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Classification and Identification of the Viridans Streptococci

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INTRODUCTION 315
THE EARLY CONTRIBUTORS 316
MIDCENTURY DEVELOPMENTS 317
THE MOLECULAR APPROACH 317
S. MUTANS 317
History and Taxonomy 317
Identification 318
S. SANGUIS* AND *S. MITIS 318
A History of Confusion 318
Recent Results 319
Identification 320
S. ANGINOSUS 320
History and Genetic Relationships 320
Identification 321
S. BOVIS 322
History 322
Genetic Relationships 322
Identification 323
S. SALIVARIUS 323
History 323
DNA and Identification 323
S. VESTIBULARIS 324
A New Species 324
CONCLUSION 324
For More Information 325
Final Thoughts 325
ACKNOWLEDGMENTS 325
ADDENDUM IN PROOF 325
LITERATURE CITED 325

INTRODUCTION

The nonhemolytic streptococci are those whose colonies do not produce clear zones on blood agar by the complete destruction of erythrocytes; that is, they are not beta-hemolytic. Because many produce greenish discoloration on blood agar (alpha hemolysis), the group is also called the viridans streptococci, although many are completely indifferent to blood. They usually do not react with Lancefield grouping sera. They are human commensals and are a major component of the oral flora. Even within this loose definition, exceptions abound: some types of *Streptococcus anginosus* are beta-hemolytic, and many react with Lancefield A, C, F, or G antiserum; *S. bovis* has some characteristics of the enterococci, including a Lancefield D reaction. The human nonhemolytic streptococci can also be defined in a practical way by the exclusion of other streptococci; the viridans group is what remains when human pathogens and commensals that are *S. pyogenes*, enterococci (a separate genus now) (106), pneumococci, Lancefield group B, and "large colony" group C and G are eliminated.

Although these bacteria are usually docile tenants of the mouth and gut, they are capable of causing diseases and death among their hosts, especially when they gain entrance to sites that are usually sterile. Nonhemolytic streptococci account for about one-half of all cases of streptococcal endocarditis, a life-threatening disease that requires long periods of expensive treatment. *S. mutans* is responsible for dental caries, a condition whose treatment accounts for great losses of time and treasure. *S. anginosus*, in its various guises, causes abscesses in the brain, liver, and joints and may also be important in neonatal infections.

The words "nonhemolytic streptococci" are nearly always preceded or followed by words such as "poorly classified." In this review, it will be shown that the taxonomy of the human nonhemolytic streptococci has improved as ever closer examination has revealed the biochemical and genetic relationships among these bacteria. Improved taxonomy has facilitated identification and has enabled laboratories to correlate species with diseases (92). Perfection has not been attained, however. Although there is considerable agreement now on which of these streptococci are related to others and which are not, there is far less unanimity on the matter of appropriate names and type strains.

This review traces the history of the six most common nonhemolytic species and presents their current taxonomic positions, with emphasis on the molecular or genetic relationships among these bacteria. These six species encompass streptococci that have been given many other names.

TABLE 1. The common viridans streptococci

Species	Comments	Test[a]				
		VP	ARG	ESC	MAN	SORB
S. anginosus	Phenotypically heterogeneous; now includes *S. constellatus*, *S. intermedius*, *Streptococcus* MG, group F, "*S. milleri*," and minute hemolytic strains; important in purulent infections	+	+	±	±	−
S. bovis	Nonenterococcal group D; variants are less reactive; common blood isolate in patients with colon cancer; animal strains may be different species	+	−	+	±	−
S. mitis	Also called "*S. mitior*," *S. oralis*, and, when raffinose positive, *S. sanguis* II; includes two genetic groups	−	−	−	−	−
S. mutans	Actually a group of seven species which are phenotypically similar; causes dental caries and endocarditis	+	−	+	+	+
S. salivarius	Rare in infections; genetically homogeneous; can resemble *S. bovis*	+	−	+	−	−
S. sanguis	Causes endocarditis; genetically heterogeneous; one genetic group contains the type strain of *S. mitis*	−	+	+	−	−
S. vestibularis	New species from the oral cavity	±	−	+	−	−

[a] VP, Voges-Proskauer; ARG, hydrolysis of arginine; ESC, hydrolysis of esculin; MAN, fermentation of mannitol; SORB, fermentation of sorbitol.

Table 1 summarizes the species, their other names, and their notable attributes along with a very simple guide to their biochemical traits. Whenever possible, the names used are those on the *Approved List of Bacterial Names* (113), and the rationale for using these names will be explained. As will be seen, the names and descriptions published by the earliest workers in this field often are the most appropriate. Most important, the phenotypic portraits of the species will be drawn so that isolates can be identified and differentiated from each other.

The nutritionally variant streptococci are not considered here. Although they have been considered forms of *S. sanguis* or *S. mitis*, they are genetically unrelated and form at least two distinct genospecies (11a).

THE EARLY CONTRIBUTORS

Three species of nonhemolytic streptococci that are still valid were named and described in 1906 by Andrewes and Horder (2). They recognized a rarely pathogenic resident of the mouth and intestine which they called *S. mitis*. For decades, this species has been poorly defined because it ferments or hydrolyzes few of the substrates commonly used to describe and identify streptococci. Often, isolates that were not clearly members of other species were relegated to *S. mitis*. This species can be more accurately described today but, as we shall see, the type strain does not fit the description and the taxonomic position of *S. mitis* is once again confused. This problem and a proposed remedy will be fully discussed below.

Andrewes and Horder also found a streptococcus that fermented sucrose, lactose, and usually raffinose and was so common in saliva that they named it *S. salivarius* (2). This species has always been well-defined, and its taxonomic position has rarely been assailed.

These two early investigators also described a hemolytic species that they associated with disease, including scarlet fever. (They were careful not to assign a definite etiologic role to the organism.) It fermented sucrose and lactose and often fermented raffinose. To this species they gave the name *S. anginosus* (2). Although it is hemolytic, we now know that there are many nonhemolytic variants of *S. anginosus* which have been described as "*S. milleri*," *S. constellatus*, *S. intermedius*, and *Streptococcus* MG. *S. anginosus* is now regarded as a member of the nonhemolytic group. This story will be told later. The observation of a hemolytic streptococcus that fermented raffinose and was associated with disease was very perceptive, and these observations give validity to both the name and the importance of *S. anginosus* today. For anyone interested in the development of taxonomy, the early application of biochemical tests, and the concept of a species, the treatise by Andrewes and Horder is recommended.

In 1919, Orla-Jensen (90) described *S. bovis*, an organism common in the bovine gut. It fermented starch, inulin, raffinose, and arabinose, but not mannitol. Similar streptococci that did ferment mannitol but not arabinose were named *S. inulinaceus* (67). Streptococci that resemble *S. bovis* are isolated from humans and are often encountered in endocarditis, and the species is more common in the bowels of people with colon disease. The significance of Orla-Jensen's work is that he recognized diversity among the bovine streptococci. Failure to attach significance to this diversity has contributed to some confusion about what species *S. bovis* really is.

Dental caries has occupied the thoughts of scientists and philosophers for centuries. Bacteria had been considered the agent of this disease since W. D. Miller's cogent argument of 1880 (81), but there was little knowledge of which of the scores of microbial forms in the mouth were responsible for the decay of teeth. J. K. Clarke sought bacteria that were associated with decayed teeth and that could produce enough acid to initiate caries. He found such an organism and named it *S. mutans* (22). It produced a pH of 4.2 in glucose broth and fermented glucose, lactose, raffinose, mannitol (mannite), inulin, and salicin. Clarke also succeeded in producing caries in extracted teeth in vitro with his *S. mutans*. His discoveries were confirmed by Maclean (79) 3 years later, and in 1928 Abercrombie and Scott (1) isolated *S. mutans* from a case of endocarditis. Until the 1960s, *S. mutans* was mentioned as a cause of dental caries in several textbooks (15, 75–77, 117, 118), but little if any research seems to have been done to explore further the lands discovered by Clarke.

In the 1930s and 1940s, Sherman and others refined the definition of several streptococci. Sherman et al. (111) reviewed the ecology and characteristics of *S. salivarius* and *S. mitis*. They noted the lack of biochemical activity of *S. mitis* and the lack of properties "uniquely its own." They suspected that the *S. mitis* "group" (as they called it) contained other entities because some members hydrolyzed arginine and esculin and fermented salicin. This observation was perceptive, because streptococci with these character-

istics were later isolated from the blood of patients with endocarditis. They attracted attention because they often resisted the penicillin and heparin endocarditis treatment which had been successful against other streptococci (74). This new species was named *S. sanguis* (127), and in addition to the characteristics noted above, it produced strong green discoloration on blood as well as an extracellular polysaccharide from sucrose identified as a glucan (86). Although *S. sanguis* was recovered from an infected tooth and an infected sinus (127), many years passed before the natural site of this species was discovered.

Sherman (110) and Smith and Sherman (114) observed that many group G streptococci could be assigned to *S. anginosus* based on the description provided by Andrewes and Horder (2). Sherman (110) also regarded *S. bovis* and *S. inulinaceus* sufficiently alike to be considered as one species, *S. bovis*.

MIDCENTURY DEVELOPMENTS

In the 1950s, the availability of germfree animals allowed scientists to show that dental caries could be induced in the presence of certain bacteria. In one case, caries was initiated with the oral introduction of an unidentified streptococcus in combination with an unknown bacillus (91). Later, a single streptococcus was found that could cause caries in gnotobiotic rats (51), and a similar bacterium would produce caries in conventional hamsters (52). Because these streptococci defied identification, interest in the taxonomy of the oral streptococci, which are mostly nonhemolytic, became intense.

This interest produced several discoveries and rediscoveries. Carlsson (18) noted the similarities between the cariogenic streptococci and Clarke's *S. mutans*. He also showed that the primary habitat of *S. sanguis* was the surfaces of teeth (17). Colman and Williams (25) further defined *S. mitis*, which they preferred to call "*S. mitior*," a similar species described by Schottmüller in 1903 (108). They also noticed that the various minute hemolytic streptococci and some nonhemolytic strains of Lancefield groups A, C, and G were more alike than different and were similar to the "*S. milleri*" of Guthof (57). Also at this time, the development of deoxyribonucleic acid (DNA) hybridization methods permitted taxonomists to test directly the genetic relationships among bacteria.

These efforts produced a large amount of information and several taxonomic ideas, but there was hardly unanimity among the workers in this field. The taxonomic systems of Carlsson (19), Colman and Williams (25), and Facklam (44) were significantly different, and the results of DNA-DNA hybridization experiments showed surprising heterogeneity within some phenotypically homogeneous species (28, 35). There developed a lack of correlation among biochemical descriptions, genetic relationships, and names. One of the objectives of this review is to present a taxonomy for these streptococci that brings concinnity to phenotypic descriptions, genetic groupings, and names.

THE MOLECULAR APPROACH

The ability to compare the genetic material of various bacteria has permitted taxonomists to classify them on the basis of genetic similarity, not merely by phenotypic similarity. Therefore, we prefer now to define a species as a group of bacteria that are genetically related. Bacteria that are not related should not be in the same species (29). When it is determined that bacteria are related and therefore form a species, then their phenotypic characteristics can be considered and those that are useful for distinguishing and identifying the species can be included in the description of the species. When two distinct genotypes have no clear phenotypic characteristics by which they could be demarcated, we have been reluctant to separate them. Thus, species should be defined genetically, described phenotypically, and, of course, named unequivocally (33). In 1987, the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics recommended that this genetic, or phylogenetic, system of taxonomy become the standard, with DNA base sequences the determinant of phylogeny (122). They recommended that DNA relatedness of about 70% or more be required to include organisms in a species. However, when genetic groups (i.e., genospecies) cannot be differentiated phenotypically, they should not be named until phenotypic differences are found. Much of the taxonomic information presented below has been derived from this phylogenetic method and as far as possible conforms to the rules set forth in the *International Code of Nomenclature of Bacteria* (72).

As we collected large numbers of reference strains and clinical isolates, the task of performing biochemical tests became onerous. To alleviate this, we began to use the API Rapid Strep system (Analytab Products, Plainview, N.Y.) to identify and characterize our streptococci. In Europe, this product is called 20S, but in the United States 20S is a different system. This product has been evaluated in several laboratories (4, 47, 103, 120). We found this scheme reproducible and easy to use. We evaluated it by testing strains whose identity had been authenticated in previous DNA studies. In recent years, we have found that Rapid Strep and DNA hybridization complement each other. In some cases, the product revealed biochemical differences between genetic groups that were phenotypically indistinguishable by the tests we had used previously. In other cases, biotypes suggested by API Rapid Strep were revealed as genotypes by DNA hybridizations. Therefore, many biochemical characteristics described below have been determined with the API Rapid Strep system. Notice is given when this is the case.

S. MUTANS

History and Taxonomy

Few streptococci have a greater number of interesting features than *S. mutans*. It was discovered and described (22), its clinical importance was documented (1), and then it was nearly forgotten. It ferments nearly all of the sugars commonly used in the identification of streptococci. It produces an extracellular polysaccharide (a glucan) (53, 55) and an intracellular starch-glycogen polysaccharide (54, 121).

So large and distinct is its biochemical repertoire that it stood out clearly in numerical taxonomic studies (19, 40), yet *S. mutans* is so genetically heterogeneous that it was divided into four species (29) and three more have been added (11, 30, 125). The literature on *S. mutans* is immense (61, 73), and only the clinical and taxonomic features can be reviewed here.

Soon after the realization that the cariogenic streptococci were none other than Clarke's long-neglected *S. mutans*, analyses of their DNAs indicated perplexing heterogeneity. The base composition of the DNA of various strains ranged

TABLE 2. Characteristics of the members of the *S. mutans* group[a]

Species	Mol% guanine plus cytosine	Serotype	Hydrolysis of arginine	Fermentation of: Raffinose	Fermentation of: Melibiose	Production of H_2O_2	Aerobic growth	Susceptibility to bacitracin	Cell wall sugars[b]	Peptidoglycan type[c]
S. mutans	36–38	c, e, f	−	+	+	−	+	−	Gluc, Rham	Lys-Ala_{2-3}
S. rattus	41–43	b	+	+	+	−	+	−	Gal, Rham	Lys-Ala_{2-3}-Ala
S. cricetus	42–44	a	−	+	+	−	−	+	Gluc, Gal, Rham	Lys-Thr-Ala
S. sobrinus	44–46	d, g	−	−	−	+	+	−	Gluc, Gal, Rham	Lys-Thr
S. ferus	43–45	c	−	−	−	−	−	+	Gluc, Rham	Lys-Ala_{2-3}
S. macacae	35–36	c	−	+	−	−	−	+	Gluc, Rham	ND[d]
S. downei	41–42	h	−	−	−	−	−	+	ND	ND

[a] Information is from references 11, 12, 27, 29, 30, 106, and 125.
[b] Gluc, Glucose; Rham, rhamnose; Gal, Galactose.
[c] Lys, Lysine; Ala, Alanine; Thr, threonine.
[d] ND, No data.

from 36 to 46 mol% guanine plus cytosine (27). The genetic diversity predicted by such a range was confirmed by DNA-DNA hybridization, which revealed four genetic groups (28). These groups correlated with the four serologic groups that Bratthall had described (12). Reexamination of the biochemical traits of several strains showed that some reactions, originally thought to be variations of little importance, correlated with genetic and serologic groups (29). For example, strains that hydrolyzed arginine accounted for one of the groups, and those unable to ferment raffinose described another. These four genetic groups were made separate species in 1977 (29). Another genetically distinct member of the mutants group had been isolated from wild rats (36), and this too was elevated to a new species, *S. ferus* (30).

Two more species have been isolated from monkeys, and these have been added to the mutans groups. *S. macacae* is very similar to *S. mutans* but is susceptible to bacitracin (11). *S. downei* (125) was first described as *S. mutans* serotype h but is now a separate species. The characteristics of the species of the mutans group are summarized in Table 2.

The most common member of this group is *S. mutans*. Most humans harbor this streptococcus in dental plaque, the microbial community that lives on tooth surfaces (73). The numbers are greater (usually) if the person has dental caries (73). A small percentage of people have *S. sobrinus* instead. *S. rattus* and *S. cricetus* can be found in some populations, but they are not common (73).

Although *S. rattus* and *S. cricetus* were first isolated from rats and hamsters, respectively, it is believed that these laboratory animals acquired them from humans (30). Similarly, the species that have been found in wild rats and laboratory monkeys have not been observed in humans.

Identification

S. mutans and its cousins in this group will be among the normal oral flora when clinical specimens are derived from the mouth or throat. *S. mutans* is not common in tissue infections (92) (although some consider dental caries an infection). However, *S. mutans* is common in bacterial endocarditis (41, 44, 92, 98) and will be recovered frequently if the nonhemolytic streptococci from blood of patients with this disease are identified to species. Most strains are entirely nonhemolytic (19, 92).

The identification of *S. mutans* is not difficult. It ferments mannitol, sorbitol, raffinose, inulin, lactose, melibiose, and the more common sugars, produces acetoin, and splits esculin (19, 25, 44, 92). When grown in broth containing sucrose, the glucans will cause the cells to adhere to the walls of the vessel (55). On agar with sucrose, such as Mitis Salivarius agar (21), colonies will produce puddles of glucan, and a droplet of glucan may appear at the top of the colony, especially if plates are allowed to stand on the bench for 24 h (65). The API Rapid Strep system easily identifies *S. mutans* but does not recognize the other species in the group. Isolates that are called *S. mutans* but which do not ferment raffinose can be assumed to be *S. sobrinus*, and those that are positive for arginine are *S. rattus*. *S. cricetus* can be differentiated from others in the mutans assemblage because it is susceptible to bacitracin (33).

S. mutans can be differentiated from other mannitol fermenters by its failure to hydrolyze hippurate and, in the case of the enterococci, by any of several routine tests. Only *S. mutans* (and its cousins) and *S. bovis* ferment mannitol and make glucan. *S. bovis* is otherwise distinguishable by its indifference to sorbitol and its action against starch. Its colonies on sucrose agar are vastly different from those of *S. mutans*.

S. SANGUIS AND *S. MITIS*

A History of Confusion

S. sanguis, like *S. mutans*, resides on the surfaces of teeth, produces extracellular glucans from sucrose, and causes endocarditis, but in other respects they are quite different. The glucans of *S. sanguis* are structurally different (53) and are not adhesive. There is no evidence that *S. sanguis* contributes to dental caries. Its biochemical profile differs considerably from that of *S. mutans* (19, 25, 44, 92).

Unfortunately, the taxonomy and identification of *S. sanguis* have become complicated because some strains of *S. mitis* produce a glucan which confers an *S. sanguis*-like morphology on colonies grown on sucrose agar. Also, a strain of *S. mitis* that splits esculin resembles a strain of *S. sanguis* that is negative for arginine and vice versa. Finally, the type strain of *S. mitis* is a strain of *S. sanguis*. Therefore, it is impossible to discuss *S. sanguis* without discussing *S. mitis*.

Recall that *S. sanguis* hydrolyzes arginine and esculin and produces a characteristic glucan. This glucan imparts to colonies a firmness and a tenacity to sucrose agar that requires the colony to be cut out of the agar if it is to be cultured further (17). There are streptococci that are negative for arginine and esculin (i.e., *S. mitis*) but produce

similar glucans and similar colonies. These have been called *S. sanguis*. Carlsson (19), for example, recognized the biochemical differences between these and typical *S. sanguis* strains but considered them *S. sanguis* based on other biochemical similarities, glucan production, and their similar ecology, i.e., the tooth surface. It was Colman and Williams (25) who suggested that these isolates were glucan-producing members of the species they preferred to call "*S. mitior*." DNA studies confirmed that the streptococci that produced glucans like *S. sanguis* but that did not split esculin or arginine were not closely related to typical *S. sanguis* strains (35) but were indeed related to typical "*S. mitior*" (or *S. mitis*) strains that did not produce glucans (34). Furthermore, there were two genetic groups within *S. sanguis* (35) and two genetic groups within "*S. mitior*" (34).

A key to separating *S. sanguis* from "*S. mitior*" was the discovery that "*S. mitior*" has ribitol, but no rhamnose, in its cell wall (24). It was also found that the peptidoglycan of these streptococci has direct lysine cross-links (105), based on analysis of ATCC 10557, a strain referred to in the American Type Culture Collection list as *S. sanguis* but which is biochemically and genetically a member of "*S. mitior*" (34, 35). Thus, "*S. mitior*" (or *S. mitis*) can be clearly described and differentiated from *S. sanguis* based on cell wall analysis.

In the taxonomic and identification system of Facklam (44), strains that produce the *S. sanguis*-like glucan and ferment raffinose are called *S. sanguis* II. These strains are otherwise similar to *S. mitis*, and DNA hybridization has shown that there is no taxonomic significance to raffinose fermentation in this species (34).

In 1980, the *Approved List of Bacterial Names* (113) accepted *S. sanguis*, with ATCC 10556 (=NCTC 7863) as the type strain. The official description is that in *Bergey's Manual of Determinative Bacteriology* (8th ed.) (39). The authors did not accept "*S. mitior*" but did accept *S. mitis*, with NCTC 3165 chosen as the type strain (113). The official description is that in *Bergey's Manual* (8th ed.) (39), a description based on that by Sherman at al. (111).

Therefore, by 1980, *S. sanguis* was an accepted species with a good description, consisting of two moderately related genetic groups, one of which contained the type strain. There were no clear phenotypic differences between the two genetic groups. *S. mitis* was an accepted species with a somewhat equivocal description, consisting of two genetic groups with no phenotypic tests to differentiate them. Unfortunately, the type strain chosen for *S. mitis* is actually a strain of *S. sanguis*. It attacks arginine and esculin and ferments raffinose and inulin; therefore, it fits the description of *S. sanguis*, not that of *S. mitis*. Its DNA hybridizes with that of *S. sanguis* strains in the genetic group that is represented not by the type strain, but by *S. sanguis* ATCC 10558 (=NCTC 7865). Its DNA is not similar to that of any *S. mitis* strain (A. L. Coykendall and P. Wesbecher, Abstr. 10th Lancefield Conf. 1987, Cologne, Federal Republic of Germany, p. 33).

Using Rapid Strep tests, we now know that the members of the *S. sanguis* genetic group that includes the type strain (ATCC 10556) and which we call genetic type 2 (group 3 in reference 35) do not produce alkaline phosphatase and usually split starch. Genetic type 1 strains, represented by ATCC 10558 and the *S. mitis* type strain, have the opposite reactions (33). Nyvad and Kilian (89) have suggested that the group represented by ATCC 10558 become a separate species named *S. gordonii*. The group that includes the type strain must, of course, remain *S. sanguis*.

This story now becomes more convoluted. In 1982, Bridge and Sneath (14) named a new species, *S. oralis*. Some of the strains of *S. oralis* had been described earlier as "*S. mitior*" (*S. mitis*) (34). The type strain of *S. oralis* (NCTC 11427) has the phenotypic characters of *S. mitis*, its peptidoglycan has direct lysine cross-links (107), and its DNA forms hybrids with strains that we recognize as *S. mitis*, such as ATCC 10557 (Coykendall and Wesbecher, Abstr. 10th Lancefield Conf. 1987). Although *S. oralis* has been reported to produce acetoin (107), we have not observed this.

This state of affairs can be resolved one of two ways, both taxonomically correct (72). Those strains of *S. sanguis* which are biochemically and genetically like the type strain of *S. mitis* could be called *S. mitis*, and what is now *S. mitis* could be renamed *S. oralis*. This has been suggested (106, 107). It is my opinion that this would lead to great confusion. Streptococci now called *S. mitis* (or "*S. mitior*") would be called *S. oralis*. About half of what are called *S. sanguis* would be renamed *S. mitis*, but except for DNA analysis and the few biochemical differences noted above (starch and alkaline phosphatase), *S. mitis* and *S. sanguis* would be difficult to differentiate. Also, the official descriptions would have to be rewritten. It is unlikely that such a change would meet with approval.

A much simpler solution would be merely to reject the current type strain of *S. mitis* and select a new type strain that fits the description of *S. mitis* and whose DNA is homologous with DNAs of other strains that are recognized as *S. mitis*. The rules of taxonomy provide for this, and a request for an opinion has been published (31). The request proposes that the type strain of *S. mitis* be rejected and *S. oralis* be considered a later synonym of *S. mitis*. The current type strain of *S. oralis* would become the type strain of *S. mitis*.

Recent Results

Recent examination of some unusual *S. sanguis* strains uncovered two additional genetic groups (Coykendall and Wesbecher, Abstr. 10th Lancefield Conf. 1987). A group of three strains that bore tufts of fibrils on their walls (62) was closely related by DNA homology but less related to the two *S. sanguis* genogroups described above. These strains split arginine but gave weak esculin reactions. They did not produce alkaline phosphatase or ferment raffinose.

A fourth group was genetically quite distinct and was represented by five strains which produced alkaline phosphatase (four of which also fermented raffinose) and hydrolyzed arginine. Four gave weak or negative esculin reactions.

Thus, the viridans streptococci that typically split arginine and esculin and do not produce acetoin form at least four genetic groups. We have continued to call them all *S. sanguis*. The groups are sufficiently disparate to be considered separate species (89), but we have been reluctant to propose new species because there are so few constant biochemical tests that would serve to discriminate among them. (See reference 122, cited above.) In other words, new species can be defined genetically but cannot be described phenotypically. The same applies to the two genetic groups in *S. mitis*.

This complicated story of *S. sanguis*, "*S. mitior*," *S. sanguis* II, and *S. oralis* has a simple conclusion. *S. sanguis* is a valid species with four genetic groups. Most strains hydrolyze arginine and esculin. There are some small phenotypic differences between some of the genotypes. The

genetic groups could become separate species, especially if enough phenotypic differences were uncovered to facilitate their descriptions and identification. *S. mitis* is a valid species. Although "*S. mitior*" is a good name for these streptococci, it was not accepted in 1980. *S. sanguis* II is the same as *S. mitis*. *S. oralis* is also a synonym for *S. mitis*. There are two genetic groups within *S. mitis*, and to date there are no phenotypic differences by which they can be discriminated.

Identification

Typical strains of *S. sanguis* can be identified by tests for arginine and esculin. Other nonhemolytic species such as *S. anginosus* have similar reactions, but *S. sanguis* does not produce acetoin (19, 25, 92). Although some isolates split starch, none attack glycogen. Many ferment inulin, and a few acidify sorbitol (23, 92). The API Rapid Strep system has no difficulty with such typical strains. Both *S. sanguis* and *S. mitis* produce strong alpha hemolysis (19, 25, 44, 92).

Typical *S. mitis* strains hydrolyze neither arginine nor esculin and almost never ferment inulin. *S. mitis* isolates are less likely to ferment trehalose and salicin compared with *S. sanguis* (19, 24, 44, 92). In the Facklam scheme (44), the fermentation of raffinose separates *S. sanguis* II (positive) from *S. mitis* (negative). As noted above, we found no taxonomic significance to raffinose among these streptococci and consider them all *S. mitis*.

When an isolate of one of these two species splits arginine but not esculin, or vice versa, identification can be difficult, especially when inulin is not fermented. Facklam (44) did not assign to *S. mitis* or *S. sanguis* II any strain that splits esculin but allowed arginine- or esculin-negative strains into *S. sanguis*. In their 1972 study (25), Colman and Williams included in *S. sanguis* 3 (of 35) strains that were arginine negative and 15 that were esculin negative. In "*S. mitior*," they accepted two that split esculin and three that attacked arginine. In a later study, Colman and Ball (23) used arginine hydrolysis as a strict requirement for *S. sanguis*. Of 174 isolates, 34 did not hydrolyze esculin. Conversely, indifference to arginine was a criterion for "*S. mitior*" (*S. mitis*) and, of 189, 47 attacked esculin. Price et al. (94) tested 48 strains that were *S. sanguis* or *S. mitior* and found that all but one strain with rhamnose in the walls (and therefore not *S. mitis*) split arginine.

Among the strains that we call *S. sanguis* based on DNA hybridization with the type strain, reference strains, or strains that fit the description of *S. sanguis*, only one was arginine negative. Among *S. mitis* strains whose identities were authenticated by DNA hybridization, only two split arginine, and two others hydrolyzed esculin. Therefore, while most strains of *S. sanguis* split arginine and most *S. mitis* ("*S. mitior*") isolates do not, DNA studies prove that exceptions can occur and will present difficulties for identification. In these cases the fermentation of inulin and salicin would assist the identification; *S. mitis* rarely ferments inulin, and salicin and trehalose are acidified by only 20 to 30% of isolates (19, 25, 44, 92). The API manual (3) suggests that the fermentation of amygdalin by many *S. sanguis* strains can help separate these from *S. mitis*. The API Rapid Strep system is helpful in separating these two species, but it too has difficulty with some strains that are arginine positive and esculin negative or vice versa.

Both *S. sanguis* and *S. mitis* strains can produce glucans. When grown on agar with 5% sucrose, colonies will be hard and adherent, with the texture and consistency of polyethylene. The agar may be depressed and form a groove or moat around the colony (17). The production of glucans is by no means a constant trait. Colman and Ball (23) found that 81 of 174 *S. sanguis* isolates did not produce glucans and only 37 of 189 "*S. mitior*" (*S. mitis*) isolates made these polymers.

S. sanguis and *S. mitis* are normal residents in the mouth and will be found in any sample contaminated with dental plaque or saliva. They are both important pathogens for the endocardium. Together they account for a high percentage of streptococcal endocarditis cases (10, 41, 44, 92, 98). There are statistical (92) and experimental (93, 52, 104) indications that the production of glucans enhances attachment of streptococci to the endocardium, but many glucan-negative isolates of *S. mitis* and *S. sanguis* have been recovered from the blood or heart valves of patients with endocarditis (A. L. Coykendall, A. Efstratiou, and G. Colman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, B37, p. 30).

In the future, a new type strain should be selected for *S. mitis*. Also, efforts should be made to find phenotypic differences by which the four genetic groups of *S. sanguis* and the two genotypes of *S. mitis* could be distinguished. This may prove useful for epidemiology and for studying the etiology of the diseases with which they are associated.

For example, *S. sanguis* strains that did not produce alkaline phosphatase and seldom split starch (reactions which seem to describe genotype 2) were more common among all *S. sanguis* isolates sent to a reference laboratory, but when only isolates from endocarditis cases were considered, strains characteristic of genotype 1 predominated (Coykendall et al. Abstr. Annu. Meet. Am. Soc. Microbiol. 1986). Finally, a rapid method to clearly distinguish *S. mitis* from *S. sanguis* would be a welcome development. A simple technique to detect ribitol in cell walls would serve the purpose nicely.

S. ANGINOSUS

History and Genetic Relationships

We now regard "*S. milleri*," *S. constellatus*, *S. intermedius*, Lancefield F streptococci, and *S. anginosus* as one species whose appropriate name is *S. anginosus*. This species embraces streptococci that can be hemolytic or nonhemolytic; some have Lancefield antigen A, C, F, or G; and some ferment several sugars, while others have a limited biochemical repertoire.

Recall that Smith and Sherman (114) recognized that some Lancefield group G streptococci fit the description of *S. anginosus*, and *Bergey's Manual* (39) regards group F as *S. anginosus*. Colman and Williams (25) realized that the minute hemolytic streptococci of Lancefield groups F and G and nonhemolytic streptococci of Lancefield groups A, C, and G had many common traits. They also perceived similarities between these bacteria and "*S. milleri*," which had been described and named in 1956 (57). They adopted this name for strains that fermented lactose, sucrose, trehalose, and salicin, hydrolyzed arginine and esculin but not hippurate, and produced acetoin. Parker and Ball (92) used this classification and also included in the species all group F streptococci and any hemolytic strain that otherwise fit the description of "*S. milleri*." Ball and Parker (6) expanded the species to include isolates that fermented several other sugars such as mannitol.

Around the same time, Facklam (44) noticed the similarity between *Streptococcus* MG (82) and *S. intermedius* (63) and

found it impossible to distinguish his reference strains of *S. anginosus* from *S. constellatus* (63). Group F streptococci and hemolytic strains with no Lancefield group antigen were also biochemically like *S. constellatus*. He realized that these bacteria could all be called "*S. milleri*" but separated the taxon for epidemiologic reasons. To isolates that fermented lactose, the name "*Streptococcus* MG-*intermedius*" was applied. Lactose-negative strains were called "*S. anginosus-constellatus*," although *S. anginosus* had been described as fermenting lactose (2). In 1984, Facklam (45) amended this scheme to give the name *S. anginosus* to the minute beta-hemolytic streptococci of groups A, C, and G and to eliminate hyphenated names. In the meantime, Moore et al. (84) recognized that *S. intermedius* was indistinguishable from *S. anginosus* and regarded it as a later synonym for that earlier valid species.

The first genetic evidence that these streptococci were indeed alike was offered by Welborn et al. (123), who found a high degree of DNA base sequence similarity among the type strains of *S. intermedius* and *S. constellatus*, two group F strains, and "*Streptococcus* MG-*intermedius*." Farrow and Collins (49) and Ezaki et al. (42) also found genetic homology among various strains in this phenotypically complex group. However, Kilpper-Bälz et al. (68) could distinguish two genetic groups, one represented by the type strain of *S. anginosus* and the other represented by the type strain of *S. constellatus*. They found no strains homologous with the type strain of *S. intermedius*.

We collected 76 clinical isolates that the API Rapid Strep system identified as "*S. milleri*," plus 35 type or reference strains that others considered to be "*S. milleri*," group F, *S. constellatus*, *S. intermedius*, or *S. anginosus* (37). These 111 strains represented nearly every combination of hemolysis, serologic group, and phenotype. Forty of these strains were compared by DNA-DNA hybridization. There was considerable homology among nearly all strains, including the type strains of *S. anginosus* and *S. constellatus*, although the type strain of *S. intermedius* was notably less related to all other strains. We did not see the distinct division between *S. anginosus* and *S. constellatus* observed by Kilpper-Bälz et al. (68). (The hot formamide method that they used may be much more stringent than our dimethyl sulfoxide technique [see the discussion in reference 37].) Strains that were beta-hemolytic and esculin and lactose negative were less related to the strains which were not hemolytic but positive for esculin and lactose plus mannitol and raffinose. These two phenotypic types represented the extremes of a range of biochemical and serologic patterns and could be considered different species. Yet there are so many phenotypic patterns in between that erecting distinct species borders would be impossible. We concluded that the members of this large group are sufficiently alike both biochemically and genetically to be called one species. The name "*S. milleri*" would be useful since it is now in common use. However, the oldest valid species name is *S. anginosus*. The type strain of *S. anginosus* is genetically homologous with strains in this complex and fits the species description. Because it is so unusual for hemolytic streptococci to ferment raffinose, the bacteria that Andrewes and Horder (2) called *S. anginosus* could not have been anything else.

Identification

Despite the unusual phenotypic diversity of its members, *S. anginosus* has a group of characteristics which seldom vary. These traits describe the species and facilitate its identification. The production of acetoin and alkaline phosphatase, the hydrolysis of arginine, and the fermentation of trehalose, with failure to split hippurate or ferment ribose, set the species apart from other streptococci (3, 6, 37). Ruoff and Ferraro (100) showed that this species could be separated from all other viridans species by just three tests: acetoin (always positive), arginine (always positive), and sorbitol (always negative). In the API system (3), failure to produce β-glucuronidase and pyrrolidonylarylamidase fortifies the identification. *S. anginosus* does not produce hydrogen peroxide (126) or make extracellular polysaccharides from sucrose (6, 37). Cultures on blood agar plates often produce a sweet smell described as caramel (7, 99) or, we think, honeysuckle. This is quite noticeable, especially when plates sit on the bench for a day or two. The appearance of hemolysis before the colony is visible is a recognized trait of the hemolytic varieties (39), and the hemolytic zones become much larger than the colonies.

Among our 111 strains were 26 different biochemical profiles. Yet only 3 of the 40 strains whose DNAs were tested failed to join the species based on genetic homology (37). Two failed to ferment trehalose and two produced hydrogen peroxide. Although these three strains appeared to be *S. anginosus*, the API system rejected two of them. Thus, there seems to be a rigid core of characteristics in this species.

The Rapid Strep system recognizes three biotypes of *S. anginosus* (3). Biotype 1 strains are usually hemolytic and negative for esculin and usually lactose. Isolates of biotype 2 ferment lactose and often starch, split esculin, and may or may not be hemolytic. Strains that also ferment mannitol and raffinose form biotype 3. As one would expect, the borders between the biotypes are indistinct. In our collection we found that biotype 1 was often associated with a Lancefield group, usually F. Within biotype 2, hemolysis was associated with Lancefield C and nonhemolytic strains were often group F. All group G isolates were hemolytic, and all hemolytic strains of biotypes 2 and 3 had a Lancefield group (37).

S. anginosus is a normal oral, intestinal, and probably vaginal resident (7, 56). However, its ability to produce serious purulent infections in liver, brain, joints, and other spaces has been thoroughly documented and often reviewed (56, 99, 112). *S. anginosus* should always be suspected in such infections. Although few cases have been reported, neonatal sepsis from *S. anginosus* has an aggressive course and a sad outcome (26, 115). Since neonatal viridans infections seem to be increasing (58, 117) and since *S. anginosus* has been found often in the vagina (7, 56), this species should be suspected in these cases.

S. anginosus is the most pathogenic and most phenotypically variegated of the viridans streptococci, and it has the most aliases. Its pathogenic mechanisms and its role in neonatal infections deserve investigation. Production of hydrolytic enzymes, which could contribute to virulence, is as heterogeneous as other characteristics in this species (101). In a recent review, Gossling (56) summarized what is known and unknown about pathogenic mechanisms and also drew attention to the fact that, at least in neonatal and vaginal infections, the streptococcal classification method used could make a significant difference in the conclusions. If the Voges-Proskauer test for acetoin is not used, *S. anginosus* could be misidentified as *S. sanguis*, for example. In the study of infectious disease, good taxomomy leads to good conclusions and good therapy.

S. BOVIS

History

The discovery that a correlation exists between chronic bowel disease and *S. bovis* illustrates the benefits of good taxonomy. Facklam (43) noticed that, among Lancefield group D organisms from endocarditis, many were *S. bovis* and urged that they be identified. There followed a series of articles attesting to the frequency of this nonenterococcal group D species in endocarditis and the need to identify it to avoid the more aggressive antibiotic treatment demanded for true enterococcal infections (59, 64, 73, 83, 96). Then in 1977, Klein et al. noticed that two patients that had *S. bovis* endocarditis also had adenocarcinoma of the colon (70). A survey of patients with colon cancer and other bowel diseases (70) and healthy controls showed that 56% of the cancer patients had *S. bovis* in their feces compared with 10% in controls and 28% in people with other inflamatory bowel diseases (70). Since then, additional studies (69, 85, 97) have confirmed this association. Patients with *S. bovis* bacteremia are now examined for colon disease. Thus it is important, even imperative (102), to identify correctly *S. bovis* in clinical material.

History shows that the association of a streptococcus with bowel diseases is not a new idea. In the 1920s, Bargen (8, 9) thought that ulcerative colitis might be caused be a streptococcus. Sherman (110) reviewed this question and concluded that the "Bargen streptococcus" was *S. bovis*.

It is important to remember that *S. bovis* was originally described as a bovine bacterium that fermented arabinose, raffinose, and starch, but not mannitol (90). Another bovine bacterium, *S. inulinaceus*, fermented mannitol and inulin, but not arabinose (67). Sherman (110) at first agreed and then disagreed with this separation after finding some isolates that hydrolyzed starch but did not acidify arabinose. Since then, the name *S. inulinaceus* has essentially disappeared. Although Niven et al. (87) found that "a few" *S. bovis* strains produced large mucoid colonies through the production of a dextran (glucan) synthesized from sucrose or raffinose, most human strains produce copious glucans (43, 92). Typical strains ferment mannitol and inulin (92), but most never acidify sorbitol or arabinose (43, 92). There is a variant biotype that neither ferments mannitol nor produces glucans. Facklam (43) called these *S. bovis* variant; Parker and Ball (92) used the term biotype II for these and biotype I for the more active type. Like *Enterococcus* spp., isolates of *S. bovis* have Lancefield D antigen and grow at 45°C and in 40% bile, but they do not succeed at 10°C or in alkaline pH (9.6), so they are referred to as nonenterococcal group D streptococci (43).

Animal isolates of *S. bovis* are more often mannitol negative and arabinose positive, and glucan production is associated with arabinose rather than with mannitol (38, 80), although not always (5); there are three cell wall types within the current species (106). When both animal and human isolates are considered, it can be seen that *S. bovis* is distinctly heterogeneous.

Genetic Relationships

The results of DNA hybridizations confirm the heterogeneity predicted by phenotypic variety. Farrow et al. (50) found considerable genetic heterogeneity among *S. bovis* strains. They found six DNA "homology groups" within a large collection of *S. bovis* and *S. equinus* strains. The type strain of *S. bovis*, NCTC 8177 (=ATCC 33317), was homologous with the type strain of *S. equinus*, NCDO 1037 (=ATCC 9812), and joined a group whose members did not ferment mannitol. Members of group 4 also were mannitol negative, but were quite unrelated genetically.

Strains in group 2 were mannitol positive and associated with bovine mastitis but included one human isolate. Group 3 strains shared genetic homology with some *S. suis* strains of group S under less than stringent hybridization conditions, but not under stringent conditions. These bacteria did not produce acetoin (negative Voges-Proskauer), which is unusual for *S. bovis*. From the remaining two groups, two new species were created: *S. saccharolyticus* and *S. alactolyticus* (50).

There were no phenotypic differences which could delineate the four groups except negative acetoin in group 3 and the fact that group 4 strains (of which there were but four in the table of biochemical reactions) never fermented trehalose and group 3 strains always did (50). Because the *S. bovis* and *S. equinus* type strains were homologous, Farrow et al. proposed that the earlier name, *S. equinus*, become the species name for all organisms in homology group 1. (*S. equinus* was described by the now familiar Andrewes and Horder in 1906 [2].)

Among *S. bovis* from humans, Knight and Shlaes (71) found good genetic relatedness. Five typical strains that were positive for mannitol, starch, and (except one) inulin and five variant strains (negative for the above) were all >60% homologous by DNA hybridization. However, the type strain of *S. bovis* (ATCC 33317, NCDO 597), which is starch and inulin positive but mannitol negative and which was isolated from cow dung, was not related to any human isolate (71).

We also compared the DNAs of several human isolates (32). We too found homology among typical strains and some, but not all, variant strains. Some variants formed a separate genetic group which was about 40 to 60% related to the others. The Rapid Strep system assigns typical *S. bovis* to a biotype I and the variants to a biotype II. Biotype II is subdivided to II/1 and II/2 (3). Our variants that were genetically related to the typical biotype I strains were biotype II/2. Those that were not closely related to biotype I were in biotype II/1. When compared with biotype II/1, biotype II/2 strains were more likely to produce β-glucuronidase and β-galactosidase and to ferment trehalose and less likely to split starch. In the case of this genetic study, correlation with the biotypes of the API Rapid Strep resolved what was a perplexing genetic heterogeneity, and it exemplifies the benefits of looking carefully at both biochemical and molecular data.

Regrettably, the type strain was not included in our hybridizations. When it was characterized in the API Rapid Strep test battery, it was identified as a member of biotype II/1.

The available data suggest that Orla-Jensen was correct. There is *S. bovis* (or *S. equinus*), and there is another species, *S. inulinaceus*. Human strains now called *S. bovis* (biotypes I and II/2) are perhaps *S. inulinaceus*. Some animal isolates and the biotype II/1 strains may be *S. bovis*. The irksome array of biotypes and genotypes among animal isolates (50) and the relationship of some human strains to the type strain yearn for clarification. Biochemical tests associated with a numerical data base, nucleic acid hybridizations, and other molecular comparisons such as cell wall analysis should be applied to a large group of human and

animal strains to untangle the taxonomy of these streptococci.

Identification

If patients who have *S. bovis* bacteremia will undergo tests for colon malignancy, then it is important to identify the organism accurately. There are viridans streptococci that can mimic *S. bovis*, but use of adequate tests should eliminate these. Typical isolates of *S. bovis* grow on bile-esculin and at 45°C, but not in 6.5% NaCl or at 10°C. They ferment mannitol, lactose, raffinose, and salicin. To these, Parker and Ball (92) added melibiose, trehalose, and inulin. Arabinose and sorbitol are almost never fermented (43, 92). Facklam (43) found that all split starch but only half fermented inulin and none fermented glycerol. Acetoin is produced and arginine is rarely attacked (23, 92). Most are completely nonhemolytic (43, 92). In the API system, isolates of biotypes I and II/I acidify starch and glycogen (3).

On sucrose agar, typical strains produce great amounts of watery glucans. This promotes very large colonies which can run together even if originally well spread. Colonies are not as firm as those of *S. salivarius*. When sucrose plates are incubated in the unusual inverted position, *S. bovis* glucans sometimes drip onto the cover.

Variant or biotype II strains neither ferment mannitol nor produce glucans; Facklam (43) further distinguished them by failure to split starch. Otherwise they are quite similar. In the API system, mannitol-negative strains that acidify starch are usually identified as biotype II/1 (the type not closely related to the typical sort). When neither substrate is fermented, the strains will usually be called biotype II/2. Other tests in Rapid Strep such as trehalose (see above) are important for discriminating II/1 from II/2 isolates (3).

The group D reaction, of course, helps in the identification of *S. bovis* but cannot be relied upon absolutely. Parker and Ball (92) used the formamide method, as well as hot acid extracts, and in some cases they resorted to concentration of extracts by alcohol (109). In only 57% of their 67 typical isolates was the D antigen detected; in the case of variant strains, the proportion was 67% of 24 isolates. Ruoff et al. (102) used concentration methods on strains which did not give a positive test and succeeded in showing a D antigen, which was confirmed by gel diffusion. Serologic tests which are technically easier, such as latex bead agglutination, often fail to detect the D antigen in *S. bovis* (20, 46, 102).

The production of glucan, fermentation of mannitol, and tolerance of bile can make *S. mutans* look like *S. bovis*. However, *S. bovis* almost never ferments sorbitol, and *S. mutans* does not ferment starch or glycogen or give a group D reaction (3, 19, 23, 92). The variant *S. bovis* can be mistaken for *S. salivarius*. Separation of these two is detailed below.

S. SALIVARIUS

History

S. salivarius has endured as a species since it was described by Andrewes and Horder (2). In the early part of the century, it was the only well-demarcated human nonhemolytic species. All others were called *S. mitis*. Use of the species *S. mitis* as a receptacle for "others" was one reason it was, and still is, not well described. This reputation for imprecision was a motive for the adoption of the name "*S. mitior*" espoused by Colman and Williams (25). *S. salivarius* was further defined by Sherman et al. (111), and Niven et al. (88) analyzed the extracellular material that it produced from sucrose and found that it was a levan. On sucrose agar, typical colonies are large and tall and have a soft viscous texture. Compared with *S. bovis*, they are taller and firmer and do not flow across the surface. However, some strains produce a glucan and their colonies look like those of *S. bovis* (32, 66). *S. salivarius* produces acetoin, hydrolyzes esculin but not arginine, and usually ferments lactose, raffinose, trehalose, and salicin (3, 19, 23, 25, 44, 92). Many strains ferment inulin and starch. Mannitol, sorbitol, and melibiose are rarely, if ever, fermented. The proportion of positive and negative reactions varies among different studies (3, 19, 23, 25, 44, 92). Many isolates produce urease (78): 7 of 16 in our study (32) and 14 of 20 in another (102).

DNA and Identification

Its clear definition and its rarity in infections, including endocarditis, have sheltered *S. salivarius* from inquisitive taxonomists. Nevertheless, it can resemble the more interesting *S. bovis* because it can react with group D antiserum, grow in bile-esculin, and present a phenotypic profile resembling those of *S. bovis* variants; also, a few strains produce glucan instead of levan. Therefore, we examined 16 strains to compare them with each other and with *S. bovis* (32). Although some were biochemically unusual, all shared extensive DNA base sequence homology. There was no genetic similarity between *S. salivarius* and *S. bovis*.

Some DNA studies have shown a close relationship between *S. salivarius* and *S. thermophilus* (48). However, Schleifer and Kilpper-Bälz (106) state that their unpublished results show only moderate (60%) DNA homology between these two species and a much lower (30%) hybridization under stringent conditions, which limit the formation of imperfect hybrid duplexes. They concluded that *S. salivarius* and *S. thermophilus* should remain as two species.

Although this species is rarely isolated from infections (92), its ability to mimic *S. bovis* obligates the clinical laboratory to identify *S. salivarius* accurately. At least 20% of isolates will grow in 40% bile (102) and nearly all strains split esculin (19, 23, 92), so it is not unusual to see this species on bile-esculin medium (102). The typical *S. bovis* isolates will ferment mannitol, but *S. salivarius* will not. The effect of *S. salivarius* on starch varies. Colman and Williams (25) and Ruoff et al. (102) found no hydrolysis, but Facklam (44) found a few (7%) positive strains. In the API system (3), acid is produced from starch in about one-half of all strains, and Colman and Ball (23), who used that product, found that 76% (31 of 41) of their *S. salivarius* isolates fermented starch. *S. bovis* of biotypes I and II/1 usually produce acid from glycogen in the Rapid Strep scheme, but *S. salivarius* does not. As usual, the variants are most troublesome. *S. bovis* isolates that ferment neither mannitol nor starch (i.e., biotype II/2) will be difficult to differentiate from *S. salivarius*. In our study (32), strains that were proven by DNA analysis to be *S. bovis* variants were identified with some equivocation by Rapid Strep. Their biochemical patterns indicated that they were *S. bovis* but with a chance that they could be *S. salivarius*. This was often the case when the β-glucuronidase or β-galactosidase test was negative in biotype II/2 or when glycogen was not fermented by biotype II/1 strains. Also, in this study, we included strains of both species that produced a polysaccharide opposite to the one expected, i.e., strains of *S. bovis* which produced a polymer (assumed to be levan) that gave colonies the morphology of

TABLE 3. Characteristics of viridans streptococcus species[a]

Species with biotype or genotype	Acetoin (V-P)	Hydrolysis of:		Production of:			Acid from:								Hemolysis		
		Esculin	Arginine	Alkaline phosphatase	β-Glucuronidase	H_2O_2	Mannitol	Sorbitol	Raffinose	Inulin	Trehalose	Salicin	Starch	Glycogen	Beta	Alpha	None
S. anginosus																	
1	+	−	+	+	−	−	−	−	−	−	+	+	−	−	+		
2	+	+	+	+	−	−	−	−	−	−	+	+	V	−			+
3	+	+	+	+	−	−	+	−	+	−	+	+	+	−			+
S. bovis																	
I	+	+	−	−	−	ND	+	−	+	90	+	+	+	+			+
II/1	+	+	−	−	−	ND	−	−	+	−	20[b]	+	+	+			+
II/2	+	+	−	−	V	ND	−	−	+	−	80[b]	ND	−	−			+
S. mitis	−	−	−	V	−	+	−	−	V[c]	−	30	20	V	−		+	
S. mutans	+	+	−	−	−	−	+	90	+	90	+	+	−	−			+
S. salivarius	+	+	−	V	−	−	−	−	90	V[c]	80[d]	+	70	−			+
S. sanguis																	
1	−	+	+	+	−	+	−	−	V	V	+[e]	+	−	−		+	
2	−	+	+	−	−	+	−	10	V	V[c]	+[e]	+	+	−		+	
S. vestibularis	V	+	−	ND	ND	+	−	−	−	−	V	+	+	−		+	

[a] +, Always or nearly always positive; −, negative or nearly always negative; V, variable, or about half of isolates positive. Numerals represent percent positive when enough information is available from literature to make an estimate. V-P, Voges-Proskauer; ND, no data. For hemolysis, + indicates only the usual type; exceptions are frequent.
[b] Data from API Rapid Strep. In reference 32, the percentages were 0 and 100.
[c] More often positive if glucan is produced (23, 92).
[d] 93% in reference 32.
[e] More often negative in strains than do not make glucan (23).

S. salivarius and vice versa. Thus, colony morphology cannot be relied upon to differentiate these two species. See reference 32 for a comparison of phenotypic traits, colony morphology, API identification, and DNA identification of *S. bovis* and *S. salivarius* strains.

Although an occasional isolate may react with group D antiserum, and more can be shown to have traces of a D-reactive material (102), this antigen is not identical to the authentic D antigen (102). Neither Parker and Ball (92) nor Bratthall and Carlsson (13) found *S. salivarius* isolates that reacted with group D reagents. However, at least half (92) or more (13) joined Lancefield group K.

When faced with the task of identifying an isolate that may be either *S. bovis* or *S. salivarius*, the advice of Ruoff et al. (102) is appropriate: a broad range of tests should be used. They recommended careful serology, bile-esculin, mannitol, inulin, starch, and urease tests. They suggested use of a commercial system for biochemical tests and advised that fatty acid analysis would show a greater amount of eicosenoic acid in *S. salivarius* and could be used to support identification.

S. VESTIBULARIS

A New Species

As this review approached completion, a new, oral, alpha-hemolytic species was described and named *S. vestibularis* (124). The species includes strains that were studied by Carlsson (18, 19) but that defied assignment to any described species. These strains split esculin, starch, and urea like *S. salivarius*, but produced hydrogen peroxide like *S. mitis* and *S. sanguis*. They synthesized neither glucans nor levans.

DNA hybridization showed that these strains were not related to *S. anginosus*, *S. mitis*, *S. mutans*, or *S. sanguis* but shared some base sequences with *S. salivarius* and with *S. thermophilus*, which the authors considered a subspecies of *S. salivarius*. These streptococci also contained eicosenoic acid in common with *S. salivarius*. Yet the extent of DNA hybridization indicated that these strains were more closely related to each other than to either *S. salivarius* or *S. thermophilus* and should be considered a separate species, which was named *S. vestibularis*. Electrophoresis of the cellular polypeptides of strains within these species implied that *S. vestibularis* was more closely related to *S. salivarius* than to *S. thermophilus* (124).

Because DNA relatedness of any two of these three species was roughly 50%, while relatedness was <16% (and often <10%) with any one of them and the other oral streptococci, they could be considered a trio of microbial cousins similar to the genospecies within the current *S. sanguis* and *S. mitis*.

The hydrolysis of both starch and urea and the production of hydrogen peroxide distinguish *S. vestibularis*. The failure to ferment raffinose and the lack of extracellular polysaccharide production further separate the new species from *S. salivarius*. The clinical importance of this species awaits determination. *S. vestibularis* may account for some of the atypical *S. salivarius* strains in a large collection of clinical isolates identified by Colman and Ball (23), so it is likely that the species will occasionally be encountered and identified by the alert microbiologist.

CONCLUSION

Table 3 presents the biochemical characteristics of the common viridans streptococci. *S. mutans* is included, but characteristics of the other members of the mutans group shown in Table 2 are not repeated, nor does Table 3 incorporate the reactions of the unusual *S. sanguis* strains discussed above. The information was compiled from reports that tested many strains and that have been cited above, including the work of Carlsson (19), Colman and Williams (25), Parker and Ball (92), and Facklam (44). Our

own experience is included, and the API Rapid Strep information was used for alkaline phosphatase and β-glucuronidase, starch, and glycogen reactions.

The day will come when nucleic acid probes (119) will be available for the identification of these streptococci. The development of these tools depends on good taxonomy. Probes detect bacteria that are genetically related to the organism from which the probe was developed. Thus, if a DNA probe for *S. mutans* was derived from a fragment of the genome of a streptococcus proven to cause caries in animals, then we must know whether the strain is *S. mutans* or *S. sobrinus*. It is essential that probes for the viridans streptococci be developed from a foundation of molecular taxonomy. Those who would embark on a project involving gene probes must be cognizant of the taxonomic pitfalls.

For More Information

Considering the vast literature of the nonhemolytic streptococci, this review is a mere abstract. For additional information, there are many detailed reviews and other taxonomic treatises. For historical interest, Sherman's 1937 review (110), Andrewes and Horder (2), and Clarke's work (22) are instructive. The detailed surveys cited just above (19, 23, 25, 44, 92) are valuable. For *S. mutans*, the proceedings of a 1985 conference (60) and a review by Loesche (73) are good places to start. With its 600 references, an earlier review by Hamada and Slade (61) is still a bibliographic treasure. *S. anginosus* ("*S. milleri*") was reviewed twice in 1988 (56, 99). The genetic relationships and cell wall compositions of the entire genus, including the now separate genera *Enterococcus* and *Lactococcus*, were compiled in detail by Schleifer and Kilpper-Bälz (106). Although I disagree with their use of the name *S. oralis* for *S. mitis* and *S. mitis* for some *S. sanguis*, as discussed above, their paper represents a valuable handbook of streptococcal molecular taxonomy.

Final Thoughts

Science endeavors to free mankind from the anarchy and oppression of ignorance. In explaining complexity and mystery, it often seems to expand complexity. Thus it is with taxonomy. Newer methods have elucidated the natural genetic relationships among bacteria and have made the complex simple, such as in *S. anginosus*. Yet new strata of complexity have been uncovered in the cases of *S. sanguis* and the mutans group. Still we benefit, for who would want a vaccine against dental caries based on *S. rattus* when most children have *S. mutans* (73)? Who would develop a nucleic acid probe for human intestinal *S. bovis* by cloning a gene from the unrelated type strain (71)? I hope that in this review elucidation has outweighed obfuscation and that the correlation of genetic types and phenotypes and the application of reasonable, valid names is more useful than theoretical, for a clearer, more logical taxonomy should serve identification, help explain disease, and guide therapy.

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ADDENDUM IN PROOF

At a meeting of the Streptococcus Working Group (London, 16 April 1989), the members present endorsed the division of *S. sanguis* into *S. sanguis* and *S. gordonii* and supported the rejection of the *S. mitis* type strain. Also recommended was the maintenance of *S. oralis*, with the name *S. mitis* reserved for the second genetic group (34) of *S. mitis* ("*S. mitior*") only (M. Kilian, manuscript in preparation).

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