Molecular Relationships and Classification of Some Viridans Streptococci as *Streptococcus oralis* and Emended Description of *Streptococcus oralis* (Bridge and Sneath 1982)

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Nucleic acid hybridization studies together with a chemical cell wall analysis and physiological data indicated that some strains designated as *Streptococcus sanguis* II, *Streptococcus* sp. ("S. mitior"-*S. sanguis*), and "S. viridans" I, II, and IV are closely related to the type strain of *S. oralis*. These strains contained a directly cross-linked peptidoglycan with lysine as a diamino acid and ribitol and choline as characteristic cell wall constituents. We propose that these strains be classified as members of *S. oralis*. An emended description of *S. oralis* is given. *S. oralis* strains are genetically related to *S. pneumoniae* strains, and representatives of both species contain ribitol and choline in their cell walls.

Viridans streptococci are a heterogeneous group of bacteria which are found predominantly in the upper respiratory tract (17). However, they are also an important cause of endocarditis and bacteremia (8, 15, 18, 23). The fact that viridans streptococci cannot be classified by group antigens or other serological methods led to many attempts to separate them on the basis of different physiological and biochemical characteristics (3, 10-14, 24). However, the results have not been satisfactory for two reasons. First, most authors only studied certain strains of viridans streptococci, and second, there is much confusion in the nomenclature of these organisms. There are, for example, species such as "*Streptococcus viridans*" and "*S. mitior*" that are not validly described; therefore, no type strains are available. The same strains may even be allocated to different species depending on the author. Thus, strains designated by Colman and Williams (3) as "*S. mitior*" were classified by Facklam (10) as *S. mitis* or *S. sanguis* II. A recently described new species, *S. oralis*, shares typical physiological characteristics with some strains designated as "*S. mitior*" and *S. sanguis* II (1). The subdivision of "*S. viridans*" and *S. sanguis* into various biotypes caused further confusion in the classification of viridans streptococci.

Other oral streptococci, such as "*S. milleri*," *S. mutans*, and related organisms, are also often regarded as viridans streptococci (17). However, recent studies from our laboratory have revealed that strains designated as "*S. milleri*" belong to either *S. anginosus* or *S. constellatus* (22). Moreover, we confirmed the finding of Coykendall (4, 5) that strains of *S. mutans* can be divided into five species, namely, *S. cricetus*, *S. ferus*, *S. mutans*, *S. rattus*, and *S. sobrinus*, which can easily be differentiated (29).

In this study, strains of "*S. mitior*," *S. mitis*, *S. oralis*, *S. pneumoniae*, *S. salivarius*, *S. sanguis* I and II, group-O *Streptococcus* spp., and "*S. viridans*" I, II, III, and IV (32) were compared with previously studied oral streptococci. Relationships were determined primarily by nucleic acid homology experiments and cell wall analysis.

MATERIALS AND METHODS

The strains included in this study are shown in Table 1. They were cultivated in CASO-bouillon (E. Merck AG, Darmstadt, Federal Republic of Germany) at 35°C without

aeration. Cells were harvested at the end of the exponential growth phase for the isolation of deoxyribonucleic acid (DNA) and at the stationary growth phase for the preparation of cell walls. Isolation of DNA and ribosomal ribonucleic acid, radioactive labeling, and nucleic acid hybridization experiments were carried out by the filter method as described previously (19, 20, 22). DNA base composition was determined by thermal denaturation with a Gilford model 2600 spectrophotometer (16). DNA from *Escherichia coli* K-12 was used as the standard. The guanine-plus-cytosine (G+C) contents (in moles percent) were calculated by the method of De Ley (7) and were corrected with the published value for *E. coli*, 51.7 mol% (7). Isolation of cell walls and determination of peptidoglycan types and cell wall sugar compositions were carried out as previously described (26, 27). Cell wall teichoic acids were analyzed by the method of Endl et al. (9). Choline was determined in cell wall hydrolysates (2 mg of cell wall, 0.2 ml of 2 M HCl, 3 h, 100°C) by thin-layer chromatography (33) and enzymatically with choline oxidase by the method of Takayama et al. (34), except that for the determination of hydrogen peroxide, 2-hydroxy-3,5-dichlorobenzene sulfonate (35) was used instead of phenol. Physiological characteristics were determined by conventional tests as described by Facklam and Wilkinson (11) and Bucher and von Graevenitz (2).

RESULTS AND DISCUSSION

Cell wall analysis and DNA base composition. Determinations of peptidoglycan types showed that *S. oralis*, "*S. viridans*" I, II, III, and IV (32), strains designated as *S. sanguis* II, strains obtained as "*S. mitior*"-*S. sanguis*, and group-O *Streptococcus* spp. contained the peptidoglycan type Lys-direct (Table 1). This type of peptidoglycan is not widespread among streptococci and has been found so far only among certain viridans streptococci (27; J. Hladny, Ph.D. thesis, Technische Universität München, Munich, Federal Republic of Germany, 1971) and group-R and -S streptococci (21). All the other "greening," "oral," or "milleri" streptococci contained different peptidoglycan types (22, 29). The most frequent one was Lys-Ala₁₋₃, but in the "mutans-like" streptococci, Lys-Thr-Ala also was found (30). Further characteristic cell wall components of the viridans streptococci containing the Lys-direct peptidoglycan type were ribitol and choline (Table 1). In this respect, they were similar to *S. pneumoniae*. The presence of ribitol

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TABLE 1. Organisms studied, G+C contents, and cell wall data

Organism	Strain ^a	G+C content of DNA (mol %)	Peptidoglycan type	Ribitol teichoic acid	Sugar and amino sugar constituents of cell walls ^b					Choline bound to the cell wall
					Rhamnose	Glucose	Galac- tose	GlcNH ₂ ^c	GalNH ₂ ^d	
<i>Streptococcus acidominimus</i>	NCDO 2025 ^T	ND ^e	ND	—	+	—	+	(+)	+	—
<i>S. anginosus</i>	DSM 20563 ^T	37	Lys-Ala ₁₋₃	—	+	+	+	—	+	—
<i>S. bovis</i>	DSM 20480 ^T	38	Lys-Thr-Ala	—	+	+	+	+	—	—
<i>S. constellatus</i>	DSM 20575 ^T	37	Lys-Ala ₁₋₃	—	+	+	—	+	—	+
<i>S. cricetus</i>	ATCC 19642 ^T	40	Lys-Thr-Ala	—	+	+	+	ND	ND	—
<i>S. ferus</i>	ATCC 33477 ^T	42	Lys-Ala ₂₋₃	—	+	+	—	ND	ND	ND
<i>S. iniae</i>	DSM 20576 ^T	37	Lys-Ala ₁₋₃	—	+	+	+	+	—	—
<i>S. intermedius</i>	DSM 20573 ^T	37	Lys-Ala ₁₋₃	—	+	+	—	—	—	—
<i>S. lactis</i>	DSM 20481 ^T	36	Lys-D-Asp	—	+	+	+	ND	ND	—
<i>S. mitis</i>	DSM 20568 ^T	38	Lys-Ala ₁₋₃	—	+	+	+	+	(+)	—
<i>S. morbillorum</i>	DSM 20572 ^T	ND	Lys-Ala ₁₋₃	—	+	(+)	+	(+)	—	—
<i>S. mutans</i>	ATCC 25175 ^T	36	Lys-Ala ₂₋₃	—	+	+	—	—	—	—
<i>S. oralis</i>	NCTC 11427 ^T	37	Lys-direct	+	(+)	(+)	+	(+)	+	+
<i>S. pneumoniae</i>	DSM 20566 ^T	36	Lys-Ala ₂ (Ser)	+	—	+	+	—	+	+
<i>S. pneumoniae</i>	R6 ^f	38	Lys-Ala ₂ (Ser)	+	—	+	—	—	+	+
<i>S. pneumoniae</i>	Cur ^g	37	Lys-Ala ₂ (Ser)	+	—	+	—	—	+	+
<i>S. pyogenes</i>	NCTC 8198 ^T	38	Lys-Ala ₁₋₃	—	+	—	—	+	—	ND
<i>S. rattus</i>	ATCC 19645 ^T	40	Lys-Ala ₂₋₃	—	+	—	+	ND	ND	ND
<i>S. salivarius</i>	DSM 20560 ^T	39	Lys-Ala ₁₋₃	—	+	+	+	—	+	—
<i>S. sanguis</i> I	ATCC 10556 ^T	43	Lys-Ala ₁₋₃	—	+	+	—	+	—	—
<i>S. sanguis</i> I	Kiel 12700	43	Lys-Ala ₁₋₃	—	+	+	—	+	—	—
<i>S. sanguis</i> I group H	Kiel 75360	ND	Lys-Ala ₁₋₃	—	+	+	+	+	—	—
<i>S. sanguis</i> II	DSM 20066	39	Lys-direct	+	(+)	+	+	—	+	+
<i>S. sanguis</i> II	Kiel 61162	38	Lys-direct	+	—	(+)	+	+	+	+
<i>S. sobrinus</i>	ATCC 33478 ^T	43	Lys-Thr-Ala	—	+	+	+	ND	ND	ND
<i>S. uberis</i>	NCTC 3858 ^T	ND	Lys-Ala ₁₋₃	—	+	+	—	+	—	—
" <i>S. viridans</i> " I	Kiel 50493	38	Lys-direct	+	—	+	+	—	+	+
" <i>S. viridans</i> " II	Kiel 60973	38	Lys-direct	+	—	+	+	+	+	+
" <i>S. viridans</i> " III	Kiel 45527	36	Lys-direct	—	+	+	+	(+)	+	—
" <i>S. viridans</i> " IV	Kiel 42841	38	Lys-direct	+	—	—	+	+	(+)	+
<i>Streptococcus</i> sp. group O	Kiel C119/48	39	Lys-direct	+	—	+	+	+	(+)	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S229 ^g	40	Lys-direct	+	(+)	(+)	+	+	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S410 ^g	40	Lys-direct	+	—	(+)	+	+	(+)	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S426 ^g	39	Lys-direct	+	—	—	+	—	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S438 ^g	40	Lys-direct	+	—	+	+	+	(+)	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S440 ^g	40	Lys-direct	+	—	—	+	+	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S444 ^g	40	Lys-direct	+	(+)	+	+	+	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S447 ^g	39	Lys-direct	+	(+)	(+)	+	—	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S526 ^g	39	Lys-direct	+	(+)	(+)	+	—	+	+
<i>Streptococcus</i> sp. ("S. mitior"- <i>S. sanguis</i>)	S662 ^g	39	Lys-direct	+	—	—	+	(+)	+	+
<i>Enterococcus faecalis</i>	DSM 20478 ^{Tg}	39	Lys-Ala ₂₋₃	+	+	+	+	+	+	—
<i>Staphylococcus aureus</i>	ATCC 12600 ^T	35 ^h	Lys-Gly ₅₋₆	+	—	—	—	+	—	—

^a NCDO, National Collection of Dairy Organisms, Shinfield, Reading, United Kingdom; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; Kiel, Streptokokkenzentrale, Kiel, Federal Republic of Germany.

^b —, Absent; +, present; (+), present in minor amounts.

^c GlcNH₂, Glucosamine.

^d GalNH₂, Galactosamine.

^e ND, Not determined.

^f Received from R. Hakenbeck, Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany.

^g Received from F. Gehring, Lehrstuhl Experimentale Zahnheilkunde, Würzburg, Federal Republic of Germany.

^h From reference 20.

teichoic acid and choline in their cell walls distinguished the viridans streptococci from group-R and -S streptococci that also possessed the Lys-direct peptidoglycan type but lacked ribitol and choline in their cell walls (Table 1). The ribitol-containing polymer of *S. oralis* NCTC 11427^T was studied in more detail. Cell wall hydrolysates (2 M HCl, 3 h, 100°C) showed that ribitol and phosphorus were present in about equimolar amounts. Only very low amounts of ribitol were released from cell walls by treatment with 70% hydrofluoric acid at 0°C for 3 h, and maximum yields were obtained only after acid hydrolysis of the hydrofluoric acid degradation products. Ribitol and sugars were simultaneously released, indicating the presence of ribitol teichoic acid. Galactose and galactosamine were found as major sugar constituents. Minor amounts of glucose and rhamnose also were present. The cell wall of *S. oralis* NCTC 11427^T was extracted with 60% hydrofluoric acid for 16 h at 0°C. Released glycosides were separated on a Bio-Gel P2 column and analyzed as previously described (9). An oligomer consisting of equimolar amounts of ribitol, galactose, and galactosamine was identified.

"*S. viridans*" III Kiel 45527 differed from the other viridans streptococci in that it lacked ribitol teichoic acid and choline and, in this respect, resembled group-R and -S streptococci.

Choline does not seem to be as good a criterion for the identification of *S. oralis* as ribitol teichoic acid, since choline also was found in some strains of *S. constellatus* (unpublished results). Cell walls of viridans streptococci can also contain rhamnose polysaccharides, but in significantly smaller amounts than are usually found in cell walls of streptococci.

The moles percent G+C contents are also shown in Table 1. Significantly different values were found only for *S. sanguis* I strains. Such high G+C contents (43 mol%) are, with the exception of *S. sobrinus*, rather unusual for streptococci. The moles percent G+C contents of the mutanslike streptococci (see Tables 1 and 5) were corrected with the value for *E. coli* K-12, as was done for all the other strains studied. For that reason they differ slightly from the values in a former publication (29).

Nucleic acid hybridization studies. The results of DNA-ribosomal ribonucleic acid hybridization studies (Table 2) showed that most of the oral streptococci formed a group of related species with differences in the melting point between homologous and heterologous hybrids [$\Delta T_{m(e)}$] of <4°C. The closer relationship of "*S. viridans*" strains to *S. sanguis* II than to *S. sanguis* I was indicated by $\Delta T_{m(e)}$ values below 1.0°C. Some organisms, such as *S. acidominimus* and *S. salivarius*, which were also classified as viridans streptococci by different authors (3, 10–12, 24, 25), had $\Delta T_{m(e)}$ values above 4°C, indicating no close genetic relationship with either *S. sanguis* I or *S. sanguis* II. Similar results were found for "*S. viridans*" III (Table 2). Moreover, the rather high $\Delta T_{m(e)}$ value of 8.6°C for *S. morbillorum* is in the range of those for *Enterococcus faecalis* (28) and *S. lactis* (K. H. Schleifer, J. Kraus, C. Dvorak, R. Kilpper-Bälz, M. D. Collins, and W. Fischer, Syst. Appl. Microbiol, in press). Further studies are necessary to show whether *S. morbillorum* should be excluded from the genus *Streptococcus*, as was proposed for *S. lactis* and related organisms (Schleifer et al., in press).

DNA-DNA hybridization studies were carried out to obtain information about the genetic relationships within the true viridans streptococci. Table 3 shows a group with very high DNA homology values consisting of *S. oralis*, *S.*

TABLE 2. Results of DNA-ribosomal ribonucleic acid hybridization studies

Source of DNA	Labeled 23S ribosomal ribonucleic acid from:			
	<i>S. sanguis</i> I ATCC 10556 ^T		<i>S. sanguis</i> II DSM 20066	
	$T_{m(e)}$ ^a	$\Delta T_{m(e)}$	$T_{m(e)}$ ^a	$\Delta T_{m(e)}$
<i>S. sanguis</i> I ATCC 10556 ^T	78.0	0.0	75.7	2.6
<i>S. sanguis</i> I Kiel 75360	77.6	0.4		
" <i>S. viridans</i> " IV Kiel 42841	75.8	2.2	77.5	0.8
<i>S. constellatus</i> DSM 20575 ^T	74.9	3.1		
" <i>S. viridans</i> " I Kiel 50493	74.9	3.1	77.6	0.7
<i>S. mitis</i> DSM 20568 ^T	74.8	3.2		
<i>S. sanguis</i> II DSM 20066	74.5	3.5	78.3	0.0
<i>S. mutans</i> ATCC 25175 ^T	74.2	3.8	75.3	3.0
<i>S. iniae</i> DSM 20576 ^T	74.2	3.8		
<i>S. bovis</i> DSM 20480 ^T	74.0	4.0		
" <i>S. viridans</i> " III Kiel 45527	74.0	4.0	73.8	4.5
<i>S. cricetus</i> ATCC 19642 ^T	73.5	4.5		
<i>S. salivarius</i> DSM 20560 ^T	73.0	5.0		
<i>S. acidominimus</i> NCDO 2025 ^T	72.5	5.5		
<i>S. morbillorum</i> DSM 20572 ^T	69.4	8.6		
<i>E. faecalis</i> DSM 20478 ^T	68.3	9.7		
<i>S. lactis</i> DSM 20481 ^T	68.3	9.7		
<i>S. aureus</i> ATCC 12600 ^T	67.5	10.5	69.1	9.2

^a Temperatures at 50% of the bound rRNA was eluted from the hybrids.

sanguis II, "*S. viridans*" I and II, and "*S. mitior*"-*S. sanguis* strains. The high homology values indicated a relationship at the species level, which is in accord with the results of the cell wall analysis and physiological data (see Tables 1 and 4). Three strains ("*S. viridans*" II Kiel 60973 and "*S. mitior*"-*S. sanguis* S526 and S662) hybridized slightly less with the other strains of this group, but additional hybridization studies with stringent conditions again revealed high homology values (>70%). This result indicates that these strains should also be classified as the same species.

On the average, there was still a high degree of homology (56%) between *S. oralis* and *S. pneumoniae* as well as between group-O *Streptococcus* spp. and "*S. viridans*" IV. However, DNA homology values below 70% justify a separate species status (31). This is obvious in the case of *S. pneumoniae*, but it is not possible to classify the other two organisms as a separate species until more strains have been studied.

All the viridans streptococci shared slightly higher DNA homology (25%) with some of the other oral streptococci, such as *S. anginosus*, *S. constellatus*, *S. intermedius*, *S. mitis*, and *S. sanguis* I, than with others (Table 3). They may

TABLE 3. DNA homology values of various oral streptococci^a

	% Homology with labeled DNA from:												
Source of filter-bound DNA	<i>S. oralis</i> NCTC 11427 ^T	<i>S. sanguis</i> II DSM 20066	<i>S. sanguis</i> II Kiel 61162	" <i>S. viridans</i> " I Kiel 50493	<i>Streptococcus</i> sp. S438	<i>Streptococcus</i> sp. S526	<i>Streptococcus</i> sp. S662	<i>S. pneumoniae</i> R6	<i>Streptococcus</i> sp. group O Kiel C119/48	<i>S. sanguis</i> I ATCC 10556 ^T	<i>S. mitis</i> DSM 20568 ^T	<i>S. anginosus</i> DSM 20563 ^T	<i>S. constellatus</i> DSM 20575 ^T
<i>S. oralis</i> NCTC 11427 ^T	100	92	72	84	98	98	82	44	60			19	18
<i>S. sanguis</i> II DSM 20066		100	98	97	100	100		51					
<i>S. sanguis</i> II Kiel 61162		80	100	100									
" <i>S. viridans</i> " I Kiel 50493	91	84	74	100	86	92	75	48	60				
<i>Streptococcus</i> sp. S229		100			75	85							
<i>Streptococcus</i> sp. S410		82			81	83							
<i>Streptococcus</i> sp. S426		72			83	89							
<i>Streptococcus</i> sp. S438		80			100	100							
<i>Streptococcus</i> sp. S440	100	87			100	92	86		66				
<i>Streptococcus</i> sp. S444					74	95							
<i>Streptococcus</i> sp. S447		78			86	100							
<i>Streptococcus</i> sp. S526	74	79			76	100	72		59				
<i>Streptococcus</i> sp. S662	75	75			72	76	100		59				
" <i>S. viridans</i> " II Kiel 60973		77	60	75				43					
" <i>S. viridans</i> " IV Kiel 42841	42	58	54	57	42	41	43	43	42			22	
<i>S. pneumoniae</i> DSM 20566 ^T								90					
<i>S. pneumoniae</i> R6	53	62	50	68		50	50	100	65			18	17
<i>S. pneumoniae</i> Curi								92					
<i>Streptococcus</i> sp. group O	58					57	56		100				
<i>S. sanguis</i> I ATCC 10556 ^T	26	29	30		26	23	21		22	100	45	25	23
<i>S. sanguis</i> I Kiel 12700		25	26	29						79	41	22	20
<i>S. sanguis</i> I Kiel 75360			31							77		26	
<i>S. mitis</i> DSM 20568 ^T			38							41		29	27
<i>S. anginosus</i> DSM 20563 ^T												100	57
<i>S. constellatus</i> DSM 20575 ^T												66	100
<i>S. intermedius</i> DSM 20573 ^T												55	62
" <i>S. viridans</i> " III Kiel 45527	20	18	19		17	20			18				
<i>S. salivarius</i> DSM 20560 ^T		16	15										
<i>S. mutans</i> ATCC 25175 ^T		18	15	21								17	17
<i>S. pyogenes</i> NCTC 8198 ^T	17	18	16			15	13		15				
<i>E. faecalis</i> DSM 20478 ^T		8	7	8								8	

^a The values were determined under optimal hybridization conditions (25°C below the melting temperature of DNA). Boxed values indicate relatedness at the species level.

be considered to be members of a species group within the streptococci similar to certain species groups within the staphylococci (30). "*S. viridans*" III was the only exception among the viridans streptococci, since it did not have a close genetic relationship with the other viridans streptococci. However, this strain also differed from the typical viridans streptococci by the lack of ribitol teichoic acid and choline in its cell wall.

S. sanguis I and *S. mitis*, which contained a peptidoglycan of the Lys-Ala₁₋₃ type, also were genetically different from the *S. oralis*-*S. pneumoniae*-"*S. viridans*" group (DNA homology values ≈25%). The DNA homology value of 40% for *S. sanguis* I and *S. mitis* indicates that their classification as different species is justified. However, more strains should be investigated to find suitable differentiating characteristics for these two species. The genetic diversity of *S. sanguis* I, *S. sanguis* II, and *S. mitis* (Table 3) is in good

agreement with the data reported by Coykendall and Specht (6) and Welborn et al. (36).

Physiological differentiation. Physiological tests were carried out with the genetically well-defined strains to find reliable differentiating characteristics for these species (Table 4). The results were generally in accord with those of other studies. *S. oralis* was the only validly described species within the group of strains that were genetically and physiologically highly related; therefore, they should be regarded as members of the same species. Differential characteristics for the routine identification of *S. oralis* in laboratories are shown in Table 5. The most characteristic feature of *S. oralis* is, besides the unique cell wall composition, the inability to hydrolyze esculin or produce acid from inulin. The physiological tests were usually sufficient to distinguish *S. oralis* from other oral streptococci. However, in a few cases, such as "*S. viridans*" I Kiel 50493 and *S.*

TABLE 4. Physiological reactions of viridans streptococci^a

Strain	Acetoin production	Esculin hydrolysis	Arginin hydrolysis	Acid production from:							
				Salicin	Inulin	Sorbitol	Mannitol	Cellobiose	Trehalose	Ribose	Raffinose
<i>S. oralis</i> NCTC 11427 ^T	+	—	+	+	—	—	—	—	+	+	—
<i>S. sanguis</i> II DSM 20066	+	+	+	+	—	—	+	+	+	+	+
<i>S. sanguis</i> II Kiel 61162	+	—	—	—	—	—	—	—	—	—	+
" <i>S. viridans</i> " I Kiel 50493	+	+	+	+	+	—	+	+	+	+	+
<i>Streptococcus</i> sp. S229	+	—	—	+	—	—	—	—	—	ND	+
<i>Streptococcus</i> sp. S410	+	—	—	—	—	—	—	—	—	ND	+
<i>Streptococcus</i> sp. S426	(+)	—	—	—	—	—	—	—	—	ND	—
<i>Streptococcus</i> sp. S438	+	—	—	+	—	—	—	—	—	ND	+
<i>Streptococcus</i> sp. S440	+	—	—	—	—	—	—	—	+	ND	+
<i>Streptococcus</i> sp. S444	+	—	—	—	—	—	—	—	—	ND	+
<i>Streptococcus</i> sp. S447	+	—	—	—	—	—	—	+	+	ND	+
<i>Streptococcus</i> sp. S526	+	—	—	—	—	—	—	—	—	ND	—
<i>Streptococcus</i> sp. S662	(+)	—	—	—	—	—	—	—	—	ND	+
" <i>S. viridans</i> " II Kiel 60973	+	—	+	—	—	—	—	—	—	—	+
<i>S. pneumoniae</i> DSM 20566 ^T	—	+	+	—	—	—	—	—	+	—	+
<i>S. pneumoniae</i> R6	ND	+	—	ND	—	ND	ND	ND	ND	ND	ND
<i>S. pneumoniae</i> Curi	ND	+	—	ND	—	ND	ND	ND	ND	ND	ND
<i>Streptococcus</i> sp. group O Kiel C119/48	(+)	—	—	+	—	—	—	+	—	—	—
" <i>S. viridans</i> " IV Kiel 42841	—	—	—	—	—	—	—	—	—	—	—
<i>S. sanguis</i> I ATCC 10556 ^T	—	+	—	—	+	—	—	—	+	—	—
<i>S. sanguis</i> I Kiel 75360	—	—	—	+	+	—	—	+	+	—	—
<i>S. mitis</i> DSM 20568 ^T	(+)	+	+	+	+	—	—	+	+	+	+
<i>S. anginosus</i> DSM 20563 ^T	+	+	+	+	—	—	—	+	+	+	+
<i>S. constellatus</i> DSM 20575 ^T	+	+	+	+	—	—	—	—	+	—	+
<i>S. intermedius</i> DSM 20573 ^T	—	+	+	+	—	—	—	+	+	—	+
" <i>S. viridans</i> " III Kiel 45527	—	+	—	—	+	—	—	+	+	—	—
<i>S. salivarius</i> DSM 20560 ^T	+	+	—	+	+	—	—	ND	ND	ND	+
<i>S. mutans</i> ATCC 25175 ^T	—	+	—	+	+	+	+	+	+	—	+
<i>S. uberis</i> NCTC 3858 ^T	+	+	+	+	+	+	+	+	+	+	—
<i>S. acidominimus</i> NCDO 2025 ^T	+	+	+	+	+	+	+	+	+	+	+
<i>S. pyogenes</i> NCTC 8198 ^T	(+)	+	—	+	—	—	—	ND	+	—	—

^a +, Positive; (+), weakly positive; —, negative; ND, not determined.

sanguis II DSM 20066, cell wall analysis was necessary to classify them as *S. oralis*. Based on the emended description of *S. oralis*, many strains previously designated as *S. sanguis* II, "*S. viridans*" I and II, and "*S. mitior*" should be

transferred to *S. oralis*. *S. mitis*, on the other hand, which is often confused with "*S. mitior*," has a separate species status and can be separated from *S. oralis* on the basis of the characteristics shown in Table 5.

TABLE 5. Differentiating characteristics of oral streptococci^a

Organism (no. tested)	Murein type	Cell wall teichoic acid	Choline in cell walls	G+C content (mol%)	Production of:		Hydrolysis of esculin	Acid production from:					
					Acetoin	NH ₃ from arginine		Salicin	Inulin	Mannitol	Sorbitol	Trehalose	Raffinose
<i>S. oralis</i> (14)	Lys-direct	Ribitol	+	37–40	+	(—)	—	v	—	—	—	v	(+)
<i>S. pneumoniae</i> (3)	Lys-Ala ₂ (Ser)	Ribitol	+	36–37	—	v	+	—	—	—	—	+	+
<i>S. sanguis</i> (2)	Lys-Ala ₁₋₃	—	—	43	—	—	v	v	+	—	—	+	—
<i>S. mitis</i> (1)	Lys-Ala ₁₋₃	—	—	38	+	+	+	+	+	—	—	+	+
<i>S. anginosus</i> (5)	Lys-Ala ₁₋₃	v	+	37–39	+	+	+	+	—	—	—	+	—
<i>S. constellatus</i> (6)	Lys-Ala ₁₋₃	v	+	36–37	+	+	+	+	—	—	—	+	—
<i>S. intermedius</i> (1)	Lys-Ala ₁₋₃	—	—	37	—	+	+	+	—	—	—	+	+
<i>S. salivarius</i> (3)	Lys-Ala ₁₋₃	—	—	39	+	v	+	+	+	—	—	v	+
<i>S. mutans</i> (6) ^b	Lys-Ala ₁₋₃	—	—	35–36	—	—	+	+	+	+	+	+	+
<i>S. cricetus</i> (4) ^b	Lys-Thr-Ala	—	—	40–41	+	—	ND	—	+	—	—	+	+
<i>S. sobrinus</i> (4) ^b	Lys-Thr-Ala	—	ND	41–43	—	—	—	—	+	+	+	+	—
<i>S. ferus</i> (1)	Lys-Ala ₂₋₃	—	ND	42	+	—	+	+	+	+	+	—	—
<i>S. rattus</i> (4) ^b	Lys-Ala ₂₋₃	Glycerol	ND	40–41	—	+	+	+	+	+	+	—	+
<i>S. uberis</i> (1)	Lys-Ala ₁₋₃	—	—	ND	+	+	+	+	+	+	+	+	—
<i>S. acidominimus</i> (1)	ND	—	—	ND	+	+	+	+	+	+	+	+	+

^a +, More than 80% positive; —, more than 80% negative; v, variable; (+), more than 70% positive; (—), more than 70% negative; ND, not determined.

^b The physiological reactions of this species were only studied for the type strain.

The following emended description of *S. oralis* is based on the description given by Bridge and Sneath (1) and our results.

Cells are gram-positive cocci in short chains and have no capsules. They are nonmotile, nonsporeforming, aerobic, facultatively anaerobic, fermentative, and catalase negative. They are hemolytic on horse blood agar and produce pronounced greening (alpha hemolytic). They reduce 0.1% tetrazolium and nitrite and decarboxylate lysine. They grow with 0.0004% crystal violet but not with 3% sodium chloride. The Voges-Proskauer reaction is positive. The methyl red reaction, growth with sodium azide, and dextran production are variable. Gluconate is usually not oxidized. More than 80% produce acid from ribose, *N*-acetylglucosamine, lactose, and sucrose. Acid production from glycerol, salicin, D-(+)-melibiose, D-(+)-trehalose, D-(+)-melezitose, D-(+)-raffinose, dextrin, *meso*-inositol, arbutin, D-(+)-cellobiose, starch, and glycogen is variable. Less than 20% produce acid from erythritol, D-(+)-arabinose, D-(+)-arabinose, amygdalin, L-(-)-sorbitol, rhamnose, mannitol, sorbitol, and inulin. Tetrathionate reductase is usually produced, lipase production is variable, and deoxyribonuclease production is negative. There is usually no hydrolysis of esculin or production of ammonia from arginine. The G+C content is 37.6 to 40.5 mol%. The cell walls contain a peptidoglycan of the Lys-direct type, ribitol cell wall teichoic acid, and choline. Galactose or glucose or both and galactosamine or glucosamine or both are present as major sugar constituents. Rhamnose is absent or present only in minor amounts. The habitat is the human mouth. The type strain is NCTC 11427.

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