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Physiological Differentiation of Viridans Streptococci

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Twelve hundred and twenty-seven clinical isolates and eighty stock strains of viridans streptococci were tested for serological and physiological characteristics. Because the serological reactions of these strains varied, a differentiation scheme could not be based on these reactions. For the same reason, there could be no correlation of serological characteristics with physiological characteristics. Nearly 97% of the clinical isolates were speciated by differences in physiological characteristics. Ten different physiological species were recognized. The physiological speciation scheme was based on stable enzymatic reactions rather than on results of tolerance tests. The study included air-tolerant anaerobic streptococcal strains as well as viridans streptococcal strains not normally found in humans. The differentiation scheme and nomenclature of the author are related to those of other investigators. Differences in the distribution of species isolated from different clinical sources and human infections were also noted. A key for the differentiation of human isolates of viridans streptococci is proposed.

The viridans streptococci are a heterologous group of very poorly defined organisms belonging to the genus *Streptococcus*. Fifty-four percent of all bacterial endocarditis in humans is caused by viridans streptococci (40), and in a recent survey of streptococcal bacteremia, the viridans streptococci were identified in 54% of the cases (35). Despite the common occurrence of these strains in human systemic infections, satisfactory differentiation schemes are not available. The most authoritative taxonomy text does not recognize all the viridans species, and the schemes recommended for differentiation of the accepted species are not practical (18).

The viridans streptococci have several common characteristics. (i) They are generally susceptible to penicillin, and patients with systemic infections are generally treated with penicillin rather than with the combined antibiotic therapy used for enterococcal infections. Thus, a patient can be treated effectively regardless of whether the viridans *Streptococcus* causing the infection has been specifically identified. This has been one of the major factors delaying the development of differentiation schemes by clinical microbiologists.

(ii) They are not beta-hemolytic on blood agar plates. An occasional strain of *Streptococcus mutans* may give a very atypical beta-hemolytic reaction on blood agar (23, 53), but most laboratories would interpret the reaction as alpha-hemolytic. (iii) Most strains do not have defined carbohydrate antigens (group antigens). This is in distinct contrast to the vast majority (99.5%) of beta-hemolytic streptococci from human infections, which have demonstrable group antigens (R. R. Facklam, unpublished data). These antigens are of considerable importance in differentiating the beta-hemolytic streptococci. With the exceptions of the group D streptococci, however, serological studies of the viridans streptococci have not yielded satisfactory identification schemes.

Lancefield attempted to use nucleoprotein and carbohydrate antigens in several serological tests (44, 45). She found cross-agglutination and cross-precipitation among the carbohydrate antigens. The nucleoprotein antigens were apparently homologous among the four strains tested. Lancefield concluded, however, that viridans streptococci could not be serologically differentiated. Soloway (59) investigated 107 blood isolates and 98 throat isolates of viridans streptococci. She placed 70% of each of these isolates in 14 serogroups. However, a physiologically defined species, *Streptococcus salivarius*, was identified in all serogroups. There was no observed correlation between physiological and serological characteristics (59). Selbie et al. (56) also reported on a serological analysis of blood, throat, and dental plaque isolates of viridans streptococci. Twenty antisera were prepared, and five serological "groups" were defined according to the patterns of serological reactions. The strains from different sources were evenly distributed throughout the five groups, but the physiological charac-

teristics did not correlate with the serological reactions.

Williamson (66) presented a serological classification system of viridans streptococci isolated from the respiratory tract. He eliminated *S. salivarius, Streptococcus pneumoniae,* and the enterococci by physiological characteristics and proposed seven serotypes of *Streptococcus mitis*. He used slide agglutination tests instead of the precipitin test used by Soloway (59) and Selbie et al. (56). He found numerous cross-reactions among his antisera and pneumococcal antisera. The results of his study are difficult to interpret because he did not take into account already established antigens shared among these and related streptococci. The possibility that these strains contain one or more of the antigens of *Streptococcus sanguis* (20, 26, 27, 32, 33, 46, 54, 61, and 62), *S. salivarius* (57, 65), and *Streptococcus* MG and the indifferent streptococci (48, 52) was not considered. Williamson also observed no correlation between the serological results and the physiological characteristics of his strains.

Recently, Austrian and his colleagues (1, 37–39) proposed extending the pneumococcal typing system to include the viridans streptococci. Their results are very difficult to interpret because they described very few physiological or hemolytic properties of the strains. Strains of group M and K streptococci were shown to possess capsular antigens similar to those of the pneumococci. Some of the strains (optochin resistant, bile insoluble) cross-reacted with established pneumococcal types, but others did not. Seventy-five percent of 248 strains that did not cross-react with pneumococcal antisera reacted with one or another of 24 new serotypes.

All of the attempts to use serological systems to differentiate the viridans streptococci have failed to show a correlation between the serological and physiological characteristics of these strains. One of the problems has been that most investigators have not sufficiently investigated the physiological properties to achieve differentiation. A large number of physiological characteristics must be determined if the species are to be separated on that basis.

Carlsson (5, 6), Colman and Williams (9–13), and Hardie and Bowden (30) have extensively investigated the physiological characteristics of the viridans streptococci. Colman and Williams used transformation studies and cell wall analysis to supplement their studies. They also used computer analysis of their data to show relationships between the species, as did Carlsson. Although Carlsson did not claim to have established a differentiation scheme, Colman and Williams (13) and Hardee and Bowen (30) observed some correlation between their results and the phenons Carlsson described.

Carlsson (5) tested 89 strains of oral streptococci for 70 different characteristics. The tests are too numerous to list, but they included all the common physiological tests used to differentiate the viridans streptococci. Carlsson tested the strains for: acid reactions in mannitol, sorbitol, lactose, inulin, raffinose, and trehalose broths; hydrolysis of esculin, arginine, and hippurate; hemolytic reaction on blood agar, glucan formation on 5% sucrose agar; tolerance to various pH limits of growth; and tolerances to salt and bile concentrations in culture media. Carlsson found the tolerance tests the least reproducible. He described seven phenons; five were related to known physiologically described species, *S. mutans*, *S. salivarius*, *S. sanguis*, *S. mitis*, and a second division of *S. sanguis*; the other two phenons showed no correlation with known species.

Colman and Williams (13) used the following major characteristics to differentiate 364 strains: hemolysis on blood agar; formation of glucans; hydrolysis of arginine, esculin, and starch; acid formation in inulin, trehalose, salicin, mannitol, and sorbitol broths; tolerance to growth at 45 and 10°C and on 10 and 40% bile; susceptibility to bacitracin and nitrofurazone; production of acetoin from glucose; and the presence of rhamnose and ribitol in the cell walls.

Colman and Williams (13) proposed five species of viridans streptococci on the basis of physiological characteristics; *S. salivarius, S. milleri, S. mitior, S. sanguis,* and *S. mutans*. They included *S. pneumonia* in the viridans group, but these strains can be easily differentiated by their susceptibility to optochin and solubility in bile salts. None of the viridans species are susceptible to optochin, and none are soluble to bile.

Hardie and Bowden (30) used nearly the same physiological tests that Colman and Williams described. They did not include susceptibility to bacitracin and nitrofurazone or cell wall analysis. One hundred and fourteen oral strains were tested for 20 physiological characteristics. They concluded that *S. mutans, S. sanguis, S. mitior, S. milleri,* and *S. salivarius* could be presumptively identified by seven characteristics: acid formation in mannitol and sorbitol broth; hydrolysis of arginine and esculin; production of acetoin from glucose; hydrogen peroxide production on chocolate agar; and glucan production on sucrose agar.

These three groups of investigators did not include two species of viridans streptococci, *S.*

acidominimus and *S. uberis*, in their studies. Although these two species are rarely found in humans, their characteristics should be included in differentiation schemes.

Recently, Holdeman and Moore (34) proposed that the air-tolerant anaerobic streptococci should be classified as streptococci. The relationship of the air-tolerant anaerobic streptococci to the viridans streptococci has not been studied. Certain physiological characteristics of the air-tolerant streptococci are similar to those of known viridans streptococcal species. This led us to believe that these strains could be identified in a physiological differentiation scheme as members of the viridans streptococci.

Our study is retrospective, i.e., the key for differentiating species was designed after serological and physiological data were accumulated on more than 1,000 strains. We will show that the key can be used, in a practical way, to differentiate clinical isolates of viridans streptococci.

MATERIALS AND METHODS

Strains. Stock strains of microorganisms used in the study are listed in Table 1. In addition, stock strains of beta-hemolytic streptococci groups A, B, C, D, E, F, G, L, M, U, and V, taken from the culture collection of the Center for Disease Control (CDC), were tested for physiological characteristics. Stock strains of alpha-hemolytic strains of groups D, H, K, N, Q, R, S, and T (CDC culture collection) were also tested for physiological characteristics. The 1,227 clinical isolates were received from various city and state public health laboratories and private institutions in the United States. They comprise all the human viridans strains received for identification at the *Streptococcus* Laboratory, Bacteriology Division, CDC between January 1969 and December 1975.

Hemolysis. Hemolysis was determined by the pour plate method. The method of preparation (25) and reading (60) have been previously defined.

Grouping. Lancefield extracts were prepared from glucose-supplemented Todd-Hewitt broth (21). The inverted capillary precipitin (capillary ring) test was used to determine group reactions (31). Antisera for the streptococcal grouping of groups A, B, C, D, E, F, G, H, K, and O were obtained from the Biological Reagents Section of CDC. Antiserum for variant A was prepared in the CDC *Streptococcus* Laboratory. Extracts of stock strains of groups L, M, N, P, Q, R, S, T, U, and V streptococci were reacted with group-specific antisera obtained from C. E. De Moor, Utrecht, Netherlands; B. Perch, Copenhagen, Denmark; and R. Shuman, Ames, Iowa.

Physiological tests. The method for determining arginine hydrolysis used in the study was described by Niven et al. (51). The preparation and interpretation of all other tests have been previously described (21-25).

TABLE 1. *Stock strains of viridans streptococci*

Species	Sender's identification	Reference
S. acidominimus	M-13573	19
	M-13582	19
S. anginosus	SS-1111	CDC[a]
	SS-1120	CDC
S. constellatus	ATCC-27823	34
Streptococcus MG	ATCC-9895	67
	ATCC-15910	67
	ATCC-15912	67
	ATCC-15913	67
	RJ-1	Thomson[b]
S. intermedius	ATCC-27823	34
S. mitis	SS-429	CDC
	ATCC-15911	67
	CHT	36
	ATCC-9811	Thomson
S. morbillorium	ATCC-27824	34
	SS-1087	CDC
S. mutans	32 strains, see reference 23	23
S. salivarius	SS-262	CDC
	HHT	36
	SS2	28
	RBA	Thomson
S. sanguis biotype I	ATCC-10556	7, 15, 43
	ATCC-10558	7, 15, 43
	NCTC-10231	7
	NCTC-7863	7
	Blackburn	7
	Challis	7
	Wicky	7
	Channon	7
	ATCC-8144	CDC
	ATCC-12396	CDC
S. sanguis biotype II	JC-67	6
	JC-74	6
	S3	Thomson
	ATCC-15909	67
	ATCC-15914	67
	ATCC-10557	7, 8, 15, 43
	NCTC-7864	7
S. uberis	010	16, 17
	011	16, 17
	0100	16, 17
	0102	16, 17
	0217	16, 17
	0237	16, 17
	0241	16, 17
	153	59
	322	59
G. hemolysans	ATCC-10379	3

[a] Strains were taken from the CDC collection.

[b] Obtained from L.A. Thomson, National Institute of Dental Research, Bethesda, Md.

RESULTS

Serological reactions of stock strains. Lancefield extracts were prepared from all of the viridans stock strains listed in Table 1.

Some of the reactions developed rapidly (visible within 1 to 2 min), but others developed very slowly (visible only after 25 to 30 min). None of the extracts reacted with group B or D antisera.

The extract of strain ATCC-15914 (*S. sanguis* II; ATCC, American Type Culture Collection) reacted weakly with group A antisera. No precipitin line formed in an Ochterloney gel diffusion test; thus, its true relationship to group A streptococci (*S. pyogenes*) was not determined. Extracts that reacted weakly in capillary precipitin tests did not react in gel diffusion tests and were thus interpreted as cross-reactions due to heterologous antibodies in the antisera for *Streptococcus* grouping.

Extracts of *S. sanguis* I strain Channon reacted strongly with group C antiserum. A line of homology formed between the extracts of strains Channon and the group C vaccine strain (*S. equisimilis*) with group C antiserum. We interpreted these results as indicating that strain Channon possessed the antigenic determinate of group C streptococci.

Extracts of *S. mutans* (LM-7 and P-2) and *S. uberis* (0100, 0237, 153, and 322) reacted with group E antiserum. Cullen (16, 17) attributed the reactions of *S. uberis* extracts with group E antiserum to the fact that some *S. uberis* strains possessed the hapten portion of the group E antigen. Bratthall and Pettersson (4) regarded the reaction of extracts of *S. mutans* with group E antiserum as a result of there being closely related but not identical antigens in *S. mutans* and group E streptococci. The degree of relatedness between *S. mutans* type e and group E streptococci was dependent on the antiserum used. Some antisera gave partial identity between the two antigens, but others did not. We investigated these reactions only in capillary precipitin test.

The extract of *S. sanguis* I strain ATCC-10558 reacted weakly with group F antiserum in the capillary precipitin test. Extracts of *Streptococcus* MG strains ATCC-9895 and RJ-1 reacted strongly with group F antiserum. The group F reactions were not investigated in gel diffusion.

Extracts of *S. sanguis* II strains (JC-74 and S-3) and *Streptococcus* MG strain (ATCC-15912) reacted with group G antiserum. Strong reactions were observed in the capillary precipitin test but were not further investigated.

The extract of *S. sanguis* I strain ATCC-8144 reacted with its homologous antiserum. Lancefield gave this strain the designation K208, which CDC used for group H antiserum production. Unfortunately, no extracts of any other *S. sanguis* I strains reacted with K208 antiserum. One commercial company also uses this strain for vaccine to prepare group H antiserum, but several other companies use the strain Blackburn (strain no. NCTC-7863, National Collection of Type Cultures [NCTC]).

The extract of *S. sanguis* II strain ATCC-15909 reacted with group K antiserum. This was not totally unexpected, because the CDC stock strains of group K streptococci (Prague 3/50, CDC SS-806, SS-807, and De Moor's K/CN477) are physiologically identical to the *S. sanguis* II strains. The group K strains do not produce extracellular polysaccharides, but about 50% of the *S. sanguis* II strains do. The CDC group K antiserum was produced with *S. salivarius* strain SS-262, which does produce extracellular polysaccharide. Extracts of all the *S. salivarius* strains reacted with the group K antiserum. Extracts of *S. mitis* strain CHT and *Streptococcus* MG strain ATCC-15910 also reacted with group K antiserum and are presumably antigenically related to the group K, *S. salivarius*, and *S. sanguis* II streptococci.

Extracts of two *S. sanguis* II strains (ATCC-15914 and JC-67) and one *S. mitis* strain (ATCC-15911) reacted with the grouping antiserum for variant A. These reactions were very weak and probably represent cross-reactions in the antiserum for variant A.

All of the stock strains of groups H, K, and O are alpha-hemolytic in poured blood agar plates. The group H streptococci are physiologically *S. sanguis* I. The group K streptococci may be either *S. sanguis* II, as most of the stock strains are, or *S. salivarius* physiologically. All the stock strains of group O streptococci are physiologically similar to *Streptococcus* MG. CDC strains SS-533, SS-808, SS-809, and ATCC-12391 are strains that Mukasa and Slade (50) considered to belong to group O, and CDC strains SS-669 and DS-1234, which Mukasa and Slade demonstrated to have a type antigen but not the group O antigen, are all physiologically homologous.

Serological results of clinical isolates. Table 2 shows the results of the grouping reactions of 1,227 clinical isolates of viridans streptococci. The heterology of serological reactions indicated that serological characteristics could not be correlated with physiological characteristics. Strains in column 11 are strains that did not fit into any of the 10 physiological patterns; they will be discussed later. No serological reactions were uniquely related to any set of physiological characteristics. Twenty-four percent of the strains reacted with the nine antisera. Group F and K antisera were the most reactive; 8.2% (F) and 6.7% (K) of the extracts of viridans strains

TABLE 2. *Number of viridans streptococcal clinical isolates giving serological reactions*

Grouping reaction	Clinical isolates												
	S. uberis	*S. mutans*	*S. sanguis* I	*S. salivarius*	*Streptococcus* MG-*intermedius*	*S. sanguis* II	*S. mitis*	*S. anginosus-constellatus*	*S. morbillorium*	*S. acidominimus*	Not differentiated	Total	%
A	0	0	0	0	9	6	4	2	0	0	0	21	1.7
C	0	0	4	0	6	6	4	1	0	0	1	22	1.8
E	1	11	0	0	0	0	0	0	0	0	0	12	1.0
F	0	3	22	3	53	4	2	8	0	1	5	101	8.2
G	0	0	0	0	3	2	2	2	0	0	0	9	0.7
H	0	0	0	0	0	1	1	0	0	0	0	2	0.1
K	2	2	5	35	2	18	16	0	0	0	3	83	6.7
O	0	0	0	0	0	3	1	0	0	0	0	4	0.3
Avar	0	1	1	0	6	11	6	0	0	0	0	25	2.0
C/K	0	0	0	0	0	3	0	0	0	0	0	3	0.2
A/C/G	0	0	0	0	0	4	0	0	0	0	0	4	0.3
A/C/G/ Avar	0	0	0	0	0	10	1	0	0	0	0	11	0.9
None	4	135	170	43	152	163	140	42	0	4	30	929	75.7
Total	7	152	202	81	231	231	177	55	46	5	40	1,227	

reacted with each antisera. Unfortunately, seven different physiological types reacted with group F, and six different physiological types reacted with K. Multiple reactions with more than one antiserum were observed in 18 instances (1.4%). These facts invalidated serogrouping as a useful tool for differentiating the viridans streptococci.

Physiological reactions of stock strains. Ten distinct physiological patterns were noted among the stock strains of the viridans cultures (Table 3). All of the strains are characterized as not having the group B or D antigen and give either alpha-hemolytic or nonhemolytic reactions on blood agar, except for the atypical beta-hemolytic reaction of *S. mutans*. Most strains did not grow at 10°C (the only exceptions were strains of *S. uberis*) or in 6.5% NaCl broth (several strains of *S. mutans* and *S. uberis* tolerated 6.5% NaCl). None of the strains tolerated 0.04% tellurite or formed acid in arabinose or glycerol broths.

The physiological characteristics of *S. mutans* are shown in column 1 of Table 3. The distinguishing characteristics were acid formation in mannitol broth, glucan production on 5% sucrose agar and broth, and failure to hydrolyze hippuric acid.

The physiological characteristics of *S. uberis* are shown in column 2 of Table 3. The distinguishing characteristics were acid formation in mannitol broth, failure to form glucan in 5% sucrose broth and agar, and hydrolysis of hippuric acid. Although *S. mutans* and *S. uberis* share a number of physiological characteristics, they are easily differentiated by their morphology on blood agar plates and by differences in the hydrolysis of hippurate and glucan production. *S. mutans* strains grew scantly on the surface of blood agar plates and often required additional CO_2 for growth, but *S. uberis* grew luxuriously, as did the group D streptococci.

The physiological characteristics of typical *S. sanguis* (designated *S. sanguis* I) are listed in column 3 of Table 3. The distinguishing characteristics were acid formation in inulin, lactose, and sucrose broths, and most strains were alpha-hemolytic, hydrolyzed arginine, and formed glucans in both 5% sucrose broth and agar; none of the strains formed acid in mannitol broth. Glucan production in 5% sucrose broth caused the medium to gel completely or partially. A partial gel reaction was described as a gelled button of growth at the bottom of the tube. These strains produced adherent refractile or adherent dry colonies on 5% sucrose agar, a characteristic trait of dextran production.

The physiological characteristics of *S. salivarius* are listed in column 4 of Table 3. The distinguishing characteristics were acid formation in inulin, lactose, sucrose, and raffinose broth, nonhemolytic reaction on blood agar, formation of glucan on 5% sucrose agar but not broth, and failure to hydrolyze arginine or to form acid in mannitol broth. One strain (S-2) did not form acid in lactose broth, but this was accepted as an atypical reaction when all other characteristics were typical. The glucan formation on 5% sucrose agar was characterized by

TABLE 3. *Percentage of viridans streptococcal stock strains giving physiological reactions*[a]

Test	Stock strains									
	S. mutans	*S. uberis*	*S. sanguis* I	*S. salivarius*	*Streptococcus* MG-*intermedius*	*S. sanguis* II	*S. mitis*	*S. anginosus-constellatus*	*S. mirbillorium*	*S. acidominus*
Hemolysis										
Alpha-	31	54	100	0	50	86	100	67	67	100
Beta-	3	0	0	0	0	0	0	0	0	0
None	66	44	0	100	50	14	0	33	33	0
Tolerance to:										
Bile esculin	53	0	0	0	17	0	0	0	0	0
Methylene blue milk	0	0	10	0	17	0	0	0	0	0
10°C	0	89	0	0	0	0	0	0	0	0
45°C	90	89	100	50	67	86	75	0	0	0
6.5% NaCl	16	67	0	0	0	0	0	0	0	0
4.0% NaCl	50	100	0	0	50	0	0	33	0	0
2.0% NaCl	97	100	100	100	100	100	100	100	0	50
10% Bile	34	100	0	50	100	43	75	100	0	100
40% Bile	22	22	30	25	50	14	0	100	0	100
0.04% Tellurite	0	0	0	0	0	0	0	0	0	0
0.1% Tetrazolium	37	56	0	25	35	14	0	0	0	0
Growth in litmus milk	100	100	100	100	100	100	100	100	0	50
Hydrolysis of:										
Arginine	0	100	90	0	50	29	25	33	0	0
Esculin	88	100	80	100	100	0	0	100	0	50
Hippurate	0	100	0	0	0	0	0	0	0	100
Starch	0	33	50	25	17	29	50	33	0	0
Acid from broths of:										
Mannitol	100	100	0	0	0	0	0	0	0	0
Sorbitol	91	100	0	0	0	0	0	0	0	0
Inulin	100	100	100	100	0	0	0	0	0	0
Lactose	97	100	100	75	100	100	100	0	0	0
Raffinose	78	33	20	100	17	100	0	0	0	0
Sucrose	100	100	100	100	100	100	100	100	100	100
Trehalose	100	100	0	50	83	71	25	67	0	50
Salicin	100	100	80	100	100	43	75	100	0	0
Melibiose	33	0	0	0	17	71	33	0	0	0
Arabinose	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0
Reaction in 5% sucrose broth:										
Gel	0	0	50	0	0	14	0	0	0	0
Partial gel	97	0	30	0	17	29	25	0	0	0
None	3	100	20	100	83	57	75	100	100	100
Reaction on 5% sucrose agar:										
Adherent, refractile	80	0	75	0	0	43	25	0	0	0
Adherent, dry	20	0	5	0	0	0	0	0	0	0
Nonadherent, gummy	0	0	0	100	0	0	0	0	0	0
Nonadherent, mucoidal	0	100	20	0	100	57	75	100	100	100

[a] The same strains are listed in Table 1.

the formation of large, fleshy, gummy, nonadherent colonies, typical of levan formation.

The *S. mitis* strains of Williamson (66) were differentiated into three reaction patterns that were basically associated with three physiological species: *S. intermedius* or *Streptococcus* MG, dextran-forming *S. mitis* (designated here as *S. sanguis* II), and non-dextran-forming *S. mitis*. The three species had similar but recognizably different physiological characteristics. The physiological characteristics of *Streptococcus* MG and *S. intermedius* were not recognizably different from the set of physiological reactions listed in Table 3. The physiological characteristics of most dextran-forming *S. mitis* were not different from *S. sanguis* (ATCC-10557). Dextran-forming and non-dextran-forming strains were placed in the described set of characteristics designated as *S. sanguis* II; the set includes glucan production as a variable characteristic.

The physiological characteristics of *Streptococcus* MG and *S. intermedius* are listed in column 5 of Table 3. The distinguishing characteristics were the formation of acid in lactose and sucrose broth and the failure of these strains to form acid in mannitol or inulin broth. The hydrolysis of esculin by these strains distinguished them from the *S. sanguis* II and *S. mitis* strains. None of these strains formed glucans on 5% sucrose agar or broth. Half of the strains were alpha-hemolytic and half were nonhemolytic on blood agar.

All the stock strains of group O streptococci had physiological characteristics identical to those listed for *Streptococcus* MG-*intermedius*.

The physiological characteristics of *S. sanguis* II are listed in column 6 of Table 3. The distinguishing characteristics were formation of acid in lactose, sucrose, and raffinose broths and failure to form acid in mannitol and inulin broths. These strains did not hydrolyze esculin, which distinguished them for *Streptococcus* MG-*intermedius*, but did form acid in raffinose broth, which distinguishes them from *S. mitis*. Most strains were alpha-hemolytic, and about half of the strains formed glucan (dextran) on 5% sucrose agar and broth. Stock strains of group K streptococci did not form glucans but had all the other physiological characteristics of the *S. sanguis* II strains.

The physiological characteristics of *S. mitis* are listed in column 7 of Table 3. The distinguishing characteristics of *S. mitis* were the formation of acid in lactose and sucrose broths and the failure of strains to form acid in mannitol, inulin, and raffinose broths. All stock strains were alpha-hemolytic on blood agar and did not hydrolyze esculin. One strain hydrolyzed arginine (ATCC-15911), and one strain (ATCC-9811) formed glucan on 5% sucrose agar and broth. Beta-hemolytic group A (the majority), group C (*S. equisimilis* and *S. equi*), group L, and group M streptococci had physiological characteristics (except for the hemolytic reaction) that place them with *S. mitis*. Beta-hemolytic group C streptococci (*S. zooepidemicus*) had unique physiological characteristics that differentiated them from other beta- and alpha-hemolytic species. Strains of beta-hemolytic group G streptococci had physiological characteristics similar to those of *Streptococcus* MG-*intermedius*, *S. mitis*, and *S. anginosus*.

Three species of viridans streptococci did not form acid in mannitol, sorbitol, inulin, and lactose broths. These three species were differentiated by hydrolysis of esculin and hippurate, growth in litmus milk, and tolerance to 10 and 40% bile media. The physiological characteristics of *S. anginosus* are listed in column 8 of Table 3. *S. constellatus* was physiologically identical to *S. anginosus*. All stock strains of beta-hemolytic group F streptococci also had the physiological characteristics listed in column 8, except for the hemolytic reaction. Some taxonomy texts list group F and G streptococci as *S. anginosus*. Strains of *S. anginosus* and *S. constellatus* were characterized by acid formation in sucrose broth and failure to form acid in mannitol, sorbitol, inulin, and lactose broths. They grew very slowly in litmus milk, and reduction was often not observed until 72 h. Most strains hydrolyzed esculin, and some strains hydrolyzed arginine. None formed glucans in 5% sucrose broth or agar.

The physiological characteristics of *S. morbillorium* are listed in column 9 of Table 3. The *Gemella hemolysans* strain (3) was physiologically identical to *S. morbillarium*. Gram stains showed that *G. hemolysans* cells were usually arranged in packets of four or in clumps, but *S. morbillorium* cells were usually arranged in pairs or very short chains (three to six cells). Sucrose was the only carbohydrate broth that was fermented. Tolerance to 10 and 40% bile was nonexistent, and none of the strains hydrolyzed arginine, esculin, or hippurate or produced glucans.

The physiological characteristics of *S. acidominimus* are listed in column 10 of Table 3. These strains were also characterized by their failure to form acid in most carbohydrate broths except sucrose. They hydrolyzed hippurate and tolerated 10 and 40% bile concentrations, thus differentiating them from *S. morbillorium* and *S. anginosus*. The most useful characteristics that differentiates *S. acidominimus* from the other viridans streptococci is the hydrolysis of

hippurate. Among the viridans streptococci, only *S. uberis* and *S. acidominimus* hydrolyze hippurate; however, *S. uberis* forms acid in mannitol broth, but *S. acidominimus* does not. Alpha-hemolytic and nonhemolytic varieties of group B streptococci may be erroneously identified as *S. uberis* or *S. acidominimus* unless additional characteristics are determined, because they also hydrolyze hippurate. Demonstrating the group and type antigens of the alpha-hemolytic and nonhemolytic group B streptococci is the best way to differentiate them from the viridans species.

The physiological characteristics of group D, Q, and N streptococci have been previously discussed (21). Each species of these streptococci has unique physiological characteristics or combinations of characteristics that differentiate them from the viridans species. None of 30 group D, 6 group Q, or 6 group N streptococci had physiological characteristics similar to any of the 10 reaction patterns listed in Table 3 except for the strains termed "*S. bovis* variants" (21), which have similar characteristics to *Streptococcus* MG-*intermedius*. *S. bovis* variants were included in the group D streptococci only when these strains were shown to have the group D antigen.

Beta-hemolytic groups E, P, U, and V streptococci are not found in humans. They have similar physiological characteristics and can be differentiated only by serological characteristics. In addition, the physiological characteristics of these streptococci were different from those in any of the 10 patterns in Table 3.

The hemolytic properties of group R, S, and T streptococci were difficult to define. Strains appeared to be a mixture of alpha- and beta-hemolytic colonies in subsurface growth. Generally, the strains were judged as alpha-hemolytic when the retesting of single-colony isolates did not resolve the problem. These strains are rarely found in humans; only group R strains have been reported in a few cases of meningitis in humans. If any of these strains were encountered, they would have been identified as *S. sanguis* I or *S. salivarius*, depending upon hydrolysis of arginine and hemolysis. If encountered, they probably would have been identified as non-glucan-forming *S. sanguis* I.

Physiological reactions of clinical isolates. Table 4 shows the percentage of positive physiological reactions of 1,187 clinical isolates of viridans streptococci. Forty (3.3%) of the strains did not fit the reaction patterns listed in Tables 3 and 4. The 10 reaction patterns are identical to those listed in Table 3. Only seven *S. uberis* and five *S. acidominimus* strains were identified from the 1,227 viridans isolates. The other eight reaction patterns had between 46 and 231 isolates each.

Of the 30 physiological characteristics, the hemolytic reaction, the bile-esculin reaction, growth at 10°C, tolerance to 6.5% NaCl broth, reduction of litmus milk, hydrolysis of esculin and hippurate, acid formation in mannitol, inulin, lactose, and raffinose broths, and glucan formation in 5% sucrose broth and agar were the most useful. Growth in methylene blue milk, tolerance to 40% bile, hydrolysis of arginine, and acid formation in sorbitol, sucrose, and melibiose were useful but less critical characteristics. Characteristics that were of little or no value in differentiating the strains were grown at 45°C, tolerance to 2 and 4% NaCl broth, tolerance to 10% bile, 0.04% tellurite, and 0.1% tetrazolium chloride, hydrolysis of starch, and acid formation in trehalose, salicin, arabinose, and glycerol broths.

The hemolytic reaction in poured blood agar plates helped to differentiate between the *S. sanguis* I strains (94% alpha-hemolytic and the *S. salivarius* strains (90% nonhemolytic). All hemolytic reactions were read with the aid of a microscope by focusing on the edge of subsurface colonies. By using a microscope and the definition of hemolysis according to Brown (60), an objective rather than a subjective description can be applied to streptococcal hemolysis. The hemolytic reactions of strains other than *S. sanguis* I and *S. salivarius* were about equally divided between alpha-hemolytic and nonhemolytic.

The bile-esculin reaction, tolerance to methylene blue milk, growth at 10°C, and tolerance to 6.5% NaCl are useful because these are characteristics of the group D, Q, and N streptococci (21). All group D and Q strains give positive bile-esculin reactions. The enterococcal group D strains grew at 10°C and in 6.5% NaCl broth and reduced methylene blue milk. Group N streptococci grow at 10°C and reduce methylene blue milk but do not give positive bile-esculin reactions. Group N streptococci are not found in human infections. Only two species, *S. mutans* and *Streptococcus* MG-*intermedius*, showed any degree of reactivity on bile-esculin medium (Table 4). Most other species were nonreactive. One strain of *S. uberis* grew at 10°C, but no other strains of that or any other species did. A few strains of several species reduced methylene blue milk and tolerated 6.5% NaCl broth; these strains were, however, a very minor portion of the collection.

Because of the variability of positive reactions, growth at 45°C was of no value in differentiating between the group D and the various viridans species of streptococci. About 50% of

TABLE 4. *Percentage of 1,187 viridans streptococcal clinical isolates giving physiological reactions*[a]

Test	Clinical isolates									
	S. mutans	*S. uberis*	*S. sanguis* I	*S. salivarius*	*Streptococcus* MG-*intermedius*	*S. sanguis* II	*S. mitis*	*S. anginosus-constellatus*	*S. morbillorium*	*S. acidominimus*
Hemolysis:										
Alpha-	59	57	94	10	45	95	92	40	50	40
Beta-	11	0	0	0	0	0	0	0	0	0
None	29	43	6	90	55	5	8	60	50	60
Tolerance to:										
Bile esculin	27	0	2	1	15	0	0	0	0	0
Methylene blue milk	1	0	9	2	11	7	7	7	0	0
10°C	0	14	0	0	0	0	0	0	0	0
45°C	42	0	63	69	48	60	40	22	13	0
6.5% NaCl	4	57	0	0	2	2	3	0	0	0
4.0% NaCl	44	86	28	21	34	18	14	18	1	0
2.0% NaCl	97	86	95	90	94	91	79	87	33	20
10% Bile	71	100	73	59	68	34	28	49	0	100
40% Bile	50	100	42	28	52	16	14	38	0	100
0.04% Tellurite	0	0	0	0	0	0	0	0	0	0
0.1% Tetrazolium	26	0	2	2	14	1	2	4	0	10
Reduces litmus milk	100	100	99	94	100	100	100	100	0	16
Hydrolysis of:										
Arginine	1	40	64	0	26	21	16	24	0	33
Esculin	90	86	77	91	100	0	0	73	0	0
Hippurate	0	57	0	0	0	0	0	0	0	100
Starch	3	57	56	7	23	30	33	20	9	0
Acid from broths of:										
Mannitol	100	100	0	0	0	0	0	0	0	0
Sorbitol	98	86	12	0	0	0	0	0	0	0
Inulin	99	71	100	100	0	0	0	0	0	0
Lactose	99	100	94	89	100	100	100	0	0	0
Raffinose	85	86	45	95	18	100	0	9	0	0
Sucrose	100	100	99	100	100	100	100	100	62	20
Trehalose	100	86	95	68	73	38	25	64	11	40
Salicin	92	100	91	88	84	28	28	60	4	0
Melibiose	38	86	38	19	18	82	1	6	0	0
Arabinose	0	57	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0
Reaction in 5% sucrose broth:										
Gel	0	0	38	0	3	17	3	0	0	0
Partial gel	88	0	34	0	7	26	7	0	0	0
None	12	100	28	100	90	57	90	100	100	100
Reaction on 5% sucrose agar:										
Adherent, refractile	75	0	80	11	10	44	12	0	0	0
Adherent, dry	21	0	4	0	2	5	1	0	0	0
Nonadherent, gummy	0	0	0	55	1	0	0	0	0	0
Nonadherent, mucoidal	4	100	16	34	87	51	87	100	100	100

[a] Same number for each species listed in Table 2.

the viridans strains grew at 45°C. Tolerance to 4.0% NaCl broth was also variable among the viridans species. About 25% of all strains were tolerant to 4.0% NaCl broth. Nearly all of the strains grew in 2.0% NaCl broth; thus, neither test was of value in differentiating the species. Tolerance to 10 and 40% bile was of value only when used with other physiological characteristics. Bile tolerance was of value when differentiating *S. morbillorium* from *S. acidominimus* and *S. anginosus*. None of the viridans strains tolerated tellurite; thus, this characteristic had no value as a differential. Only a few strains of several species reduced tetrazolium; thus, this characteristic too had no value as a differential. Reduction of litmus milk was of some help in differentiating *S. morbillorium* strains from *S. anginosus-constellatus* strains. None of the morbillorium strains reduced litmus milk, but all the *S. anginosus-constellatus* strains did.

The hydrolysis of arginine by 66% of the *S. sanguis* I strains (Table 4) helped to differentiate these strains from *S. salivarius* strains. None of the *S. salivarius* strains hydrolyzed arginine. The hydrolysis of esculin by all of the *Streptococcus* MG-*intermedius* differentiated these strains from both *S. mitis* and *S. sanguis* II. None of the *S. mitis* or *S. sanguis* II strains hydrolized esculin. The hydrolysis of esculin by 73% of the *S. anginosus-constellatus* strains helped to differentiate these strains from *S. morbillorium* and *S. acidominius* strains, none of which hydrolyzed esculin. The hydrolysis of hippurate by 57% of the *S. uberis* and 100% of the *S. acidominimus* strains helped to differentiate these two species from all viridans species, none of which hydrolyzed hippurate. The hydrolysis of hippurate was especially useful in distinguishing the *S. acidominimus* strains (100% positive) from the *S. morbillorium* strains (non-positive). The hydrolysis of starch by the viridans streptococci is a variable characteristic. The reaction is much weaker than that exhibited by the group D *Streptococcus, S. bovis* (22). The hydrolysis of starch by 57% of the *S. sanguis* I strains was of some value as a differential characteristic, because only 7% of the *S. salivarius* strains hydrolyzed starch. The hydrolysis of starch, however, was not as useful as the hemolytic reaction, hydrolysis of arginine, or glucan formation on 5% sucrose broth and agar as a differential characteristic of *S. sanguis* I strains and *S. salivarius* strains.

Acid formation in mannitol was useful because all the strains of *S. mutans* and *S. uberis* gave acid reactions, whereas none of the other viridans species did (Table 4). Acid formation in sorbitol was a useful but not critical indicator of the same two species. Twelve percent of *S. sanguis* I formed acid in sorbitol broth, thus limiting its usefulness as an indicator of *S. mutans* and *S. uberis*. Acid formation in inulin was a useful characteristic that helped to differentiate the mannitol-negative strains. All of the *S. sanguis* I and *S. salivarius* strains formed acid in inulin broth, but none of the other mannitol-negative species did. Acid formation in lactose broth was a useful characteristic that helped to differentiate the mannitol-, sorbitol-, and inulin-negative strains. All the strains of *Streptococcus* MG-intermedius, S. sanguis II, and *S. mitis* gave acid reactions in lactose broth, but none of the strains of *S. anginosus-constellatus, morbillorium,* and *acidominimus* did. Acid formation in raffinose broth was a useful characteristic that helped differentiate *S. sanguis* II strains from *S. mitis*. All the *S. sanguis* II strains formed acid, but none of the *S. mitis* strains did. Acid formation in sucrose broth was of very little value as a differential characteristic. Nearly all the isolates gave an acid reaction in sucrose broth. Acid reactions in trehalose and salicin broths were also of little value as differential characteristics. Most *S. morbillorium* and *S. acidominimus* strains did not form acid in either trehalose or salicin, but 64 and 60% of the *S. acidominimus* strains, respectively, formed acid in both broths. The hydrolysis of hippurate and bile tolerance were more accurate differential characteristics for these species. Acid reaction in melibiose broth was a useful characteristic of *S. sanguis* II strains, since 82% gave acid reactions; only 1% of the *S. mitis* strains, however, formed acid in melibiose broth. This helped to differentiate between the two species. Only 57% of the *S. uberis* strains produced acid reactions in arabinose broth. Thus, this characteristic had limited value as a differential. Glucan formation and colonial morphology were more useful differential characteristics of *S. uberis* and *S. mutans*.

Glucan formation in 5% sucrose broth was useful in differentiating *S. mutans* strains (88% positive) from *S. uberis* strains (none positive) (Table 4). It was also helpful in differentiating *S. sanguis* I strains (72% positive) from *S. salivarius* strains (none positive). Glucan production in 5% sucrose broth by *S. sanguis* II strains was a variable characteristic (43% positive), but very few strains of *Streptococcus* MG-*intermedius* (10% positive) and *S. mitis* (10% positive) and no strains of *S. anginosus-constellatus, S. morbillorium,* and *S. acidominimus* formed glucans.

Glucan production on 5% sucrose agar was similar to glucan production in 5% sucrose broth, except for the production of levan by *S. salivarius*. Fifty-five percent of the *S. salivarius* strains produced large, fleshy, nonadherent colonies typical of levan production. Two strains of *Streptococcus* MG-*intermedius* were the only other strains to grow in this fashion on 5% sucrose agar. Most *S. mutans* (96%) and most *S. sanguis* I (84%) produced adherent refractile or dry colonies, typical of dextran production, on 5% sucrose agar. Half (49%) of the *S. sanguis* II strains also produced dextran-like colonies, but only a few *Streptococcus* MG-*intermedius* (13%) and *S. mitis* (13%) produced glucans on this agar. None of the strains of *S. uberis, S. anginosus-constellatus, S. morbillorium,* or *S. acidominimus* produced glucans. Glucan production was most useful in differentiating between *S. uberis* and *S. mutans* and between *S. sanguis* I and *S. salivarius*.

The key to the differentiation of the viridans streptococcal species (Table 5) was designed from the data in Tables 3 and 4. We are not proposing this key as a rigid taxonomic tool, but as a guide for clinical microbiologists to use in differentiating the viridans streptococci from one another. It is important for the microbiologist to eliminate the beta-hemolytic streptococci and the alpha-hemolytic and nonhemolytic group B and D streptococci from the key. This is done by determining the correct hemolytic reaction, extraction, and serogrouping of the strains. Strains with group B or D antigens do not belong to the viridans streptococcal group. If extraction and serogrouping are not used, non-beta-hemolytic strains with two of the following characteristics should be excluded from the viridans streptococcal group: tolerance to bile-esculin, growth in methylene blue milk, tolerance to 6.5% NaCl broth, or growth at 10°C.

The tests outlined in Table 5 (i.e., acid formation in mannitol, lactose, inulin, and raffinose broths; hydrolysis of hippurate, arginine, and esculin; growth in litmus milk; tolerance to 40% bile; glucan production on 5% sucrose agar and broth; and hemolysis [alpha- or none]) were used to differentiate the species in the viridans group. Other acid reactions in carbohydrate broths, such as trehalose, salicin, and melibiose, were helpful but not necessary in differentiating the species.

Unspeciated strains. Among the 40 unspeciated strains, 4 of 5 had extracts that reacted with group F antiserum and had uniform physiological characteristics. All the strains formed acid in mannitol, lactose, sucrose, raffinose, salicin, trehalose, and melibiose. None of the strains formed acid in sorbitol, inulin, glycerol, or arabinose broth. All hydrolyzed esculin, grew in litmus milk, tolerated 40% bile and 2% NaCl broth; none tolerated 6.5% NaCl broth, hydrolyzed hippurate, or formed glucans on 5% sucrose agar or broth. Acid formation in mannitol was an unusual property of viridans species;

TABLE 5. *Key to the identification of viridans streptococci*[a]

Test used	Clinical isolate
Acid formed in mannitol and lactose broths	
Hippurate hydrolyzed, glucans not produced on sucrose agar or broth	*S. uberis*
Hippurate not hydrolyzed, glucans produced on sucrose agar and broth	*S. mutans*
Acid formed in lactose but not mannitol broth	
Acid formed in inulin broth	
Glucans (dextrans) produced on both sucrose agar and broth, NH_3 split from arginine, alpha-hemolytic	*S. sanguis* I
Glucans (levans) produced on sucrose agar only, no NH_3 from arginine, nonhemolytic	*S. salivarius*
Acid not formed in inulin broth	
Esculin hydrolyzed	*Streptococcus* MG-*intermedius*
Esculin not hydrolyzed	
Acid formed in raffinose broth	*S. sanguis* II
Acid not formed in raffinose broth	*S. mitis*
No acid formed in mannitol or lactose broth	
Hippurate hydrolyzed	*S. acidominimus*
Hippurate not hydrolyzed	
Esculin hydrolyzed, growth in 40% bile broth, litmus milk reduced weakly	*S. anginosus-constellatus*
Esculin not hydrolyzed, no growth in 40% bile broth, litmus milk not reduced	*S. morbillorium*

[a] Not beta-hemolytic. Extracts do not react with group B or D antisera. Occasional positive reaction on bile-esculin or methylene blue milk. Growth in 6.5% NaCl or at 10°C is rarely observed.

except for *S. mutans* and *S. uberis*, no other known member of the viridans group formed acid in mannitol broth. Seven other strains formed acid in mannitol, but did not resemble *S. mutans* or *S. uberis* in other physiological properties, i.e., glucans were not formed on 5% sucrose media and hippurate was not hydrolyzed. The Lancefield extract of one mannitol-positive strain reacted with group K antiserum, but the strain did not resemble the stock strains of group K or *S. salivarius* in physiological properties.

Twelve strains formed acid in sorbitol but not mannitol broth. The Lancefield extract of one of these strains reacted with group C antiserum. Acid formation in raffinose broth prevented these strains from being identified as the group C streptococcal species *S. dysgalactiae* (18). Extracts of two other strains reacted with group K antiserum. Acid formation in sorbitol was not observed among the stock strains of group K or *S. salivarius* streptococci, thus eliminating these stains as members of these two groups. One of the sorbitol-positive, group K-positive strains formed *S. salivarius*-like glucan (levan) on 5% sucrose agar.

Six satelliting streptococci were induced to grow in laboratory media after several transfers in thioglycolate broth. All strains grew poorly on laboratory media but were closely related to one another by formation of acid in inulin broth. These strains were excluded from the *S. sanguis* and *S. salivarius* species because of their satelliting property on initial isolation. Extracts of all of these strains did not react with any of the antisera used.

Ten additional strains that had very heterologous physiological properties were investigated. The extract of one of these strains reacted with group F antiserum. None of the extracts of the other nine reacted with the antisera used. The physiological reactions of these strains could not be correlated with those of any known species of streptococci.

Sources of clinical isolates. Table 6 shows the distribution of the viridans species isolated from various human sources. This by no means represents a species distribution of organisms found in various human infections, but the frequencies of occurrence of the various species are indicated. For example, among the endocarditis isolates, *S. sanguis* I is the most commonly isolated strain (29% of the subacute bacterial endocarditis isolates). *S. sanguis* II (21%) was the next most commonly identified strain, followed by *S. mutans* (18%) and *Streptococcus* MG-*intermedius* (14%). The distribution of species from the blood of patients with sepsis, pneumonia, and meningitis was somewhat different. Information submitted with these isolates indicated that many patients had debilitating diseases, such as tuberculosis, cancer, and diabetes. The most common species identified were *S. sanguis* II and *Streptococcus* MG-*intermedius* (21% each), followed by *S. mitis* (16%), *S. salivarius* (14%), and *S. sanguis* I (11%). *S. mutans*, the third most commonly isolated strain among the endocarditis patients, was the sixth most common strain (6%) among these patients.

TABLE 6. *Number of viridans streptococcal species identified from various human sources*

Source	Clinical isolate											
	S. uberis	*S. mutans*	*S. sanguis* I	*S. salivarius*	*S. sanguis* II	*S. mitis*	*Streptococcus* MG-*intermedius*	*S. anginosus-constellatus*	*S. acidominimus*	*S. morbillorium*	Not identified	Total
Blood[a]												
SBE	0	64	106	13	76	37	50	6	0	8	4	364
Sep Pn Men	1	16	29	32	57	43	56	13	0	13	6	266
NS	1	16	20	12	26	19	14	8	0	13	10	139
Brain abscesses	0	0	0	0	0	3	20	3	0	1	1	28
Body fluid I[b]	0	0	3	4	4	8	18	5	0	1	2	45
Body fluid II[c]	2	2	6	8	18	5	31	12	1	3	3	91
Urine-Gyn[d]	2	2	3	2	11	6	9	2	4	4	7	52
Plaque	1	50	25	2	24	22	22	3	0	0	3	152
Sputum, throat	0	2	10	8	15	34	11	3	0	3	4	90
Total	7	152	202	81	231	177	231	55	5	46	40	1,227

[a] Abbreviations: SBE, Subacute bacterial endocarditis; Sep, sepsis; Pn, pneumonia; Men, meningitis; NS, not stated.

[b] Body fluids I consisted of pleural and peritoneal fluids and internal abscesses.

[c] Body fluids II consisted of wounds, cysts, and external abscesses.

[d] Obtained from urine, vaginal, urethral, and cervical sources.

A different distribution of species was observed from brain abscesses, body fluids I (pleural and peritoneal fluids and abscesses of the appendix, liver, etc.), and body fluids II (wounds, cysts, and other external abscesses). *Streptococcus* MG-*intermedius* predominated from these sources and was found in 20 of 28 (71%) of the brain abscesses, 18 of 45 (40%) of the body fluids, I, and 31 of 91 (34%) of the body fluids II. No particular species predominated among the genitourinary isolates. Among the plaque isolates, *S. mutans* predominated (50 of 152, 33%), followed by *S. sanguis* I and *S. sanguis* II (25 of 152 and 24 of 152, respectively, 16% each). *S. mitis* and *Streptococcus* MG-*intermedius* strains (22 of 152 each, 14%) were also identified among the plaque isolates. The sputum and throat isolates were of very little significance as opposed to most of the other isolates. They were not from a randomized study and were not sufficiently numerous to represent a true spectrum of the species distribution found in throats.

Only 40 (3.3%) specimens could not be identified as 1 of the 10 species of known viridans streptococci.

DISCUSSION

Because nearly all the viridans streptococci are susceptible to penicillin, differentiating these strains into species is of little value to clinical microbiologists. One possible use of a differential procedure is to determine the identity of strains isolated from the blood of patients having recurring endocarditis. Speciation of isolates would help determine if the isolates indicated treatment failures or infections by another strain. We identified similar organisms (*S. sanguis* II) from the blood of a patient with sepsis recurring over a 6-month period. This indicated a hidden internal abscess and alerted the physician to search for the abscess. Several investigators have used physiological differentiation schemes in the epidemiology of the various viridans species involved in dental caries.

The body of evidence gathered by early investigators (44, 45, 56) and later investigators (13) and my own results indicates that viridans streptococci cannot be serologically differentiated. There are no well-defined group antigens that will clearly differentiate between the physiological species. Although of little value in differentiating the viridans species, serological methods can be used to eliminate alpha-hemolytic and nonhemolytic strains of group B, D, N, and Q streptococci from the viridans group. Alternatively, a battery of physiological tests can be used, but groups B, D, N, and Q can be more accurately differentiated from the viridans streptococci by serological methods.

The possibility of the viridans strains possessing the Ottens type and Z antigens described by Willers et al. (63, 64) should be thoroughly investigated. The relationship of these antigens to the antigens carried by *S. salivarius* and *Streptococcus* MG-*intermedius* has been studied (42, 53, 63), but additional studies, including those of antigens carried by strains of *S. mitis, S. sanguis* I, and *S. sanguis* II, are needed. Investigators should include strains of streptococci that have been previously described as having various antigenic characteristics. Resolving the antigenic heterogeneity of these strains will not be a simple task. In addition to the two strains of viridans streptococci used to produce group H antiserum (K208 and Blackburn), Rosan (56) proposed still another strain for group H antiserum production. The controversy is far from settled, but it is apparent that strain K208 should not be used for group H vaccine strain. Montague and Knox (49) described *S. salivarius* strains having two distinct antigens; some strains had one, some had the other, and still other strains had either both or none. Kothari et al. (42) found antigens among the *S. salivarius* strains that were related to the Ottens F-type and z-antigens described by Willers and Alderkamp (63).

The antisera produced by CDC for groups A through G are produced and tested for cross-reactions with extracts of beta-hemolytic strains of streptococci group A through G, except for several group D alpha- and nonhemolytic strains. These sera are not tested for cross-reactions with all of the other streptococci, including the viridans species. Thus, it is not surprising to find cross-reacting antibodies in the grouping antisera A through G. The recommendations for the use of antisera produced by CDC are that, except for group D, the antisera should be used to identify only beta-hemolytic strains.

It is my belief that the physiological differentiation of the species offers the best method of classifying the human isolates. I dealt only with human strains in this study and cannot be sure that my methodology will be satisfactory for differentiating strains from other sources. The key in Table 5 will enable laboratorians to physiologically differentiate the human isolates of viridans streptococci.

In Table 7 the nomenclatures for the viridans species used by Carlsson (5, 6), Colman and Williams (13), and myself are compared. Overall, the three nomenclatures were in good agreement. Two factors were primarily responsible for the differences. First, each investiga-

TABLE 7. *Comparison of viridans species nomenclature as identified by Carlsson, Colman and Williams, and Facklam*

Nomenclature used		
Carlsson (reference 5)	Facklam	Colman and Williams (reference 13)
S. sanguis Ia	*S. mitis*	Dextran + *S. mitior*
S. sanguis Ib	*S. sanguis* II	Dextran + *S. mitior*
S. sanguis Ic	*S. sanguis* I	*S. sanguis*
S. sanguis Id	*S. sanguis* I	*S. sanguis*
S. sanguis Ie	*S. sanguis* I	*S. sanguis*
S. sanguis If	*S. sanguis* I	*S. sanguis*
S. sanguis Ig	*Streptococcus* MG-*intermedius*	*S. sanguis*
S. sanguis Ih	*S. sanguis* I	*S. sanguis*
S. mutans Ii	*S. mutans*	*S. mutans*
S. mutans II	*S. mutans*	*S. mutans*
S. salivarius III	*S. salivarius*	*S. salivarius*
Unknown IVa (?)	*Streptococcus* MG-*intermedius*	(*S. milleri*)[a]
Unknown IVb (?)	*S. mitis*	*S. mitior*
S. mitis Va	*S. mitis*	*S. mitior*
S. mitis Vb	*S. sanguis* I	*S. sanguis*
S. mitis Vc	*S. sanguis* II	(*S. mitior*)
S. mitis Vd	*S. sanguis* I	(*S. sanguis*)
S. mitis Ve	*S. mitis*	(*S. milleri*)
S. mitis Vf	*S. sanguis* II	(*S. mitior*)
S. mitis Vg	*S. sanguis* I	(*S. sanguis*)
S. mitis Vh	*Streptococcus* MG-*intermedius*	(*S. milleri*)
S. mitis Vi	*S. sanguis* I	(*S. sanguis*)

[a] Taxa in parenthesis are my interpretations of probable interpretations by Colman and Williams according to characteristics outlined in reference 13.

tor had determined physiological characteristics that the others had not. Second, each investigator accepted certain physiological characteristics as variable reactions, whereas the others had not. Carlsson accepted glucan production (dextran and levan) as a stable characteristic, but Colman and Williams and I did not. Colman and Williams (11, 13) used cell wall analysis as the stable differentiating characteristic, but the other investigators did not perform cell wall analysis. I used enzyme-related physiological characteristics, acid formation in carbohydrate broths, and hydrolytic reactions as stable differentiating characteristics, but Carlsson and Colman and Williams used these reactions as variable characteristics. Still, there is overall agreement, and the major variations can be easily explained.

All three groups of investigators agree on the physiological characteristics of *S. mutans*, *S. salivarius*, and typical *S. sanguis*. There is general agreement on the physiological characteristics of *S. mitis*. Colman and Williams prefer to designate *S. mitis* strains as *S. mitior*. In my system, most dextran-producing *S. mitior* strains are differentiated as *S. sanguis* II by acid formation in raffinose broth. Other investigators have claimed that *S. sanguis* II (ATCC-10557) should not be included in the *S. sanguis* taxon (8, 15, 43). These opinions were based on physiological (8, 54, 62), serological (43), and homology of deoxyribonucleic acid (15) differences between typical *S. sanguis* and *S. sanguis* II strains. I believe that the dextran-producing *S. mitior* (raffinose positive) and *S. sanguis* II strains represent a new taxon which, at present, has no taxonomic designation.

Several investigators have accepted non-glucan-producing strains into *S. sanguis* I (13, 19, 20); therefore, I feel justified in including non-glucan-producing strains in *S. sanguis* II. The methodology of determining glucan production differs somewhat in each laboratory, and the results are subject to variation in media composition and incubation atmospheres. Porterfield (54) advocated anaerobic incubation of the 5% sucrose agar plates for determining glucan production, but most investigators use normal atmospheres (as we did) or candle jar atmospheres for incubation of 5% sucrose agar. The most refined technique for determining glucan formation was described by Hehre and Neill (33). In this technique, alcohol precipitation of the 5% sucrose broth is used to indicate the presence of either dextran or levan; the technique is probably the most accurate available. Carlsson (6) and Colman and Williams (13) used this method, but I did not.

I placed the mannitol-negative, nonhemolytic, inulin-positive, arginine-negative, nonglucan-forming strains in the *S. salivarius* category because these strains resembled *S. salivarius* more closely than they did *S. sanguis*. These strains were also required to form acid in raffinose, which is a more common characteristic of *S. salivarius* (95%) than of *S. sanguis* (45%).

The other discrepancy between my system and that of Colman and Williams is in the recognition of *Streptococcus* MG-*intermedius* and *S. milleri*. Colman and Williams (13) used Guthof's (29) description of *S. milleri*. Basically, these strains are mannitol-, sorbitol-, and inulin-negative and hydrolyze both esculin and arginine, according to Colman and Williams. I agree with this description but divided these strains into two categories; lactose-positive strains (*Streptococcus* MG-*intermedius*) and lactose-negative strains (*S. anginosus-constellatus*). If acid formation in lactose broth among the viridans streptococci is plasmid associated, as Cords et al. (14) suspect it is for the group N streptococci, this differentiation may not be valid. Carlsson (5), however, found acid formation in lactose broth to be a very stable characteristic of the viridans strains he studied. The antigenic composition of these strains and their physiological characteristics indicate that they are undoubtedly closely related. Many of the clinical strains of *Streptococcus* MG-*intermedius* (23%) and *S. anginosus-constellatus* (15%) reacted with group F antiserum. *Streptococcus* Mg has been shown to possess the group F antigen (64), and beta-hemolytic group F streptococci are termed *S. anginosus* (18). Most of the beta-hemolytic streptococci of human origin that do not have demonstrable group antigens have physiological characteristics that resemble either *S. anginosus-constellatus* or *Streptococcus* MG-*intermedius* (R. R. Facklam, unpublished data). Ottens and Wrinkler (52) also described strains of beta-hemolytic streptococci that did not possess the group F antigen but were related to the group F strains by their type antigens. All of these strains (beta-hemolytic streptococci with and without the group F antigen and alpha-hemolytic and nonhemolytic strains with and without the group F antigen) that have physiological characteristics of *S. anginosus-constellatus* or *Streptococcus* MG-*intermedius* in my system are termed *S. milleri* by Colman and Williams. Although these strains are closely related, I think that they can be differentiated for epidemiological purposes by lactose fermentation.

Colman and Williams (13) did not complete the comparison of their identifications to those of Carlsson (6). Table 7 compares our identification with those of both groups of investigators. The species in parentheses are my interpretations of the probable interpretations by Colman and Williams according to the physiological characteristics they outlined (13). Colman interpreted the species identified by Carlsson as *S. sanguis* Ia and Ib as dextran-positive *S. mitior*, but by my methodology the species identified by Carlsson as *S. sanguis* Ia was *S. mitis* and his *S. sanguis* Ib was *S. sanguis* II. We did not accept inulin-negative strains as typical *S. sanguis* I; therefore, the species identified by Carlsson as *S. sanguis* Ig was *Streptococcus* MG-*intermedius*. I include, as do Colman and Williams, inulin-positive, alpha-hemolytic, non-glucan-producing strains in *S. sanguis* I. Therefore, the species identified by Carlsson as *S. mitis* Vb, Vd, Vg, and Vi are *S. sanguis* by both my methodology and that of Colman. The non-glucan-producing, inulin-negative, raffinose-positive, and esculin-negative strains of the species identified by Carlsson as group V (Vc and Vf) are non-glucan-producing strains of *S. sanguis* II by my system but *S. mitior* according to the system of Colman and Williams. The mannitol- and inulin-negative, esculin-positive strains identified as such by Colman (Ig, IVa, and Vh) are identified as *Streptococcus* Mg-*intermedius* by my system and probably as *S. milleri* by Colman and Williams. Mejare and Edwardson (47) have recently described oral isolates of *S. milleri* that physiologically resemble strains IV and Vh identified according to the system of Carlsson, *S. milleri* identified according to the system of Colman and Williams, and *S.* MG-*intermedius* strains according to my system. The only difference in the identifications used by these investigators is the terminology, not the descriptions, of the physiological characteristics.

Other investigators have not included *S. uberis* and *S. acidominimus* in their studies. These strains are normal inhabitants of domestic animals (2, 17, 58). Although rare, they are found in humans and stock strains and should be included in taxonomic studies. The relationship of *G. hemolysans* to *S. morbillorium* certainly merits further study. The homology of deoxyribonucleic acid and cell wall analysis are two areas that may resolve any differences or add to the similarity of the strains.

The air-tolerant anaerobic strains (34) used in this study showed physiological similarities to the accepted viridans species. It is difficult to suggest a convenient method for differentiating between the aerobic and anaerobic streptococci,

but, as a general rule, if the organisms grow in an atmosphere that is not anaerobic, they cannot be anaerobes by definition. In general, poorly growing strains that adapt to candle jar or normal atmospheres are not anaerobic and should be identified as aerobic streptococci.

The distribution of species in different human infections is interesting. For example, 68% of the *S. sanguis* I and 67% of the *S. mutans* blood isolates were associated with endocarditis, but only 21% of the *S. salivarius* blood isolates were associated with endocarditis. On the other hand, 60% of the *S. salivarius* blood isolates were associated with sepsis, meningitis, and pneumonia, but only 19 and 17% of the *S. sanguis* I and *S. mutans*, respectively, were associated with these diseases. The other commonly occurring blood isolates, *S. sanguis* II, *Streptococcus* MG-*intermedius*, and *S. mitis*, showed no particular affinity to either subacute bacterial endocarditis, sepsis, pneumonia, or meningitis. I do not know whether this represents a difference in virulence or simply a numerical species distribution at the source of entry into the blood stream. The physiological speciation of these organisms does give investigators a tool that will aid in the investigation of the ecology and virulence of these strains.

The frequency of each species in each disease may not be a representative distribution, because laboratories send problem specimens to CDC. This could account for the lower frequency of *S. sanguis* I strains in endocarditis (29%) than the 39% Loewe et al. reported (46) and the relatively high frequency of *S. mutans* (18%), an organism that is only infrequently reported in endocarditis. *S. mutans* is difficult to recognize, but *S. sanguis* I strains are easy to recognize; therefore, CDC would receive more *S. mutans* than *S. sanguis* I strains.

The observation that *Streptococcus* MG-*intermedius* and *S. anginosus-constellatus* strains were identified from abscesses of the brain, liver, and appendix and from pleural and peritoneal fluids more frequently than the other viridans species is not surprising. Other investigators have reported the frequency of beta-hemolytic group F streptococci and *S. milleri* from similar clinical sources (41, 67; N. T. Bateman, S. J. Eykyn, and I. Phillips, Lancet i:675, 1975). This finding may represent differences in the ecology or virulence of these strains, but information regarding either of these factors is very meager.

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