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)

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We examined a collection of 151 strains of the viridans type of streptococci, which were isolated mainly from human oral cavities and included several reference strains, in an attempt to revise and improve the taxonomy of this group of bacteria. Our examinations included determinations of a high number of biochemical and physiological characteristics and serological reactivity. The resulting data revealed several hitherto unrecognized characters of taxonomic significance, and several of the species can now be more accurately defined. A diagnostic key to the taxa is presented. Strains previously identified as *Streptococcus sanguis* could be divided into two clearly distinct species, *Streptococcus sanguis* sensu stricto (type strain, ATCC 10556) and a new species, *Streptococcus gordonii* (type strain, ATCC 10558). *Streptococcus mitis* was divided into two biovars, consisting of strains possessing group O antigens and strains possessing group K antigen. The group of strains assigned to *Streptococcus anginosus* was biochemically and serologically heterogeneous, but the data did not allow natural subdivisions. Based on the results of this study, emended descriptions of the species *Streptococcus oralis*, *S. mitis*, and *S. sanguis* are provided. The classification resulting from this study is in complete agreement with previously published genetic data.

The viridans type of streptococci encountered in oral cavities and pharynxes have been curiously refractory to satisfactory classification. Although several comprehensive taxonomic studies have been performed (3, 5, 8, 11, 22, 25, 41, 42, 49), international consensus on classification and nomenclature has not been obtained. As a consequence, several synonyms have been applied to the same organisms (18, 24, 29). Furthermore, several of the species are genetically heterogeneous (12, 13). However, lack of distinguishing phenotypic traits has left these problems unsolved. There is no doubt that this situation has hampered a clear understanding of the ecology of these bacteria and of some of the molecular mechanisms involved in plaque formation on teeth.

The Approved Lists of Bacterial Names published in 1980 (43) included, in addition to some of the "mutans" species, the following six species of streptococci normally encountered in oral cavities: *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*. Subsequently, Coykendall et al. (14) demonstrated that the last two names, together with the name "*Streptococcus milleri*," which is used mainly in European laboratories, are later synonyms of *S. anginosus*. Other workers have found that *S. constellatus* is sufficiently distinct from *S. anginosus* to warrant specific recognition (26).

The name "*Streptococcus mitior*" has been used mainly by workers in European laboratories for streptococci that lack the ability to hydrolyze arginine and esculin and may or may not produce extracellular polysaccharide. Although an overwhelming amount of data, from studies of nucleic acid homology and base composition (13), cell wall carbohy-

drates (10), biochemical and physiological characteristics (5, 11, 17), and serology (11, 23, 40, 46), has established that polysaccharide-producing strains deserve specific recognition, these organisms have been regularly referred to, in some parts of the world, as "*S. sanguis* II"; other synonyms are "*S. sanguis* I:A," "*S. sanguis* biotype B," and "*S. sanguis* serotype II." In addition, Colman and Williams (11) included strains otherwise referred to as *S. mitis* in "*S. mitior*" because they considered *S. mitis* a later synonym of "*S. mitior*." Although extensively used, the name "*S. mitior*" was not included on the Approved Lists, and no formal attempt has been made to revive it. Thus, it has no formal standing. In the meantime, a new species, *Streptococcus oralis*, has been described (2). Although this species appears to be heterogeneous, the type strain is a typical strain of "*S. mitior*," and the new species thus covers this taxon (29, 30). The species *S. mitis* formally exists, yet it is not clearly defined. The type strain, which was chosen for the purpose of representing the species on the Approved Lists, does not fit the authentic description of the species (1) and is reminiscent of *S. sanguis* (29, 47). Recently, Whiley and Hardie described a new species, *Streptococcus vestibularis*, which resembles *S. salivarius* phenotypically but differs from it as determined by deoxyribonucleic acid (DNA)-DNA homology and cell wall analyses (48).

Several extensive serological studies of oral streptococci have been performed (7, 11, 22, 23, 39, 40, 46). However, partly as a result of the above-mentioned situation, a clear correlation with the existing classification has not been achieved. This problem is further amplified by a lack of international consensus concerning reference strains for serology. The special problems concerning serological group H have been extensively discussed and elucidated by Cole et al. (7).

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The taxonomic study described in this paper encompassed 151 viridans streptococci, including reference strains and freshly isolated strains from oral cavities, from pharynges, and from cases of subacute bacterial endocarditis. The strains were subjected to extensive analysis by using both serology and biochemical tests, many of which were new to this group of bacteria. Most of the taxa revealed by our results were clearly defined. On the basis of these results, a new species is described, and emended descriptions of the species *S. sanguis*, *S. oralis*, and *S. mitis* are presented.

MATERIALS AND METHODS

Streptococcal strains. The designations, sources, taxonomic status, and other pertinent information on the 151 streptococcal strains used in this study are shown in Table 1. All of the strains were gram-positive, catalase-negative cocci with a tendency to form chains in broth cultures, and they lacked soluble hemolysin. Upon isolation or receipt they were subcultured on Todd-Hewitt agar (Difco Laboratories, Detroit, Mich.) plates 5 to 10 times to ensure purity and were subsequently lyophilized. A code number (beginning with the prefix SK) was assigned to each strain of the collection. Table 1 lists the strains under the taxa to which they were assigned as a result of this study.

Standard cultural conditions. Stock agar plate cultures and cultures for tests performed on agar plates were incubated at 36°C in a 5% CO₂-95% N₂ atmosphere obtained in anaerobic jars. All liquid cultures were incubated at 36°C in air. The standard inoculum used for liquid cultures was 1 drop of a 24-h Todd-Hewitt broth culture.

Colonial and cell morphology. Strains grown on mitis salivarius agar (Difco) were examined for colonial features after incubation for 24 h at 36°C in CO₂-N₂, followed by incubation for 24 h at room temperature in air. Overnight cultures in Todd-Hewitt broth were examined by phase-contrast microscopy, and the approximate lengths of chains were recorded.

Action on blood. Hemolysis was demonstrated on 5% horse blood agar, and greening was recorded on chocolate agar prepared with 5% horse blood.

Susceptibility tests. Susceptibility to antibiotics was examined on blood agar plates (Difco) supplemented with 5% horse blood. The plates were inoculated to give a dense growth of discrete colonies. Bacitracin disks were prepared to contain 0.05 U per disk. In addition, sulfafurazole and nitrofurazon disks (Neo-Sensitabs; Rosco, Copenhagen, Denmark) were used. For bacitracin a zone of inhibition indicated susceptibility, as described by Facklam (17). The inhibition zones around sulfafurazole and nitrofurazon disks were measured; zone diameters of more than 22 and 14 mm, respectively, were considered indicative of susceptibility according to the instructions of the manufacturer of the disks.

The ability to grow in broth containing 4 or 6.5% NaCl (4) was recorded after incubation for 7 days.

Biochemical tests. Production of acids from carbohydrates was determined in phenol red broth base (Difco) containing 0.05% Tween 80 (16) and carbohydrates at concentrations of 1%. Carbohydrates were added to the autoclaved medium after cooling as filter-sterilized concentrated solutions. The final pH was recorded with a pH meter (Radiometer, Copenhagen, Denmark) after incubation for 7 days. The following carbohydrates were used: glycerol, ribose, galactose, D-(+)-glucose, D-(-)-fructose, D-(-)-mannose, L-(-)-sorbitol, rhamnose, dulcitol, mannitol, sorbitol, *N*-acetylglucosamine,

amygdalin, arbutin, esculin, salicin, D-(+)-cellobiose, maltose, lactose, D-(+)-melibiose, sucrose, D-(+)-trehalose, inulin, D-(+)-melizitose, D-(+)-raffinose, and soluble starch. The carbohydrates were analytical grade and were purchased from BDH, Poole, United Kingdom, E. Merck AG, Darmstadt, Federal Republic of Germany, Sigma Chemical Co., St. Louis, Mo., and Koch-Light, Colnbrook, United Kingdom.

The presence of a number of glycoside hydrolases, peptidases, phosphatases, esterases, and lipases was demonstrated by using chromogenic substrates included in two separate kits, an API ZYM kit and an API ZYM osidase kit (API System, La Balme-Les-Grottes, France). Both sets of test preparations were inoculated and read as described by the manufacturer. The bacteria used were harvested from a 24-h Todd-Hewitt broth culture and were suspended (turbidity, between MacFarland standards 5 and 6) in distilled water for the API ZYM tests and in 0.15 M phosphate buffer (pH 7.5) containing 0.85% (wt/vol) NaCl for the API ZYM osidase tests.

Esculin hydrolysis was detected by the presence of dark coloration after 7-days of growth in a 1% (wt/vol) Trypticase-based medium containing 0.5% (wt/vol) yeast extract, 0.05% (wt/vol) ferric citrate, 0.5% (wt/vol) sodium chloride, and 0.1% (wt/vol) esculin (4). D-Arginine hydrolysis was tested with the Nessler reagent to detect ammonia after 5 days (11). Starch hydrolysis was detected on Todd-Hewitt broth supplemented with agar and 1% (wt/vol) starch. After incubation, the cultures were flooded with an iodine solution. Clear zones around colonies were considered to be indicative of starch hydrolysis. Hippurate hydrolysis was demonstrated as described by Carlsson (5), and decarboxylation of ornithine and lysine was determined as described by Møller (35). Production of the extracellular polysaccharides dextran and levan from sucrose was tested by precipitation; 1 and 3 parts of 96% ethanol, respectively, were added to the supernatants of 7-day cultures in Trypticase medium containing 5% sucrose (19).

Urease activity was detected by a micromethod (32). Production of acetoin from glucose (Voges-Proskauer test) was demonstrated in a medium composed of 1% tryptone, 0.5% yeast extract, 0.5% potassium hydrogen phosphate, and 0.5% glucose (added as a filter-sterilized solution after autoclaving) (pH 7.3). A 2-ml portion of the medium was inoculated, and the culture was incubated for 4 days. Acetoin was demonstrated by using a standard procedure (17). Production of hydrogen peroxide was detected on the medium described by Müller (36).

Immunoglobulin A1 (IgA1) protease activity was demonstrated as described previously by using purified human myeloma IgA1 as the substrate (27). Neuraminidase was detected by using 4-methylumbelliferyl- α -D-*N*-acetylneuraminate as the substrate at a concentration of 0.1 mM dissolved in 0.1 M sodium cacodylate buffer (pH 6.0) (44). One loopful of bacteria from a Todd-Hewitt agar plate culture was suspended in 0.2 ml of the substrate solution, and the preparation was incubated overnight at 36°C. After centrifugation, 0.1 ml of the supernatant was mixed with 1 ml of 0.1 M sodium bicarbonate buffer (pH 9.1). Release of the fluorescent product was demonstrated with a fluorescence spectrophotometer (excitation, 365 nm; emission, 450 nm).

Serological examinations. The streptococcal antisera were prepared at the Streptococcus Department, Statens Seruminstitut, Copenhagen, Denmark. Grouping antisera covering groups A through V, as well as antisera against "*S. milleri*," "*S. mitior*," *Streptococcus mutans*, and *S. salivar-*

TABLE 1. Strains studied, listed according to the classification resulting from this study

Study reference no.	Received as:	Isolated from:	Source, strain designation(s), and taxonomic status ^a	G+C content (mol%) ^b	Reference
<i>S. sanguis</i> biovar 1 SK1 ^T	<i>S. sanguis</i> type I	S.b.e.	ATCC 10556 ^T ; type strain of <i>S. sanguis</i>	46	
SK36, SK48 SK59	<i>S. sanguis</i> <i>Streptococcus</i> sp. group H	Dental plaque, human S.b.e.	NCTC 7863 (= ATCC 10556)	46	27
SK72, SK75, SK76, SK77, SK78, SK85	<i>S. sanguis</i>	Dental plaque, human			33
SK104 SK108, SK118, SK119	<i>S. oralis</i> <i>S. sanguis</i>	Oral cavity, human Initial dental plaque, human	Sneath, PB177		3
<i>S. sanguis</i> biovar 2 SK4 SK115, SK156, SK157, SK158, SK164	<i>Streptococcus</i> sp. <i>S. sanguis</i>	Dental plaque, human	Carlsson, 804		33
<i>S. sanguis</i> biovar 3 SK150, SK159, SK160, SK161, SK162, SK163	<i>S. sanguis</i>	Initial dental plaque, human	Nyvad		37
<i>S. sanguis</i> biovar 4 SK45, SK49, SK80 SK46 SK112	<i>S. sanguis</i> <i>S. sanguis</i> <i>S. sanguis</i>	Dental plaque, human Buccal mucosa, human Dental plaque, human			27 27 33
<i>S. gordonii</i> sp. nov. biovar 1 SK6 SK120, SK121 SK123	<i>S. oralis</i> <i>Streptococcus</i> sp.	Lancefield group H Oral cavity, human	ATCC 12396 (Lancefield F90A, Hare strain Perryer) Sneath, PB179 (SK121 is a colonial variant of SK120) Our isolate	42	3
<i>S. gordonii</i> sp. nov. biovar 2 SK3 ^T SK5 SK7 SK60 SK86 SK183 SK184, SK188, SK190	<i>S. sanguis</i> type I-II <i>Streptococcus</i> sp. group H <i>S. sanguis</i> <i>Streptococcus</i> sp. group H <i>S. sanguis</i> <i>S. sanguis</i>	S.b.e. S.b.e. S.b.e. Dental plaque, human	ATCC 10558 ^T ; type strain of <i>S. gordonii</i> sp. nov. NCTC 10231 (Blackburn) Challis NCTC 7865 (= ATCC 10558) Burroughs Wellcome Ltd. (CN2814) Handley, FT2, MJ2, Rosan M5	41 42 41 41	13 33
<i>S. gordonii</i> sp. nov. biovar 3 SK8, SK9 SK11, SK12, SK17, SK33, SK83 SK51 ^T SK186	<i>S. sanguis</i> <i>S. sanguis</i> <i>S. mitis</i> <i>S. sanguis</i>	Oral cavity, human Oral cavity, human Dental plaque, human	W. Liljemark (S7, S18) Our isolates NCTC 3165 ^T ; type strain of <i>S. mitis</i> Handley, LGR2	39	
<i>S. gordonii</i> sp. nov. aberrant strains SK42, SK44 SK43	<i>S. sanguis</i> <i>S. sanguis</i>	Dental plaque, <i>Macaca fascicularis</i> Dental plaque, human			27
<i>S. oralis</i> SK2 SK10 SK23 ^T SK92, SK100, SK105, SK107, SK109, SK111, SK134 SK122 SK133 SK140 SK143 SK144 SK146 SK153, SK155 SK225	<i>S. sanguis</i> type II <i>S. sanguis</i> type II <i>S. oralis</i> <i>Streptococcus</i> sp. <i>Streptococcus</i> sp. I:A <i>Streptococcus</i> sp. I:A <i>Streptococcus</i> sp. I:A <i>Streptococcus</i> sp. V:A <i>Streptococcus</i> sp. V:A <i>Streptococcus</i> sp. <i>S. oralis</i>	S.b.e. S.b.e. Oral cavity, human Dental plaque, human Oral cavity, human Oral cavity, human Oral cavity, human Oral cavity, human S.b.e. <i>S. oralis</i>	ATCC 10557 (= NCTC 7864) NCTC 7864 (= ATCC 10557) Sneath, PB182 ^T (= NCTC 11427 ^T); type strain of <i>S. oralis</i> Nyvad Carlsson, BU174 Carlsson, HPA1 Carlsson, MDP1 Carlsson, OTF1 Carlsson, OP51 Gutschik DSM 20066	42 42 41	33 37 5 5 5 5 5 30
<i>S. oralis</i> aberrant strains SK38, SK39	<i>Streptococcus</i> sp.	Oral cavity, human			27

Continued on following page

TABLE 1—Continued

Study reference no.	Received as:	Isolated from:	Source, strain designation(s), and taxonomic status ^a	G+C content (mol%) ^b	Reference
<i>S. mitis</i> biovar 1					
SK24	<i>Streptococcus</i> sp. group O		NCTC 8029		
SK113	<i>Streptococcus</i> sp. viridans type	Sputum	NCTC 10712 (= FW75)	40	
SK135	<i>Streptococcus</i> sp. V:A	Oral cavity, human	Carlsson, FW103	40	
SK137, SK138, SK139, SK141	<i>Streptococcus</i> sp.	Dental plaque, human	Nyvad		37
SK142	<i>Streptococcus</i> sp. V:A	Oral cavity, human	Carlsson, NS51 (= NCTC 12261); proposed neotype of <i>S. mitis</i>	41	5
SK145	<i>Streptococcus</i> sp. V:A	Oral cavity, human	Carlsson, OS51	40	5
SK226	<i>Streptococcus</i> sp. group O		Hahn (Kiel C119/48)	39	30
<i>S. mitis</i> biovar 2					
SK21	<i>Streptococcus</i> sp.		SSI 6235/64		
SK34, SK71, SK79, SK90, SK95, SK96, SK102, SK103	<i>Streptococcus</i> sp.	Oral cavity, human	Our isolates		
SK132	<i>S. mitis</i>	Ulcerated sore throat	ATCC 903	39	
SK147, SK148, SK149	<i>Streptococcus</i> sp.	Dental plaque, human	Nyvad		37
<i>S. mitis</i> aberrant strains					
SK84, SK110		Dental plaque, human			33
<i>S. anginosus</i>					
SK14, SK47	<i>S. milleri</i>		Our isolates		
SK16	<i>S. anginosus</i>		HF60R		
SK18	<i>S. milleri</i>		NCTC 10708		
SK19	<i>S. anginosus</i>		NCTC 8037		
SK22	<i>S. milleri</i>		SSI 1986/64		
SK52 ^T	<i>S. anginosus</i>		NCTC 10713 ^T ; type strain of <i>S. anginosus</i>	38	
SK53 ^T	<i>S. constellatus</i>		ATCC 27823 ^T ; type strain of <i>S. constellatus</i>		
SK54 ^T	<i>S. intermedius</i>		ATCC 27335 ^T ; type strain of <i>S. intermedius</i>	38	
SK55	<i>S. oralis</i>		Sneath, PB183		
SK57	<i>S. mitis</i>		ATCC 9895		
SK61	<i>S. milleri</i>		NCTC 10709		
SK63, SK64, SK65, SK66, SK73, SK81, SK87, SK88, SK89, SK154		Dental plaque, human			33
SK98	<i>S. oralis</i>		Sneath, PB187		
SK126	<i>Streptococcus</i> sp. group F		NCTC 10714		
<i>S. salivarius</i>					
SK13	<i>S. salivarius</i>	Human tongue	Our isolate		
SK25	<i>S. salivarius</i>		NCTC 8606	41	
SK26	<i>S. salivarius</i>		Kelstrup, Tove Ru		
SK32, SK40	<i>S. salivarius</i>	Buccal mucosa, human			27
SK56 ^T	<i>S. salivarius</i>	Cardioarthritis	NCTC 8618 ^T ; type strain of <i>S. salivarius</i>	41	
SK58	<i>S. salivarius</i>		ATCC 9759		
SK99, SK101	<i>S. salivarius</i>	Dental plaque, human			33
SK128	<i>S. salivarius</i>	Tongue, human			
<i>S. salivarius</i> aberrant strains					
SK35		Buccal mucosa, human			27
SK74		Dental plaque, human			33
<i>S. mutans</i>					
SK27	<i>S. mutans</i>	Dental plaque, human	Our isolate		
SK28 ^T	<i>S. mutans</i>	Dental plaque, human	NCTC 10449 ^T ; type strain of <i>S. mutans</i>	37	
SK30	<i>S. mutans</i>	Dental plaque, human	Gibbons, GS5		
SK62, SK68, SK69, SK70, SK91, SK93, SK97	<i>S. mutans</i>	Dental plaque, human			33

^a ATCC, American Type Culture Collection, Rockville, Md.; Carlsson, J. Carlsson, University of Umea, Umea, Sweden; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Federal Republic of Germany; Gibbons, R. J. Gibbons, Forsyth Dental Center, Boston, Mass.; Gutschik, E. Gutschik, Department of Clinical Microbiology, Bispebjerg Hospital, Copenhagen, Denmark; Hahn, G. Hahn, Institut für Hygiene, Kiel, Federal Republic of Germany; Handley, P. Handley, University of Manchester, Manchester, United Kingdom; Kelstrup, J. Kelstrup, Royal Dental College, Aarhus, Denmark; NCTC, National Collection of Type Cultures, Colindale, United Kingdom; Nyvad, B. Nyvad, Royal Dental College, Aarhus, Denmark; Sneath, P. H. A. Sneath, University of Leicester, Leicester, United Kingdom; SSI, Streptococcal Department, Statens Seruminstitut, Copenhagen, Denmark.

^b G+C values from references 12, 13, 30, and 48.

TABLE 2. Streptococcal strains used for the production of antisera

Group, type, or species designation	Strain
Group C	ATCC 12388
Group F	SSI H127
Group G	ATCC 12394
Ottens type II	H189
Ottens type III	MG216
Group H	Blackburn (= SK5)
Group H (F90A; American group H)	ATCC 12396 (= SK6)
" <i>S. mitior</i> "	ATCC 10557 (= SK2)
Group K (Levy)	NCTC 10232
Group K	CN477
Group O	2/65 Jelinkova
" <i>S. milleri</i> "	SSI 14132/70
" <i>S. milleri</i> "	SSI MG2 1132
<i>S. mutans</i> type c	NCTC 10449
<i>S. salivarius</i>	ATCC 13419
<i>S. salivarius</i>	NCTC 8606

ius and antisera against the five Ottens-Winkler antigens, were used to examine each strain (38). Those antisera that gave positive reactions with the strains which we studied are shown in Table 2; the labels of the sera are listed together with the designations of the strains used for immunization.

Antisera were prepared by immunizing rabbits with streptococci grown in Todd-Hewitt broth for about 18 h at 36°C. The cultures were killed by heating them at 60°C for 30 min, washed three times in saline, and stored in saline at a concentration of 1:100 of the original volume, with 0.5% Formalin added as preservative. The concentration of bacteria used for immunization was either 4×10^9 or 8×10^9 organisms per ml, and the course of immunization was either 4 weeks or 2 to 3 weeks, respectively. The animals were given three successive injections of 0.5 ml the first week and three injections of 1 ml the following weeks. The animals were not bled until a strong capillary precipitation reaction had developed. Bleeding generally took place 5 to 6 days after the last injection.

RESULTS

Cultures. During the initial phases of this study, it became evident that many of the cultures examined yielded biochemical reactivity patterns that appeared to be intermediate between the patterns shown by defined taxa. Repeated attempts to check the purity of such cultures by serial subcultivation revealed that two different strains could be isolated only after up to five subcultures on Todd-Hewitt agar. Thus, several cultures, including "strains" received from other laboratories, initially consisted of two different strains. Consequently, all of the strains included in the study were purified by at least five serial subcultures on Todd-Hewitt agar.

Even after careful purification, cultures on Todd-Hewitt agar often showed a mixture of colony types that could not be separated by subculturing; i.e., they were not stable. Biochemical examination of individual cultures based on different colony types revealed identical results.

No distinct colonial morphology on Todd-Hewitt agar could be associated with the individual taxa in this study. However, in most cases, species-associated differences in colonial morphology on mitis salivarius agar were evident (Table 3).

Biochemical tests. In a pilot experiment, 18 strains that were later assigned to *S. sanguis* were tested for the ability to hydrolyze esculin both in the medium described above and in a medium in which tryptic soy broth was substituted for Trypticase peptone. While 12 of the strains showed a positive reaction in the medium containing Trypticase peptone, only 3 strains were positive in the medium containing tryptic soy broth. In the final examination of strains the medium containing Trypticase peptone was used.

Several glycoside hydrolases were determined by both the API ZYM and API ZYM osidase kits. However, as shown in Table 3, the two kits did not produce identical results. For example, the majority of the *S. sanguis* strains were recorded as negative for β -D-glucosidase and β -D-galactosidase activities when they were examined by using the API ZYM kit, whereas the majority of strains were positive when they were examined by using the API ZYM osidase kit (Table 3). Furthermore, in several cases in which the API ZYM kit yielded weak and often poorly reproducible reactions (e.g., α -D-galactosidase), the reactions obtained with the API ZYM osidase kit were generally strong and produced more consistent results within the individual taxa.

Generally weak, indistinct, and poorly reproducible reactions were observed in the tests for esterase (API ZYM kit), esterase lipase (API ZYM kit), cystine aminopeptidase (API ZYM kit), trypsin (API ZYM kit), PNP- β -D-galacturonohydrolase (API ZYM osidase kit), and α -L-arabinosidase (API ZYM osidase kit). These reactions are not included in the tables.

None of the strains included in the study were inhibited by optochin, had detectable lysine or ornithine decarboxylase activity, fermented glycerol, dulcitol, L-(-)-sorbitol, or rhamnose, or exhibited α -mannosidase, α - or β -xylosidase, N-acetyl- α -D-glucosaminidase, α -fucosidase (API ZYM kit), or β -L-fucosidase (API ZYM osidase kit) activity. All of the strains fermented D-(+)-glucose, D-(-)-fructose, and D-(-)-mannose.

The pH values of uninoculated fermentation media were 7.2 to 7.4. When tests were recorded as positive, the final pH values ranged from 4.0 to 5.0, although occasional strains showed final pH values for single carbohydrates of 5.6 to 5.9. Exceptions to this pattern were strains assigned to *S. oralis*, which consistently produced final pH values of 5.6 to 6.2 in the melibiose medium. Strains assigned to other species which were recorded as positive in this medium produced final pH values of 4.3 to 5.2. The final pH values measured in glucose broth were very consistent within individual species, with deviations of only ± 0.2 U from the calculated mean (Table 3).

Characteristics of the individual taxa. The biochemical and serological characteristics of the strains which we classified are shown in Table 3. To calculate the data in the table, strains examined in duplicate that were obtained from two different sources (American Type Culture Collection, Rockville, Md., and National Collection of Type Cultures, Central Public Health Laboratory, London, England) were included only once as the duplicates always gave identical results. Strains that we considered aberrant are not included in Table 3 but are discussed individually below. The results in Table 3 are presented according to the revised classification resulting from this study. Although the names used in the tables and in the descriptions of taxa below to some extent anticipate the final taxonomic discussion (and one case is pending the response of the Judicial Commission to our proposed rejection of the present type strain of *S. mitis* [28]), it makes the presentation easier to follow.

Enzymes ^c	0/23 ^f	0	0	0	100	100	100	100	89/11	60	54/31	60/40	67/33	0
Alkaline phosphatase (z)	100	0	0	0	100	100	100	100	100	70	85	80/20	100	0/20
Acid phosphatase (z)	100	0	0	0	100	100	100	100	100	100	100	100	100	0
Leucine aminopeptidase (z)	100	100	100	100	100	100	100	100	100	100	100	100	100	0
Valine aminopeptidase (z)	100	83	100	100	100	100	100	100	100	100	100	80/20	83/13	20/80
Chymotrypsin (z)	57/8	100	100	100	100	89/11	100	100	100	100	100	90/10	79/21	70/30
Phosphoamidase (z)	0	0/100	0	0/80	60/40	0	0	0	0/72	0/80	0/54	80/10	13/63	0/100
β-D-Glucosidase (z)	0	0	0	80	100	56/22	44/66	0	0	0	0	80/10	25/25	100
β-D-Glucosidase (o)	100	100	33/67	100	100	100	100	0	0	0	92	90/10	75/17	100
β-D-Galactosidase (z)	100	0	0	0	0	44/44	11/67	94	20/40	0	69/31	50	29/21	0
β-D-Galactosidase (o)	100	0/100	0/83	100	100	100	100	100	50/40	100	100	60	33/46	100
α-D-Glucosidase (z)	0	0	0	0	0/75	67/33	22/67	100	60	100	100	60/40	63/25	100
α-D-Glucosidase (o)	0	0	0	0	0	78/22	56/44	100	60	100	100	90/0	67/13	100
α-D-Galactosidase (z)	54/31 ^c	0/100	67/17	0	75/25	0	0	17/28	80	77/16	0	0	13	20/80
α-D-Galactosidase (o)	94/0 ^f	0/100	100	0	100	67/0	0	33/33	80	100	100	0	13	100
β-Glucosaminidase (z)	0	0	0	0	100	100	100	100	100	0	100	0	17/29	0
N-acetyl-β-D-glucosaminidase (o)	100	0	0/100	100	100	100	100	100	80/20	0	100	0	38/46	0
α-Maltosidase (o)	100	100	50	100	100	100	100	94/6	0	100	100	100	54/21	0/50
β-Maltosidase (o)	0	0	0	0	0	0	0	0	0	0	0	0/30	8	0
β-Mannosidase (o)	0	0	0	0	100	100	56/33	0	0	0	0	0	33/8	0
α-L-Fucosidase (o)	0	0	0	0	100	100	100	0	0	0	0	0	8	0
β-D-Fucosidase (o)	92	0	0	0	0	0	0	39/56	0/20	0	38/31	20	4	0
β-D-Glucuronidase (z)	0	0	0	0	0	0	0	0	0	0	0	0	4	0
β-D-Glucuronidase (o)	0	0	0	0	0	0	0	0	0	0	0	0	4	0
β-D-Lactosidase (o)	15/38	0	0	40/60	100	100	100	0	0	0	46/38	60/0	17/17	100
α-L-Arabinosidase (o)	0	0	0	0	0	0	0	0	0	0	0	0	8	0
Phospho-β-galactosidase (o)	23/62	0	0	0/80	25/75	88/12	78/22	0/50	0	0	62/15	0	13/17	0/40
Reactions with antisera														
Blackburn (group H)	77	100	100	0	0	89	0	0	0	0	0	0	0	0
F90A (American group H)	0	0	0	0	25	0	22	0	0	0	0	40	0	0
ATCC 10557	0	0	0	100	75	0	78	50	0	0	0	0	0	0
SSI 14132 ("S. milleri")	0	50	100	0	0	33	0	0	0	0	0	0	4	0
Levy (group K)	0	0	0	0	0	0	0	0	0	0	46	0	0	0
CN477 (group K)	0	0	0	0	0	0	0	0	0	0	31	0	0	0
NCTC 8606 (S. salivarius group K)	0	0	0	0	0	0	0	0	20	15	0	30	0	0
ATCC 13419 (S. salivarius)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group C	0	0	0	0	0	0	0	0	0	0	0	0	13	0
Group G	0	0	0	0	0	0	0	0	0	0	0	0	17	0
Group F	0	0	0	0	0	0	0	0	0	0	0	0	33	0
Group O	0	0	0	0	0	0	0	0	90	0	0	0	0	0
Ottens type II	0	0	0	0	0	0	0	0	0	0	0	0	8	0
Ottens type III	0	0	0	0	0	0	0	0	0	0	0	0	8	0
MG2	0	0	0	0	0	0	0	0	0	0	0	0	4	0
S. mutans (serotype c, NCTC 10449)	0	0	0	0	0	0	0	0	0	0	0	0	0	100

^a n is the number of strains tested.

^b The mean terminal pH values in glucose medium were as follows: *S. sanguis* (all biovars), 4.7; *S. gordonii* biovar 1, 4.3; *S. gordonii* biovar 2, 4.4; *S. gordonii* biovar 3, 4.3; *S. oralis*, 4.5; *S. mitis* biovar 1, 4.6; *S. mitis* biovar 2, 4.4; *S. salivarius*, 4.1; *S. anginosus*, 4.2; *S. mutans*, 4.0.

^c The reaction of the type strain differed from the reaction of the majority of the strains.

^d The majority of the fermentation reactions were weaker than the reactions with the other carbohydrates (pH 5.6 to 6.2).

^e (z), Determined with an API ZYM kit; (o), determined with an API ZYM osidase kit.

^f Percentage of strong positive reactions (values of 3 to 5)/percentage of weak positive reactions (values of 1 or 2).

S. sanguis. A total of 31 strains were assigned to *S. sanguis*. On the basis of biochemical and serological differences, this species could be subdivided into four biovars, which are characterized as shown in Table 3. Biovar 1 included the type strain of *S. sanguis*, strain ATCC 10556 (= NCTC 7863). However, as shown in Table 3, the characteristics of the type strain differed in several ways from the majority reactions of the taxon. Only 1 of the 31 strains assigned to *S. sanguis* failed to produce detectable extracellular polysaccharide in 5% sucrose broth. This strain, strain SK118, also differed from other strains assigned to biovar 1 by fermenting sorbitol but not esculin.

With the exception of three strains of biovar 1 and five strains of biovar 4, all of the strains assigned to *S. sanguis*, including the aberrant strain, reacted with group H (Blackburn) antiserum. All five biovar 4 strains reacted with the antiserum against "*S. mitior*" ATCC 10557. Besides reacting with the group H (Blackburn) antiserum, the majority of the strains assigned to biovars 2 and 3 reacted with "*S. milleri*" SSI 14132/70 antiserum.

Streptococcus gordonii sp. nov. The 25 strains assigned to *Streptococcus gordonii* sp. nov. possessed many of the properties that are considered characteristic of *S. sanguis*, such as the ability to hydrolyze arginine and esculin and the ability to produce extracellular polysaccharide. However, they differed from *S. sanguis* strains in lacking IgA1 protease activity, in their ability to ferment amygdalin, and in having β -glucosaminidase, β -mannosidase, α -L-fucosidase, and strong alkaline phosphatase activities (Table 3). As discussed below, the results of DNA base composition and DNA-DNA homology studies support the point of view that this group warrants specific taxonomic status.

S. gordonii could be subdivided into three biovars on the basis of the biochemical and serological reactivities of the strains (Table 3). One of these biovars (biovar 3) included the designated type strain of *S. mitis*, strain SK51 (= NCTC 3165). However, this strain differed from all of the other strains assigned to the species by lacking extracellular polysaccharide production and by exhibiting a strong β -galactosidase reaction when the API ZYM kit was used. The taxonomic problems raised by this strain are discussed below.

Eight of the nine strains of biovar 2 reacted with group H (Blackburn) antiserum, and three showed an additional reaction with "*S. milleri*" SSI 14132/70 antiserum. With one exception, all of the other strains reacted either with the ATCC 10557 antiserum or with the American group H (F90A) antiserum.

Three strains (strains SK42, SK43, and SK44) were considered aberrant strains of *S. gordonii*. Two of these, strains SK42 and SK44, were isolated from dental plaque of *Macaca fascicularis* monkeys. These three strains differed from other strains assigned to *S. gordonii* by exhibiting IgA1 protease activity (except strain SK43) and by lacking β -glucosaminidase, β -mannosidase, α -L-fucosidase, and alkaline phosphatase activities. Several of these properties are characteristic of *S. sanguis*. However, strains SK42, SK43, and SK44 differed from strains of *S. sanguis* by fermenting amygdalin, by exhibiting β -D-lactosidase activity, and by lacking α - and β -galactosidase and acid phosphatase activities when the API ZYM kit was used. All three strains reacted in group H (Blackburn) antiserum. Although these strains appeared to be phenotypically intermediate between *S. gordonii* and *S. sanguis*, a genetic analysis of one of them (strain SK42) revealed that it is closely related to the former species but not to the latter species (20).

S. oralis. A total of 21 strains were assigned to *S. oralis*. These strains included the type strain of *S. oralis* (strain NCTC 11427) and several other reference strains previously designated "*S. sanguis* II" or "*S. mitior*" (Table 1). The majority of the strains produced extracellular polysaccharide, and all exhibited both IgA1 protease and neuraminidase activities. A detailed description of 18 of the strains is provided in Table 3; 9 of these 18 strains, including the type strain, reacted in the antiserum raised against strain ATCC 10557. The remaining strains were negative in all antisera. Two strains (strains SK38 and SK39), which were considered aberrant, differed from all of the other strains of the species by lacking β -glucosaminidase, β -galactosidase (API ZYM kit), and alkaline phosphatase activities.

S. mitis. A total of 25 strains were tentatively assigned to *S. mitis* (see below). This species was subdivided in two groups, designated biovar 1 and biovar 2. None of the strains produced extracellular polysaccharide. The two biovars were separated by having different reactions in tests for arginine hydrolysis, β -glucosaminidase, β -glucosidase, and α -mannosidase (Table 3). The detailed biochemical characteristics are shown in Table 3. Two strains tentatively assigned to biovar 1 (strains SK135 and SK136) exhibited both IgA1 protease and neuraminidase activities.

All but one of the strains assigned to biovar 1 reacted with the group O antiserum, and two, in addition, reacted with the antiserum raised against *S. salivarius* NCTC 8606. The majority of the biovar 2 strains reacted with one of the two group K antisera (Levy and CN477). One strain, strain SK132 (= ATCC 903), reacted with both of these antisera, as well as with the NCTC 8606 and Ottens type III antisera, and one strain reacted with the NCTC 8606 antiserum only.

The four strains assigned to biovar 2, including strain SK132 (= ATCC 903), which reacted with the CN477 group K antiserum, may warrant recognition as a separate biovar as they differed from other strains of biovar 2 by lacking neuraminidase and β -D-fucosidase activities and by fermenting sorbitol.

Likewise, the exact classification of two strains (strains SK84 and SK110) listed as aberrant will require further study. In contrast to the strains of biovar 2, these two strains did not hydrolyze arginine but did hydrolyze esculin and fermented trehalose. They otherwise possessed the majority of the reactions of *S. mitis* biovar 2 (Table 3), including neuraminidase activity.

S. salivarius. The 10 strains assigned to *S. salivarius* included the type strain of *S. salivarius*, strain NCTC 8618 (= SK56), and two other reference strains of *S. salivarius* (Table 1). This group of strains was relatively homogeneous and was well separated from the other taxa (Table 3). The majority of the strains reacted with antiserum against either American group H (F90A) or *S. salivarius* NCTC 8606. Two additional strains assigned to this species were considered aberrant. Strain SK35 differed from other strains in lacking the ability to ferment *N*-acetylglucosaminic acid, in fermenting melibiose and raffinose, in exhibiting a strong β -D-fucosidase reaction, and in lacking alkaline and acid phosphatase, valine aminopeptidase, and phosphoamidase activities. It reacted with the American group H (F90A) antiserum. The other strain (strain SK74) was the only strain in the collection that was capable of hydrolyzing hippurate. Furthermore, it differed from the other strains by lacking the ability to ferment *N*-acetylglucosaminic acid, by exhibiting β -mannosidase and β -glucuronidase activities, and by lacking α -maltosidase activity. It reacted with ATCC 13419 antiserum.

S. anginosus. The 24 strains tentatively assigned to *S. anginosus* included the type strains of *S. anginosus*, *S. constellatus*, and *S. intermedius*, several reference strains of "*S. milleri*," *S. mitis* ATCC 9895, and two strains classified as *S. oralis* by Bridge and Sneath (3) (Table 1). All 24 strains hydrolyzed arginine and esculin, and the majority (71%) produced acetoin. However, as revealed by the data in Table 3, this taxon was a biochemically and serologically very heterogeneous group, and no natural clustering of the 24 strains was found. The strains were separated from strains assigned to *S. sanguis* and *S. gordonii* by their lack of extracellular polysaccharide formation, by their inability to ferment inulin, by their colonial morphology on mitis salivarius agar, and by their serological reactions. None of the strains assigned to *S. anginosus* possessed a group H antigen. In contrast, the majority of the strains reacted with group C, F, or G antiserum and with antiserum to Ottens type II or III (Table 3).

S. mutans. The 10 strains assigned to *S. mutans* formed a homogeneous group with only minor variations in certain biochemical reactions (Table 3). All of the strains reacted in serotype c antiserum. The strains were clearly separated from all other strains included in this study.

Genetic relatedness between and within the species. The strain collection examined in this study was composed to include, as far as possible, strains that previously had been subjected to genetic analyses, such as determinations of DNA base composition and DNA-DNA homology and multilocus enzyme analysis. As the data from these studies formed an important background for the taxonomic decisions resulting from our study, they are summarized below with special emphasis on the species *S. oralis*, *S. mitis*, *S. sanguis*, and *S. gordonii*.

Six of the strains which we assigned to *S. oralis* (strains SK2, SK133, SK140, SK143, SK144, and SK146) and three of the strains assigned to *S. mitis* (strains SK135, SK142, and SK145) were examined in DNA-DNA hybridization experiments (S1 nuclease method) by Coykendall and Munzenmaier (12). Applied to our classification, the results of these authors showed 65 to 100% homology within *S. oralis* and *S. mitis* and 30 to 38% homology between the two species. Values compatible with this level of interspecies relatedness have been reported for *S. mitis* biovar 1 (strain SK226) and *S. oralis* (strain SK23) by Kilpper-Bälz et al. (30) and for *S. mitis* biovar 2 (strain SK132) and *S. oralis* (strain SK2) by Coykendall and Specht (13).

DNA homology data demonstrating the intra- and interspecies relationships of *S. sanguis* and *S. gordonii* as defined in this study have been published for *S. sanguis* SK1, SK4, and SK59 and *S. gordonii* SK3, SK5, SK6, SK51, and SK190 by Coykendall and Specht (13), Schmidhuber et al. (41), and Welborn et al. (47). Applied to our classification, the data from these studies showed 89 to 90% genetic homology within the species and 40 to 60% homology between the two species. Furthermore, all three studies demonstrated 20 to 30% genetic homology between *S. sanguis* and *S. gordonii* on one side and *S. oralis* on the other. These levels of intra- and interspecies relatedness among the three species are further supported by the results of our own genetic analysis of 23 strains from this study in which multilocus enzyme electrophoresis was used (20).

Information on the intra- and interspecies relationships and DNA base compositions based on studies of strains included in this study is summarized in Fig. 1. DNA base compositions compiled from previous studies (12, 13, 48) are included in Table 1 for individual strains.

FIG. 1. Genetic relatedness, DNA base compositions, and cell wall peptidoglycan types of selected *Streptococcus* species defined in this study. The information is compiled from previously published data for strains included in this study (12, 13, 30, 41, 47).

DISCUSSION

Several factors undoubtedly contribute to the difficulties that have been encountered in reaching a satisfactory taxonomy of oral streptococci. It is notoriously difficult to obtain pure cultures of these bacteria, as we experienced in the initial phases of this study. This has probably led several authors to consider oral streptococci a continuum with overlapping species and many intermediate forms (15, 22). Second, many of the biochemical tests used to examine streptococci are not sufficiently optimized for these bacteria, and small alterations in a test may give different results. This is evident when results reported by workers from different laboratories for identical strains are compared. For example, the type strain of *S. sanguis*, strain ATCC 10556 (= SK1), is often reported to be negative for esculin hydrolysis. In our study this strain was found to be clearly positive in the test substrate recommended by Carlsson (4), but negative, along with several other *S. sanguis* strains, if tryptic soy broth was substituted for Trypticase peptone in the medium. Several differences between phenotypic data reported by workers in the laboratory of Schleifer (30, 41), who used API 20 STREP (41) or "conventional tests" (30), also demonstrate the importance of the methods used to determine specific biochemical activities of streptococci. Thus, the type strain of *S. sanguis*, strain ATCC 10556 (= SK1), has been reported to be unable to hydrolyze arginine (30), whereas the type strain of *S. oralis* was found to be positive. Both results are in conflict with data from several other studies, including our study. Other examples, which are relevant for the phenotypic description of *S. oralis*, are discussed below.

Similar discrepancies in the reported abilities of strains to ferment carbohydrates may be due to the use of different pH indicators. To avoid this problem, we measured the final pH in every fermentation test culture after 7 days of incubation. Virtually all of the reactions recorded as positive reached pH values between 4.0 to 5.0, and the values were remarkably consistent within each taxon (Table 3). However, a few characteristic exceptions were found. Thus, the final pH values of *S. oralis* strains in melibiose and of *S. sanguis* biovar 1 strains in starch were consistently between 5.6 and 6.2.

The API kits with chromogenic substrates used to detect glycoside hydrolases in this study also demonstrate the significance of test optimization. API ZYM kits have been used previously in studies of oral and other streptococci (3, 9, 45). However, the reproducibility and diagnostic validity of the results have not been entirely satisfactory. The same problems were observed in this study for some of the reactions. The importance of the substrate used for propagation of the inoculum has been demonstrated previously (9). Another factor is demonstrated by the results obtained in our study, in which we used two different API kits with many overlapping tests. Table 3 shows that the results obtained for identical glycoside hydrolases with the two kits often were quite different. Generally, results obtained with the API ZYM osidase kit were more clear-cut, reproducible, and consistent within individual taxa. The two kits use different chromogenic substrates (i.e., naphtol and nitrophenol derivatives, in the API ZYM and API ZYM osidase kits, respectively), which may influence the results. However, it may also be significant that the inocula used for the API ZYM osidase kit tests were suspended in a buffer at pH 7.5, which is closer to the pH optimum of the relevant enzymes (pH 7.5 to 8.0) (unpublished data) than the pH obtained with the API ZYM kit tests, which used saline-suspended inocula.

Two of the tests used in this study, the IgA1 protease and neuraminidase tests, have not been used previously in streptococcal taxonomy. Both of these tests proved to be highly valuable in supporting the classification presented in Table 3. However, the drawback of the two tests is that they require specialized equipment and are, at the present time, not easily applicable to routine bacteriology.

The individual taxonomic groups resulting from this study are discussed below. The entity previously referred to as "*S. sanguis* II" is a priori excluded from the discussion of *S. sanguis* as "*S. sanguis* II" strains differ from the type strain of *S. sanguis* by multiple biochemical characteristics (5, 11, 24) (Table 3), by growth requirements (6), by cell wall composition (10, 34, 39), by serology (11, 23), by DNA base composition, and by exhibiting only 20 to 40% DNA homology (13, 41). "*S. sanguis* II" is discussed below under *S. oralis*.

S. sanguis. *S. sanguis* is the name given by White and Niven (49) to the viridans type of streptococci that hydrolyze arginine, usually hydrolyze esculin, ferment inulin, salicin, lactose, and trehalose, and produce extracellular dextran from sucrose (26). However, a certain heterogeneity has been suggested as a result of both biochemical and serological studies (29, 39, 49). Most convincingly, Coykendall and Specht (13) demonstrated two separate genotypes with only 30 to 60% DNA homology and significantly different DNA base compositions (guanine-plus-cytosine [G+C] contents, 44 to 46 and 41 to 43 mol%). The names "*S. sanguis* subsp. *carlssonii*" and "*S. sanguis* subsp. *sanguis*" were applied to the two genotypes, but because of a lack of correlating phenotypic differences this subdivision has never been practically employed. Our study revealed several phenotypic traits that clearly differentiate two major clusters, which, as noted above, contained several strains representing the genotypes described by Coykendall and Specht. The two clusters clearly warrant specific recognition (see below). The cluster that includes the type strain of *S. sanguis*, strain ATCC 10556 (= NCTC 7863) (Table 3), must retain the name *S. sanguis*. *S. sanguis*, thus defined, correlates with *S. sanguis* subsp. *carlssonii* of Coykendall and Specht.

S. sanguis sensu stricto was divided into four taxa that

were separated by several biochemical and serological characteristics. We provisionally label these taxa biovars 1 through 4. The type strain was assigned to biovar 1. Paradoxically, this strain was unique among the *S. sanguis* strains because it lacked the ability to ferment several carbohydrates typical of the species (Table 3). The majority of the *S. sanguis* strains reacted with the group H (Blackburn) antiserum. However, it is interesting that none of the strains reacted with the American group H (F90A) antiserum. The five strains assigned to biovar 4 were exceptional in reacting with antiserum against *S. oralis* ATCC 10557 ("*S. mitior*") but not with any of the group H antisera and by forming long chains (Table 3). Also, strains of biovar 4 have been shown to differ from other *S. sanguis* strains by possessing a distinct IgA1 protease type (Reinholdt et al., in preparation) and may belong to a separate species.

S. gordonii sp. nov. The cluster excluded from *S. sanguis* included strains previously designated "*S. sanguis* subsp. *sanguis*" by Coykendall and Specht (13). Furthermore, it contained strain NCTC 3165 (= SK51), which is the designated type strain of *S. mitis* (43). This strain differed from all other strains of *S. gordonii* by lacking detectable extracellular dextran, and we previously demonstrated a genetic distance of 20% (Gower distance coefficient) between this strain and other representative strains of *S. gordonii* based on multilocus enzyme electrophoresis (20). In our opinion, it would clearly cause confusion to apply the name *S. mitis* to this group of organisms which is characterized by arginine and esculin hydrolysis, inulin fermentation, and production of extracellular polysaccharide, and it would be in conflict with the original description of that species (1). Pending the response of the Judicial Commission to our request to replace the type strain of *S. mitis* (28), we propose the name *S. gordonii* in honor of the British bacteriologist Mervyn H. Gordon, who apparently was the first researcher to apply fermentation tests to the classification of viridans streptococci in a comprehensive way (21). Previously published DNA-DNA hybridization data on five strains which we assigned to *S. gordonii* (see above) show that it is a homogeneous taxon. It is clearly distinct from *S. sanguis* based on the following data: it exhibits only 40 to 60% DNA homology with *S. sanguis* (Fig. 1); it has a significantly lower G+C content than *S. sanguis* (42 versus 46 mol%) (13); it ferments amygdalin; it has alkaline phosphatase and β -glucosaminidase activities; and it lacks IgA1 protease activity (Table 3). Further genetic evidence to support the separation of the two species was recently obtained by a multilocus enzyme analysis of several representative strains from this study (20).

The three taxa defined within *S. gordonii* differ biochemically and serologically. Whereas the majority of strains assigned to biovars 1 and 3 reacted with the antiserum against *S. oralis* ATCC 10557 ("*S. mitior*") and some reacted with American group H (F90A) antiserum, most of the strains of biovar 2 reacted with group H (Blackburn) antiserum. Together with some of our findings for group K strains (see below), these results confirm and extend previous observations (7, 11) that streptococcal group antigens may be shared by separate species. Based on results published by Rosan (40) and Cole et al. (7) for strains common to their studies and our study, it appears that *S. gordonii* biovar 2 strains contain all of the antigens (antigens a through e) defined by Rosan (40), as does the type strain of *S. sanguis* (strain ATCC 10556). *S. gordonii* biovar 1 strain SK6 (= F90A) was previously found to possess antigens a and e, whereas *S. gordonii* biovar 3 strain SK51 (= NCTC 3165)

was found to possess antigens a, c, and d (7). The latter finding explains the reaction of *S. gordonii* biovar 3 strains with ATCC 10557 antiserum (*S. oralis*/"*S. mitior*") as strain ATCC 10557 has been found to possess antigens c and d (7). According to Rosan (40), antigen a is the group H antigen. Thus, it appears that all *S. gordonii* biovars possess the group H antigen, although only biovar 2 strains reacted with the Blackburn serum.

S. oralis. *S. oralis* is the name applied by Bridge and Sneath (2) to one of their clusters resulting from a numerical taxonomic study of streptococci (3). As defined by these authors, this taxon appears to be heterogeneous because representative strains from their study were assigned in this study to widely different species, including *S. oralis*, *S. sanguis*, *S. gordonii*, and *S. anginosus*. The taxon resulting from our study, which included the type strain of *S. oralis*, was characterized by the production of extracellular polysaccharide by most strains, by neuraminidase and IgA1 protease activities, and by a lack of arginine and esculin hydrolysis and inulin fermentation; 9 of 18 strains, including the type strain, reacted with the antiserum against ATCC 10557, which is consistent with the fact that this strain and several other strains previously referred to as "*S. mitior*" or "*S. sanguis* II" were included in the species. DNA-DNA hybridization data for six of the strains which we included in *S. oralis* (see above) confirm that this species is genetically homogeneous and well separated from other species, including *S. mitis* (Fig. 1).

Kilpper-Bälz et al. (30) have published an emended description of *S. oralis* based on data resulting from their own study and data reported by Bridge and Sneath (2). However, there are several significant discrepancies between the results of these authors and our results. Thus, both the type strain of *S. oralis*, strain NCTC 11427 (= SK23), and *S. oralis* DSM 20066 (= SK225) were found to be positive for arginine hydrolysis and acetoin production, and strain DSM 20066 was also found to be positive for esculin hydrolysis by Kilpper-Bälz et al. (30). We were unable to confirm these data, which results in *S. oralis* being phenotypically more homogeneous than reported by Kilpper-Bälz et al. (30). Furthermore, we found that none of the strains exhibits lysine decarboxylase activity as previously reported for *S. oralis* (3). On the basis of our results an emended description of *S. oralis* is presented below.

S. mitis. The name *S. mitis* was used by Andrewes and Horder (1) to designate streptococci which are rarely pathogenic, grow in short chains, and usually fail to ferment inulin and raffinose. Because of the lack of sufficient detail in this description, the definition of *S. mitis* is usually based on the description provided by Sherman et al. (42). Although, these authors noted that "*S. mitis* forms a rather heterogeneous and perhaps complex group," they found that it was well separated from other viridans species by the inability of any culture out of 147 studied to ferment inulin or to synthesize polysaccharide from sucrose. That the *S. mitis* group may contain more than one distinct unit was indicated by the fact that an appreciable proportion of the strains of this group hydrolyzed arginine with the production of ammonia (42). These characteristics concur with the definition of *S. mitis* used by Carlsson (5), Facklam (17), and workers in several other laboratories.

The use of the name *S. mitis* for the two clusters defined in Table 3 is in complete agreement with the definitions used by Sherman et al. (42), Carlsson (5), and Facklam (17). However, the formal acceptability of this name is pending the response of the Judicial Commission to our request to reject

the present type strain of *S. mitis*, strain NCTC 3165 (= SK51) (28); we propose to replace this strain with strain NCTC 12261.

S. oralis and *S. mitis*, as defined here, are related by having a cell wall composition that is distinct from that of *S. sanguis*. In contrast to *S. sanguis*, the cell walls of *S. oralis* and *S. mitis* (biovars 1 and 2) lack glycerol teichoic acid and significant amounts of rhamnose and contain a ribitol teichoic acid (10, 11, 33, 39). Accordingly, *S. oralis* and *S. mitis* were grouped together as "*S. mitior*" by Colman and Williams (11); however, the subsequent study of Coykendall and Munzenmaier (12), which included several strains which we classified as *S. oralis* and *S. mitis*, respectively, demonstrated that these two species exhibit only 40 to 60% DNA homology. As demonstrated in Table 3, the two species are also well separated by biochemical and serological differences and furthermore have different ecological properties (Kilian, manuscript in preparation). As the study of Coykendall and Munzenmaier (12) did not contain representatives of *S. mitis* biovar 2, further studies will be required to define definitively the correct affiliation of this taxon.

S. anginosus. The taxon that contained the type strain of *S. anginosus* also encompassed the type strains of *S. intermedius* and *S. constellatus* and several strains labeled "*S. milleri*." The 24 strains formed a phenotypically heterogeneous group, and no natural subdivisions were possible on the basis of the data. This finding confirms conclusions from several previous studies that many strains of *S. anginosus*, *S. constellatus*, and *S. intermedius* are closely related to each other genetically in spite of phenotypic heterogeneity (14, 31, 46). Therefore, we followed the recent proposal by Coykendall et al. (14) to use the designation *S. anginosus* for the whole group of bacteria because of the priority of that name. In the present study *S. anginosus* could be separated from *S. sanguis* by a lack of extracellular polysaccharide and IgA1 protease activity, by differences in colonial morphology, by the inability to ferment inulin, by a positive Voges-Proskauer reaction in most strains, and by serological reactivity (Table 3). Clearly, further studies will be required to explain the taxonomically unnatural situation surrounding this group of bacteria.

S. salivarius. The taxon that contained the type strain of *S. salivarius* formed a relatively homogeneous group which was well separated from other species. The 10 strains possessed the typical characteristics of *S. salivarius*, including extracellular levan production. Thus, none of the strains appears to be related to the newly described species *S. vestibularis* (48).

S. mutans. The 10 strains assigned to *S. mutans* included the type strain and pose no taxonomic problems. Strains of other mutans species were not included in the study.

Table 4 lists the phenotypic characteristics that are most useful for distinguishing the streptococcal species and their biovars as defined here. The characteristics included in this table are those that were found to be constant and most reproducible in tests.

Table 5 correlates the classification presented here with designations used previously in the literature. This table is based on previously published information concerning reference strains common to the individual studies.

Description of *Streptococcus gordonii* sp. nov. Cells are gram-positive cocci that grow in short chains in serum broth. They are nonmotile, nonsporing, aerobic and facultatively anaerobic, fermentative, and catalase negative and show α -hemolysis on horse blood agar and pronounced greening on chocolate agar. The cell wall contains glycerol teichoic

TABLE 4. Differential characteristics of the species

Characteristic	<i>S. sanguis</i>				<i>S. gordonii</i>			<i>S. oralis</i>	<i>S. mitis</i>		<i>S. salivarius</i>	<i>S. anginosus</i>	<i>S. mutans</i>
	Biovar 1	Biovar 2	Biovar 3	Biovar 4	Biovar 1	Biovar 2	Biovar 3		Biovar 1	Biovar 2			
Arginine hydrolysis	+	+	+	+	+	+	+	—	—	+	—	+	—
Esculin hydrolysis	+	—	+	+	+	+	+	—	—	—	+	+	+
Extracellular polysaccharide	+	+	+	+	+	+	d	d	—	—	+	—	+
Acetoin (VP)	—	—	—	—	—	—	—	—	—	—	d	d	+
Acid produced from:													
Amygdalin	—	—	—	d	+	+	+	—	—	—	+	d	d
Arbutin	d	+	+	+	+	+	+	—	—	—	+	+	+
Melibiose	+	—	d	—	+	—	—	+ ^b	d	+	—	d	+
Inulin	+	+	+	d	+	d	+	—	—	—	d	—	+
Mannitol	—	—	—	—	—	—	—	—	—	—	—	—	+
Sorbitol	—	—	d	—	—	—	—	—	—	d	—	—	+
Raffinose	+	—	d	—	+	—	—	d	d	+	—	d	+
β-Glucosaminidase ^c	—	—	—	—	+	+	+	+	—	+	—	d	—
α-L-Fucosidase ^d	—	—	—	—	+	+	+	—	—	—	—	—	—
Acid phosphatase ^c	+	—	—	+	+	+	+	d	d	+	+ ^b	d	+ ^b
Alkaline phosphatase ^c	d ^b	—	—	—	+	+	+	+	d	d	+	+	—
IgA1 protease	+	+	+	+	—	—	—	+	d	—	—	—	—
Neuraminidase	—	—	—	—	—	—	—	+	d	—	—	—	—

^a +, 90% or more of the strains are positive; —, 90% or more of the strains are negative; d, 11 to 89% of the strains are positive.

^b Reactions are usually weak.

^c Determined with an API ZYM kit.

^d Determined with an API ZYM osidase kit.

acid and rhamnose (10), and the peptidoglycan type is Lys-Ala₁₋₃ (30, 41). Biochemical, physiological, and serological characteristics of the species and three biovars are shown in Table 3. The G+C content of the DNA is 40 to 43 mol% (13). *S. gordonii* resembles *S. sanguis* but can be differentiated from this species by several biochemical characteristics (Tables 3 and 4), by a lack of IgA1 protease, and by a significantly different G+C content. Found in human oral cavities and pharynges. The type strain is strain ATCC 10558 (= NCTC 7865). The reactions of the type strain are the majority reactions for *S. gordonii* biovar 2 given in Table 3. The G+C content of the type strain is 41 mol%.

Emended description of *Streptococcus sanguis* White and Niven 1946, 722. Cells are gram positive and usually grow in short chains in serum broth. They are nonmotile, nonsporing, aerobic and facultatively anaerobic, fermentative, and catalase negative and show α-hemolysis on horse blood agar and pronounced greening on chocolate agar. The cell wall contains glycerol teichoic acid and rhamnose (10), and the peptidoglycan type is Lys-Ala₁₋₃ (30, 41). Biochemical,

physiological, and serological characteristics of the species and its biovars are shown in Table 3. Found in human mouths. The type strain is strain ATCC 10556 (= NCTC 7863). The reactions of the type strain are the majority reactions for *S. sanguis* biovar 1 given in Table 3, with the exceptions indicated. The G+C content of the type strain is 46 mol% (13).

Emended description of *Streptococcus oralis* Bridge and Sneath 1982, 414. Cells are gram-positive cocci and usually grow in long chains in serum broth. They are nonmotile, nonsporing, aerobic and facultatively anaerobic, fermentative, and catalase negative and show α-hemolysis on horse blood agar and pronounced greening on chocolate agar. The cell wall contains ribitol teichoic acid and lacks significant amounts of rhamnose (10). The peptidoglycan type is Lys-direct (30, 41). Biochemical, physiological, and serological characteristics of the species are shown in Table 3. Contrary to information provided in previous descriptions (2, 30), *S. oralis* does not have lysine decarboxylase activity, does not hydrolyze arginine or esculin, and is negative in the Voges-

TABLE 5. Correlation between the classification resulting from this study and designations used previously

Taxon as defined in this study	Designation used by:						
	Sherman et al. (41) ^a	Carlsson (5)	Colman and Williams (11)	Coykendall and Specht (13)	Facklam (17)	Hamada et al. (23)	Kilpper-Bälz et al. (30)
<i>S. sanguis</i>	Type I	I:B	<i>S. sanguis</i>	Genotype 3, " <i>S. sanguis</i> subsp. <i>carlssonii</i> "	<i>S. sanguis</i> I	Biotype A, serotype I	<i>S. sanguis</i>
<i>S. gordonii</i>	Type I-II		<i>S. sanguis</i>	Genotype 1, " <i>S. sanguis</i> subsp. <i>sanguis</i> "	<i>S. sanguis</i> I	Biotype A, serotype III	<i>S. mitis</i>
<i>S. oralis</i>	Type II	I:A	" <i>S. mitior</i> "	Genotype 2, " <i>S. mitior</i> "	<i>S. sanguis</i> II	Biotype B, serotype II	<i>S. oralis</i>
<i>S. mitis</i> biovar 1		V:A	" <i>S. mitior</i> "		<i>S. mitis</i>		
<i>S. mitis</i> biovar 2		V:B	" <i>S. mitior</i> "				

^a The numbers in parentheses are reference numbers.

Proskauer reaction (acetoin). The G+C content of the DNA is 39 to 42 mol% (12). Found in human oral cavities. The type strain is strain NCTC 11427. The characteristics of the type strain are the majority reactions for *S. oralis* given in Table 3, with the exceptions indicated. The G+C content of the type strain is 41 mol%.

Emended description of *Streptococcus mitis* Andrewes and Horder 1906, 712. Cells are gram-positive cocci and grow in short or long chains in serum broth. They are nonsporing, aerobic and facultatively anaerobic, nonmotile, fermentative, and catalase negative and show α -hemolysis on horse blood agar and pronounced greening on chocolate agar. The cell wall contains ribitol teichoic acid and lacks significant amounts of rhamnose (10). The peptidoglycan type is Lys-direct (30, 41). Biochemical, physiological, and serological characteristics of the species and two biovars are shown in Table 3. The G+C content of the DNA is 39 to 41 mol% (12). Found in human mouths and pharynges. The type strain is strain NCTC 12261. The reactions of the type strain are the majority reactions for *S. mitis* biovar 1 given in Table 3. The G+C content of the type strain is 41 mol%.

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