* numbering) of the 16S rRNAs of strains O-66^T and O-122^T were determined. We also determined the partial sequences of the 16S rRNA gene (about 500 bp from the 5' end, which includes V1, V2 and V3 areas) from six other strains. The three strains of group I shared complete sequence homology within this 500 bp region. The five strains of group II shared almost identical sequences with each other. Each of these

Fig. 1. Phylogenetic positions of two new similarity groups and *S. cristatus* among selected members of the genus *Streptococcus*. Distances were calculated by the neighbour-joining method. The numbers at the branching points are bootstrap values. *Lactococcus lactis* was used as the outgroup. 16S rRNA sequence accession numbers; '*S. peroris*', AB008314; '*S. infantis*', AB008315; *S. mitis*, D38482; *S. oralis*, X58308; *S. pneumoniae*, X58312; *S. gordonii*, D38483; *S. cristatus*, AB008313; *S. sanguinis*, X53653; *S. parasanguinis*, X53652; *S. anginosus*, X58309; *S. constellatus*, X58310; *S. intermedius*, X58311; *S. salivarius*, X58320; *S. thermophilus*, X68418; *S. vestibularis*, X58321; *S. bovis*, M58835; *S. lactolyticus*, X58319; *S. equinus*, X58318; *S. mutans*, X58303; *S. macacae*, X58302; *S. dysgalactiae*, X59030; *L. lactis*, M58837.

latter strains possessed only 1–4 different bases from the O-122^T sequence.

We investigated the phylogenetic position of representative strains of each group (strains O-66^T and O-122^T). The phylogenetic analysis demonstrated that the branching roots of the two new groups were located within the mitis group, as shown in Fig. 1. Group I was clustered with *S. gordonii*. Group II was clustered with the *S. mitis*–*S. oralis*–*S. pneumoniae* branch.

The highest levels of sequence homology, and therefore

Table 2. Levels of 16S rRNA sequence homology among the strains of two new similarity groups and other members of the genus *Streptococcus*

Group	Strain/species	Percentage homology with:	
		O-66	O-122 ^T
Mitis	O-66 ^T	100.0	
	O-122 ^T	97.2	100.0
	<i>S. mitis</i>	97.2	98.4
	<i>S. oralis</i>	96.9	98.3
	<i>S. pneumoniae</i>	97.1	98.3
	<i>S. gordonii</i>	96.9	97.2
	<i>S. cristatus</i>	96.4	96.8
	<i>S. sanguinis</i>	96.4	97.5
Anginosus	<i>S. parasanguinis</i>	96.1	96.8
	<i>S. anginosus</i>	94.4	93.8
	<i>S. constellatus</i>	94.6	95.0
	<i>S. intermedius</i>	95.0	94.6
Salivarius	<i>S. salivarius</i>	96.3	95.4
	<i>S. thermophilus</i>	95.6	94.8
Bovis	<i>S. vestibularis</i>	95.9	95.1
	<i>S. bovis</i>	95.9	95.0
	<i>S. alactolyticus</i>	95.2	95.7
	<i>S. equinus</i>	95.9	95.0
Mutans	<i>S. mutans</i>	93.2	92.9
	<i>S. macacae</i>	91.1	90.3
Pyogenic	<i>S. pyogenes</i>	94.2	94.1
	<i>S. dysgalactiae</i>	93.2	94.5

the closest relationships, were found to be between group I, group II and *S. mitis* (Table 2).

Biochemical characteristics

Biochemical traits were determined using two different commercially available kits (Rapid ID32 Strep and STREPTOGRAM). The biochemical reaction patterns are shown in Table 3. All strains of group I gave a positive reaction for acidification of lactose, maltose and sucrose, and production of β -D-galactosidase (only from the substrate *p*-nitrophenyl β -D-galactopyranoside) and alanine-phenylalanine-proline-arylamidase (APPA). All strains gave a negative reaction for arginine and aesculin hydrolysis with both commercial kits and the conventional tests. Only one strain gave a positive reaction for alkaline phosphatase. All strains of group II gave a positive reaction for acidification of lactose, maltose, sucrose and tagatose, and production of β -D-galactosidase (from both *p*-nitrophenyl β -D-galactopyranoside and 2-naphthyl β -D-galactopyranoside substrates), APPA and glycine-tryptophan arylamidase. All strains of group II gave a negative reaction for arginine and aesculin hydrolysis with both commercial kits and the conventional tests. Only one strain gave a positive reaction for acidification of inulin and pullulan. Three strains gave a

positive reaction for the production of β -D-fucosidase and *N*-acetyl- β -D-glucosaminidase. Only one strain gave a weak reaction for pyrrolidonyl arylamidase.

Rapid ID32 Strep kit profile numbers for O-66^T and O-122^T were 44012001100 and 40016641120, respectively.

G + C content of DNA

The DNA G + C contents of strains O-66^T and O-122^T were 39.8 ± 0.1 and 40.1 ± 0.1 mol %, respectively. The DNA G + C contents of groups I and II were 39.8–40.5 mol % (mean = 40.0 mol %) and 39.9–40.4 mol % (mean = 40.1 mol %), respectively.

DISCUSSION

From the DNA–DNA hybridization results, the strains of groups I and II formed completely separate groups, showing more than 75 % DNA similarity with their respective representative strains (O-66^T and O-122^T) even under stringent conditions. In contrast, less than 30 % DNA similarity was obtained with the seven type strains of currently recognized members of the mitis group. The genomic similarity values showed that groups I and II were closely related to each other, since they shared 40–60 % DNA similarity (Table 1). However they shared only 97.2 % 16S rRNA gene sequence homology. This value was lower than that obtained for group II with *S. mitis*, *S. oralis*, *S. pneumoniae* or *S. sanguinis* (Table 2). Furthermore, the phylogenetic tree showed that the branching roots of groups I and II were slightly separated and were clustered with the *S. gordonii* and *S. mitis*–*S. oralis*–*S. pneumoniae* branches, respectively (Fig. 1). Therefore, the genetic relationships deduced from the DNA–DNA hybridization data and from the 16S rRNA sequence data were different.

There are three registered 16S rRNA gene sequences of the type strain of *S. sanguinis* in the database (accession numbers X53653, AF003928 and AB002524). They have 4–13 base differences within the same area (about 1300 bp). We could not explain the actual reason for this sequence variation, however the possible reason is technical, leading to mis-determination of sequence or sequence determination from different copies of the 16S rRNA gene. Nevertheless, with use of any one of these registered sequence data, *S. sanguinis* is still located in the mitis group, even if the branching root is slightly different than that in Fig. 1 (data not shown).

The group I strains showed a positive β -galactosidase reaction only from the substrate *p*-nitrophenyl β -D-galactopyranoside (Table 3). At least three substrates have been used for the detection of β -galactosidase activity (2-naphthyl β -D-galactopyranoside, *p*-nitrophenyl β -D-galactopyranoside and 4-methylumbelliferyl β -D-galactopyranoside) (Beightton *et al.*, 1991; bioMérieux). Most likely, the collective data for β -galactosidase has been using the substrate 2-naphthyl

Table 3. Biochemical characteristics of the new similarity groups I and II

R, Determined with Rapid ID32 Strep kit; S, determined with Streptogram kit; C, determined by conventional methods. —, negative; +, positive; d, variable. The numbers in parentheses are the number of positive strain(s)/test strains.

Character (method used)	Group I (<i>S. peroris</i>)		Group II (<i>S. infantis</i>)	
	All isolates (3 strains)	O-66 ^T	All isolates (5 strains)	O-122 ^T
Haemolysis on sheep blood agar plate	α	α	α	α
Acidification of:				
Amygdalin (S)	—	—	—	—
L-Arabinose (R, S)	—	—	—	—
D-Arabitol (R)	—	—	—	—
Arbutin (S)	—	—	—	—
Cyclodextrine (R)	—	—	—	—
Glycogen (R)	—	—	—	—
Inulin (S)	—	—	d (1/5)	—
Lactose (R, S)	+	+	+	+
Maltose (R)	+	+	+	+
Mannitol (R, S)	—	—	—	—
Methyl β-D-glucopyranoside (R)	—	—	—	—
Melibiose (R, S)	—	—	—	—
Melezitose (R)	—	—	—	—
Pullulane (R)	—	—	d (1/5)	+
Raffinose (R, S)	—	—	—	—
Ribose (R)	—	—	—	—
Sucrose (R)	+	+	+	+
Sorbitol (R, S)	—	—	—	—
Tagatose (R)	—	—	+	+
Trehalose (R, S)	—	—	—	—
Production of:				
α-Galactosidase (R, S)	—	—	—	—
β-Galactosidase (R)*	—	—	+	+
β-Galactosidase (R, S)†	+	+	+	+
Alanyl-phenylalanyl-proline arylamidase (R)	+	+	+	+
β-Glucosidase (R)	—	—	—	—
β-Mannosidase (R)	—	—	—	—
β-D-Fucosidase (S)	—	—	d (3/5)	+
β-Glucuronidase (R, S)	—	—	—	—
Glycine-tryptophan arylamidae (R)	—	—	+	+
N-Acetyl-β-glucosaminidase (R, S)	—	—	d (3/5)	+
Alkaline phosphatase (R, S)	d (1/3)	+	—	—
Pyrollidonyl arylamidase (R, S)	—	—	d (1/5)	—
Urease (R)	—	—	—	—
VP-reaction (R, S)	—	—	—	—
Hydrolysis of:				
Arginine (R, S, C)	—	—	—	—
Aesculin (S, C)	—	—	—	—
Hippurate (R, S)	—	—	—	—

* Substrate is 2-naphthyl β-D-galactopyranoside.

† Substrate is *p*-nitrophenyl β-D-galactopyranoside.

β-D-galactopyranoside; as used in the API-ZYM and API 20 Strep identification test kits (bioMérieux). Using these commercial kits, the strains of group I

were judged to have no β-galactosidase activity, however they could degrade the substrate *p*-nitrophenyl β-D-galactopyranoside.

Table 4. Biochemical characteristics that differentiate the new species from other members of the mitis group

Data taken from Kikuchi *et al.* (1995), except that in parentheses which was our observation. +, More than 90 % of strains positive; —, less than 10 % of strains positive; d+, 50–89 % of strains positive; d–, 11–49 % of strains positive. Substrates/products: 1, arginine; 2, melibiose; 3, raffinose; 4, trehalose; 5, tagatose; 6, α -galactosidase; 7, β -galactosidase; 8, *N*-acetyl- β -glucosaminidase; 9, β -glucosidase; 10, alkaline phosphatase.

Species	Hydrolysis of:	Acidification of:				Production of:				
	5	2	3	4		6	7*	8	9	10
<i>S. peroris</i>	—	—	—	—	—	—	—	—	—	d–
<i>S. infantis</i>	—	—	—	—	+	—	+	d+	—	—
<i>S. mitis</i>	—	d–	d–	—	—	d–	—	—	—	d+
<i>S. oralis</i>	—	d+	d+	d–	d– (–)	d+	d–	d–	—	d+
<i>S. pneumoniae</i> †	d–	—	+	+	—	+	—	d+	d–	—
<i>S. gordonii</i>	+	—	—	+	d–	—	—	—	d+	+
<i>S. cristatus</i>	+	d–	d–	d+	d+	d–	d–	—	—	—
<i>S. sanguinis</i>	+	d+	d+	+	d–	d+	—	—	d+	—
<i>S. parasanguinis</i>	+	d+	d+	d–	d+	d+	d–	d–	d–	d+

* The substrate is 2-naphthyl β -D-galactopyranoside.

† Only for *S. pneumoniae*, data taken from Rapid ID32 Strep database (bioMérieux).

Strains of group II showed similar biochemical traits to *S. mitis* or *S. oralis*. However, some biochemical reactions, such as alkaline phosphatase, β -galactosidase (substrate is 2-naphthyl β -D-galactopyranoside) and tagatose acidification, are helpful for differentiating group II strains from these species (Table 4). The tagatose acidification test is only used in the Rapid ID32 Strep kit and was not performed using a conventional method. All *S. mitis* strains were negative for tagatose acidification in the Rapid ID32 Strep test kit as previously reported by Kikuchi *et al.* (18). The proportion of strains of *S. oralis* positive for this test is 30 % and 40 %, according to the Rapid ID32 Strep database and Kikuchi *et al.* (1995), respectively. However, in studies undertaken in our laboratory, only 10 % (3/30) of *S. oralis* strains which were clearly classified by quantitative plate DNA–DNA hybridization, showed a positive reaction for tagatose acidification (unpublished data), compared with all strains of group II which gave positive reactions in this test.

In view of the data presented above, we believe that these two groups should be classified as new species. We propose the names *Streptococcus peroris* sp. nov. and *Streptococcus infantis* sp. nov. for groups I and II, respectively.

Description of *Streptococcus peroris* sp. nov.

Streptococcus peroris (per.or.is. L. adj. *per* through; L. n. *oris* oral cavity; L. adj. *peroris* pertaining to the oral cavity, from where the organism was isolated).

Cells are non-sporulating, non-motile, Gram-positive, catalase-negative cocci that are approximately 0.6–0.8 mm in diameter, and grow in short chains.

Facultatively anaerobic. α -Haemolytic on Columbia blood (sheep) agar plates. The biochemical characteristics of the species are shown in Table 3. The G+C content of the DNA is 39.8–40.5 mol % (HPLC method). Strains are isolated from the human tooth surface and pharynx. The type strain is strain O-66^T (= GTC 848^T = JCM 10158^T).

Description of *Streptococcus infantis* sp. nov.

Streptococcus infantis (in.fant.is. L. n. *infans* infant; L. adj. *infantis* pertaining to human infants, from whom the organism was isolated).

Cells are non-sporulating, non-motile, Gram-positive, catalase-negative cocci that are approximately 0.6–1.0 mm in diameter, and grow singly or in short chains. Facultatively anaerobic. α -Haemolytic on Columbia blood (sheep) agar plates. The biochemical characteristics of the species are shown in Table 3. The G+C content of the DNA is 39.9–40.4 mol % (HPLC method). Strains are isolated from the human tooth surface and pharynx. The type strain is strain O-122^T (= GTC 849^T = JCM 10157^T).

ACKNOWLEDGEMENTS

This work was partially supported by a Grant-in-Aid for Scientific Research (B)-09557024 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Beighton, D., Hardie, J. M. & Whaley, R. A. (1991). A scheme for identification of viridans streptococci. *J Med Microbiol* **35**, 367–372.

- Bentley, R. W., Leigh, J. A. & Collins, M. D. (1991). Intragenic structure of *Streptococcus* based on comparative analysis of small-subunit rRNA sequences. *Int J Syst Bacteriol* **41**, 487–494.
- Collins, M. D. & Wallbanks, S. (1992). Comparative sequence analyses of the 16S rRNA genes of *Lactobacillus minutus*, *Lactobacillus rimae* and *Streptococcus parvulus*: proposal for the creation of a new genus *Atopobium*. *FEMS Microbiol Lett* **95**, 235–240.
- Devriese, L. A., Kilpper-Balz, R. & Schleifer, K. H. (1988). *Streptococcus hyointestinalis* sp. nov. from the gut of swine. *Int J Syst Bacteriol* **38**, 440–441.
- Devriese, L. A., Pot, B., Vandamme, P., Kersters, K., Collins, M. D., Alvarez, N., Haesebrouck, F. & Hommez, J. (1997). *Streptococcus hyovaginalis* sp. nov. and *Streptococcus thoralensis* sp. nov., from the genital tract of sows. *Int J Syst Bacteriol* **47**, 1073–1077.
- Eldar, A., Bejerano, Y. & Bercovier, H. (1994). *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal species causing a meningoencephalitis in fish. *Curr Microbiol* **28**, 139–143.
- Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S., Miura, H., Matsui, K. & Yabuuchi, E. (1988). Simple genetic method to identify viridans group streptococci by colorimetric dot hybridization and fluorometric hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Ezaki, T., Saidi, S. M., Liu, S.-L., Hashimoto, Y., Yamamoto, H. & Yabuuchi, E. (1990). Rapid procedure to determine the DNA base composition from small amounts of gram-positive bacteria. *FEMS Microbiol Lett* **67**, 127–130.
- Ezaki, T., Li, N., Hashimoto, Y., Miura, H. & Yamamoto, H. (1994). 16S ribosomal DNA sequences of anaerobic cocci and proposal of *Ruminococcus hansenii* comb. nov. and *Ruminococcus productus* comb. nov. *Int J Syst Bacteriol* **44**, 130–136.
- Handley, P., Coykendall, A., Beighton, D., Hardie, J. M. & Whiley, R. A. (1991). *Streptococcus crista* sp. nov. a viridans streptococcus with tufted fibrils, isolated from the human oral cavity and throat. *Int J Syst Bacteriol* **41**, 543–547.
- Kawamura, Y., Hou, X. G., Sultana, F., Liu, S., Yamamoto, H. & Ezaki, T. (1995a). Transfer of *Streptococcus adjacens* and *Streptococcus defectivus* to *Abiotrophia* gen. nov. as *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov., respectively. *Int J Syst Bacteriol* **45**, 798–803.
- Kawamura, Y., Hou, X. G., Sultana, F., Miura, H. & Ezaki, T. (1995b). Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. *Int J Syst Bacteriol* **45**, 406–408.
- Kawamura, Y., Sultana, F., Hou, X. G. & Ezaki, T. (1995c). Evaluation of a new streptococcal identification kit 'STREPTOGRAM'. *J Jpn Soc Clin Microbiol* **5**, 4–12 (in Japanese).
- Kilian, M., Mikkelsen, L. & Henriksen, J. (1989). Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis* (White and Niven 1946), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrewes and Horder 1906). *Int J Syst Bacteriol* **39**, 471–484.
- Kilpper-Balz, R. & Schleifer, K. H. (1988). Transfer of *Streptococcus morbillorum* to the genus *Gemella* as *Gemella morbillorum* comb. nov. *Int J Syst Bacteriol* **38**, 442–443.
- Kikuchi, K., Enari, T., Totsuka, K. & Shimizu, K. (1995). Comparison of phenotypic characteristics, DNA-DNA hybridization results, and results with a commercial rapid biochemical and enzymatic reaction system for identification of viridans group streptococci. *J Clin Microbiol* **33**, 1215–1222.
- Meinkoth, J. & Wahl, G. (1984). Hybridization of nucleic acids immobilized on solid supports. *Anal Chem* **138**, 267–284.
- Ohkuni, M., Todome, Y., Mizuse, M. & 9 other authors (1993). Biologically active extracellular products of oral viridans streptococci and the aetiology of Kawasaki disease. *J Med Microbiol* **39**, 352–362.
- Osawa, R., Fujisawa, T. & Sly, L. I. (1995). *Streptococcus gallolyticus* sp. nov.; gallate degrading organisms formerly assigned to *Streptococcus bovis*. *Syst Appl Microbiol* **18**, 74–78.
- Robinson, I. M., Stromley, J. M., Varel, V. H. & Cato, E. P. (1988). *Streptococcus intestinalis*, a new species from the colons and feces of pigs. *Int J Syst Bacteriol* **38**, 245–248.
- Schleifer, K. H. & Kilpper-Balz, R. (1984). Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Int J Syst Bacteriol* **34**, 31–34.
- Schleifer, K. H., Kraus, J., Dvorak, C., Kilpper-Balz, R., Collins, M. D. & Fischer, W. (1985). Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. *Syst Appl Microbiol* **6**, 183–195.
- Skaar, I., Gaustad, P., Tonjum, T., Holm, B. & Stenwig, H. (1994). *Streptococcus phocae* sp. nov., a new species isolated from clinical specimens from seals. *Int J Syst Bacteriol* **44**, 646–650.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Trüper, H. G. & de' Clari, L. (1997). Taxonomic note: necessary correction of specific epithets formed as substantives (nouns) in 'apposition'. *Int J Syst Bacteriol* **47**, 908.
- Vandamme, P., Pot, B., Falsen, E., Kersters, K. & Devriese, L. A. (1996). Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. *Int J Syst Bacteriol* **46**, 774–781.
- Whiley, R. A. & Hardie, J. M. (1988). *Streptococcus vestibularis* sp. nov. from the human oral cavity. *Int J Syst Bacteriol* **38**, 335–339.
- Whiley, R. A., Russell, R. R. B., Hardie, J. M. & Beighton, D. (1988). *Streptococcus downei* sp. nov. for strains previously described as *Streptococcus mutans* serotype h. *Int J Syst Bacteriol* **38**, 25–29.
- Whiley, R. A., Fraser, H. Y., Douglas, C. W. I., Hardie, J. M., Williams, A. M. & Collins, M. D. (1990). *Streptococcus parasanguis* sp. nov. an atypical viridans *Streptococcus* from human clinical specimens. *FEMS Microbiol Lett* **68**, 115–122.
- Williams, A. M. & Collins, M. D. (1990). Molecular taxonomic studies on *Streptococcus uberis* type I and II. Description of *Streptococcus parauberis* sp. nov. *J Appl Bacteriol* **68**, 485–490.