Characteristics of *Streptococcus mutans* Isolated from Human Dental Plaque and Blood

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A comparison of physiological characteristics of *Streptococcus mutans* isolates from human blood (54 strains) and dental plaque (50 strains) showed that there were no appreciable differences among the strains. The species can be defined and differentiated by a combination of serological and physiological properties. The strains were tested for 30 physiological characteristics. Lancefield extracts of each strain were tested for precipitin reactions with antisera for Lancefield groups A, B, C, D, E, F, G, H, K, O, and A variant. The failure of Lancefield extracts of *S. mutans* to react with streptococcal group D antisera, the formation of characteristic gelatinous deposits in 5% sucrose broth, and acid formation in mannitol broth are the three major criteria for recognizing *S. mutans*.

Several recent studies (10, 11, 17, 22) have shown that streptococci isolated from dental plaque resemble *Streptococcus mutans*. Clarke in 1924 (12) described this organism isolated from dental plaque, and Abercrombie and Scott in 1928 (1) reported this organism in a single case of infective endocarditis. Even though the organism could be differentiated from the other streptococci, textbooks and taxonomy manuals have not published descriptions of the organism. The seventh edition of *Bergey's Manual of Determinative Bacteriology* (7) does not have a description of *S. mutans*. This report describes a physiological identification scheme and a comparison of physiological characteristics of *S. mutans* isolated from human dental plaque and blood.

MATERIALS AND METHODS

Stock strains of *S. mutans* were obtained from the various investigators listed in Table 1. The human plaque isolates were kindly supplied by L. A. Thomson, National Institute of Dental Research, Bethesda, Md. The human blood isolates were submitted to the Center for Disease Control (CDC) by various state and city health departments or by private investigators with whom we have collaborative studies. The procedures for serological grouping, determining hemolytic and catalase activities, and testing for the majority of physiological reactions listed in Table 2 have been previously described (18, 19). Tests that have not been previously described are tolerance to 2 and 4% NaCl broths and determination of hydrolysis of hippurate. The 2 and 4% broths of NaCl were prepared by adding NaCl to heart infusion broth (Difco) up to the desired concentration. Dextrose

(0.1%) and an indicator (bromocresol purple) were added to the media. The medium for determining hippurate hydrolysis was prepared by adding sodium hippurate (1% final concentration) to heart infusion broth. The three media were tubed in 16 by 125 mm screw-capped tubes and sterilized by autoclaving. Tolerance to 2 and 4% NaCl broth was determined by rotating the tubes in front of an incandescent lamp and observing for growth after 24, 48, and 72 h. Hydrolysis of hippurate was determined by pipetting 0.8 ml of supernatant from a 48-h growth of hippurate broth to a Kahn tube; 0.2 ml of ferric chloride solution (12 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 100 ml of aqueous HCl) was added to the supernatant. A heavy precipitate that remained longer than 15 min indicated that hippurate had been split. An immediate clearing or no precipitate indicated that hippurate was not split.

Extracellular polysaccharide production (presumably glucan) was determined by the following methods:

(i) **Five percent sucrose agar.** Glucan production typical of *S. sanguis* and *S. mutans* resulted in highly refractile, adherent or white-dry, adherent growth on the agar. Levan production, typical of *S. salivarius*, resulted in opaque, gummy, nonadherent growth. Typical *S. bovis* growth was similar to the growth of *S. salivarius* but somewhat less gummy and rarely adherent to the medium. Large or small colonies that were mucoid and nonadherent were considered as being negative or having no extracellular polysaccharide production.

(ii) **Five percent sucrose broth.** Glucan production was indicated when the broth was partially or completely gelled, typical of *S. sanguis*. Glucan production was also indicated when gelatinous, adherent deposits formed on the bottom and walls of the growth tube, typical of *S. mutans*. An increase in viscosity of the broth indicated the production of slime (unknown polysaccharide) typical of *S. bovis*. Negative reactions occurred when no gelling, deposits, or increase in viscosity occurred.

RESULTS

Table 1 shows the biotypes of the stock strains tested. A typical *S. mutans* will grow in 2% NaCl broth, form acid in litmus milk, and form acid in the following 1% carbohydrate broths: trehalose, mannitol, sucrose, inulin, lactose, sorbitol, esculin, and raffinose. Typical strains will not grow in 0.1% methylene blue milk or at 10 C. Typical strains do not hydrolyze starch, hippurate, or gelatin nor do they form acid in 1% broths of glycerol or arabinose. The majority of strains, but not all, do not grow in broth containing 6.5% NaCl. Nearly all the typical strains form extracellular polysaccharides (glucans) in either 5% sucrose agar or 5% sucrose broth or in both.

The exceptional physiological reactions which make up the biotypes described are listed in Table 1. The majority of these exceptions involve only three reactions: failure to split esculin and failure to form acid in sorbitol and in raffinose broths.

Table 2 shows the percentage of strains exhibiting the different hemolytic reactions in

5% rabbit blood agar. The source of the specimen seemed to affect the kind of hemolysis observed. More blood isolates were non-hemolytic (74%) than were plaque isolates (38%), and more of the plaque isolates gave alpha reactions (54%) than did blood isolates (7%). The percentage of stock strains and the total percentages of all isolates that produce the three kinds of hemolysis were similar to those reported by Edwardsson (17).

The results of reacting Lancefield extracts of the *S. mutans* strains with CDC streptococcal grouping antisera are shown in Table 2. The majority of strains (89%) failed to react with any of the antisera. These antisera are standard Lancefield grouping antisera produced with Lancefield group A (*S. pyogenes*), group B (*S. agalactiae*), etc. Three stock strains, one blood isolate, and nine plaque isolates reacted with serogroup E antisera. One blood and one plaque isolate reacted weakly with serogroup F antisera. Also, one blood isolate and one strain isolated from an endocervix (other) reacted with serogroup K antisera. All these precipitin reactions (including E) were very weak and

TABLE 1. *Identification and biotype of S. mutans stock strains*

Biotype	Strain identification	Exceptional reactions
I	Carlsson's OPK-1, KPSK-2, KPK-3, and MPK-1; Jordan, Englander, and Keyes' PP-3, 4, 5, 6, 7, 8, 9, 10, 12, and 3720; Gibbons' LM-7 and GS-5. Zinner's BHT. Fitzgerald and Keyes' E49; NTCC 10449 and strain 214T.P.	None
II	No stock strains	Fails to split esculin
III	Jordan, Englander, and Keyes' PP-1 and PP-11; Fitzgerald and Jordan's HS-6, FA-1, and K1-R	Fails to form acid in 1% raffinose broth
IV	Jordan's SL-1; Fitzgerald's 6715-8; and Zinner's AHT	Fails to split esculin or form acid in raffinose broths
V	Fitzgerald and Keyes' E49-2	Fails to form acid in 1% sorbitol broth
VI	Carlsson's MEI	Fails to form acid in sorbitol and raffinose broth
VII	Carlsson's OIHI	Fails to split esculin or form acid in sorbitol and raffinose broths
VIII	DeStoppelaar's C67-25 (mutant)	Fails to form acid in 1% lactose broth

probably represent cross-reacting antibodies in the CDC grouping antisera. No attempt was made to validate these reactions as being homologous to beta-hemolytic group E, F, or K streptococci.

Table 2 also shows the results of the tests used to detect extracellular polysaccharide production by *S. mutans* (presumably glucan). All but two blood isolates and two isolates from other sources formed glucan on 5% sucrose agar. One stock strain failed to produce glucan in 5% sucrose broth. This was DeStoppelaar's (16) nondextran-forming mutant. The two blood isolates and five of the ten plaque isolates that failed to produce glucan in 5% sucrose broth also failed to grow after 3 days of aerobic incubation. Two strains from other sources (dog bite wound and jaw exudate) failed to produce glucan in 5% sucrose broth. These two strains were the only two *S. mutans* strains that failed to produce glucan in both 5% sucrose broth and agar.

Table 3 gives the results of 142 *S. mutans* strains in 29 physiological tests. The source of the specimen was found to have little or no influence on the physiological test results. All or the majority of strains were able to grow in 2% NaCl broth, form acid and clot litmus milk, and form acid in trehalose, mannitol, sucrose, inulin, lactose, sorbitol, salicin, esculin, raffinose, and melibiose broths. All or nearly all of the strains failed to grow in 0.1% methylene blue milk or at 10 C. None of the strains hydrolyzed hippurate or gelatin or tolerated 0.04% tellurite. The majority of the strains did not grow in 6.5% NaCl broth and failed to hydrolyze starch. None of the strains formed acid in glycerol, arabinose, and melezitose broths. Variable reactions were observed in bile-esculin medium, growth in 4% NaCl broth, growth at 45 C, growth on 10 and 40% bile, and reduction of 0.1% tetrazolium.

Table 4 shows the number of strains of *S. mutans* isolated from various sources that gave the biotype reactions listed in Table 1. More than 95% of the strains did not have any exceptional reactions. Only four of 54 blood isolates and 11 of 50 plaque isolates deviated from the biotype I reactions. One of six isolates from other sources deviated from biotype I reactions. In general, the biochemical patterns observed were consistent with those observed by other investigators (11, 17, 22).

DISCUSSION

At the CDC, biotyping of *S. mutans* is not considered to be necessary or valid. It is

TABLE 2. Percentage of *S. mutans* strains giving different hemolytic and serological reactions and forming dextrans on sucrose media

Reaction	Stock strains (32) ^a	Blood (54)	Plaque (50)	Other (6)	Total (142)
Hemolysis					
Alpha	31	7	54	33	30.3
Beta	3	19	8	17	11.3
None	66	74	38	50	58.4
Serogroups					
E	9	2	18	0	8.4
F	0	2	2	0	1.4
K	0	2	0	17	1.4
None ^b	91	94	80	63	88.8
Dextran on: ^c					
5% Sucrose agar	100	93	100	67	97.2
5% Sucrose broth	97	96	80	67	91.2

^a Number of strains.

^b Streptococcal grouping antisera used were specific antisera for groups A, B, C, D, E, F, G, H, K, and O streptococci.

^c All strains that failed to form dextrans were biotype I except one plaque biotype IV strain. The one stock strain that failed to produce dextran in 5% sucrose broth was DeStoppelaar's non-dextran-producing mutant. The two blood isolates and five of the 10 plaque isolates that failed to produce dextrans in 5% sucrose broth failed to grow in the medium. Two strains from other sources (dog bite wound and jaw exudate) failed to produce dextran on either the agar or broth. No other strains failed to produce dextran in both tests.

presented here as a convenient method for demonstrating atypical strains. In the description of the species, we accept the variable reactions such as splitting of esculin and acid formation in raffinose and sorbitol broths. Strains that fail to give positive reactions in one or more of these three tests, but give all the other expected reactions of *S. mutans*, are considered to be *S. mutans* strains even though they are atypical. All streptococci giving alpha and gamma reactions in blood agar are speculated by "spectrum analysis" (18). Spectrum analysis is observing the results of the reactions listed in Tables 2 and 3 by unknown strains; the resulting reactions fit known reactions of stock strains of streptococcal species.

The hemolytic activity and serogrouping (primarily for elimination of group D reaction) of the strains help to identify *S. mutans*. It is important that these characteristics be used in conjunction with the different physiological characteristics, because identification of *S.*

TABLE 3. *Percentage of S. mutans strains giving different physiological reactions*^a

Test	Stock strains	Blood	Plaque	Other	Total
Bile esculin	53	17	48	17	35.9
Methylene blue milk	0	2	4	0	0.7
6.5% NaCl broth	16	2	12	0	8.5
4.0% NaCl broth	50	30	46	50	40.8
2.0% NaCl broth	97	93	92	100	93.6
Growth at 10 C	0	0	0	0	0
Growth at 45 C	90	30	64	83	57.5
Growth on 10% bile	34	48	86	67	59.2
Growth on 40% bile	22	19	62	33	35.2
Hydrolysis of starch	0	11	0	17	4.9
Hydrolysis of hippurate	0	0	0	0	0
Hydrolysis of gelatin	0	0	0	0	0
Tolerance to 0.04% tellurite	0	0	0	0	0
Reduces 0.1% tetrazolium	37	22	56	0	36.6
Litmus milk acid	100	100	100	100	100.0
Litmus milk clot	93	81	96	67	88.7
Acid from 1% broths:					
Trehalose	100	100	100	100	100.0
Mannitol	100	100	100	100	100.0
Sucrose	100	100	100	100	100.0
Inulin	100	100	100	100	100.0
Lactose	97	98	100	100	98.6
Sorbitol	91	98	92	100	94.4
Salicin ^b	93	100	78	100	92.1
Esculin	88	96	84	83	88.7
Raffinose	78	98	84	100	88.7
Melibiose ^b	83	88	43	100	80.9
Glycerol	0	0	0	0	0
Arabinose	0	0	0	0	0
Melezitose ^b	0	0	0	0	0

^a Same number of strains as in Table 2.^b Not all strains were tested.TABLE 4. *Number of biotypes identified from various sources*

Biotype	Source			Total	Percentage
	Blood	Plaque	Other		
I	50	39	5	94	85.4
II	1	3	1	5	4.5
III	0	2	0	2	1.8
IV	1	2	0	3	2.7
V	1	0	0	1	0.9
VI	0	1	0	1	0.9
VII	0	3	0	3	2.7
VIII	1	0	0	1	0.9
Total	54	50	6	110	

mutans on hemolytic and/or serological properties is not possible.

The capacity of streptococci to form glucans helps to differentiate the streptococci. *S. sanguis* strains form glucans in both 5% sucrose agar and broth. Glucan formation by *S. sanguis* cannot be reliably differentiated from glucan

formation by *S. mutans* on 5% sucrose agar. However, most *S. sanguis* strains gel 5% sucrose broth, but *S. mutans* strains do not. *S. mutans* forms sticky gelatinous deposits on the media container (20). Also *S. sanguis* does not form acid in mannitol broth, which helps to differentiate it from *S. mutans*. In our collection of clinical isolates of streptococci giving alpha-reactions in blood agar, 150 strains are identified as *S. sanguis*. All 150 strains form acid in inulin broth and do not form acid in mannitol broth; nearly all (90%) of the strains form glucan on either one or both 5% sucrose agar and 5% sucrose broth. Forty-nine (33%) of these strains formed acid in raffinose, seven (4%) formed acid in sorbitol; and eight (5%) formed acid in both sorbitol and raffinose broths. Three raffinose-positive strains and three raffinose-negative strains produced the gelatinous deposits in 5% sucrose broth (typical of *S. mutans*). None of the sorbitol-positive strains did so.

Six strains of streptococci identified as mannitol-negative *S. mutans* (Bratthalls' type d)

were obtained. Of these strains, only one fermented raffinose and two fermented sorbitol. The raffinose-positive strain formed acid in inulin, sucrose, and lactose broth, split esculin, and produced gelatinous deposits in 5% sucrose broth. Two other "mannitol-negative mutans" strains formed acid in trehalose, lactose, sucrose, and inulin broth and produced gelatinous deposits in 5% sucrose broth. Two strains formed acid in trehalose, sorbitol, lactose, sucrose, and inulin, failed to split esculin or form acid in raffinose broth, and gave negative reactions in 5% sucrose broth. The other "mannitol-negative mutans strain" formed acid in trehalose, lactose, sucrose, and inulin, failed to split esculin or form acid in raffinose broth, and did not form glucans in either 5% sucrose broth or sucrose agar. The mannitol-negative strains could not be differentiated from typical *S. sanguis* strains except that they formed the gelatinous deposits in the 5% sucrose broth. This one reaction was not considered sufficient to differentiate streptococcal species. The reaction in 5% sucrose broth should be considered as a variable reaction for identification of streptococci. Therefore, these strains were considered *S. sanguis* regardless of serological reaction with *S. mutans* typing antisera (I. L. Shklair and H. J. Keene, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, G57, p. 35).

Glucan formation and failure to split hippurate by *S. mutans* serves to differentiate *S. mutans* from *S. uberis*. *S. uberis* forms acid in the same carbohydrate broths as does *S. mutans*. Also, extracts of some strains of *S. uberis* react with streptococcal group E antisera (15). Although *S. mutans* has antigenic (probable group E antigen) relationships and certain physiological (mannitol and sorbitol fermentation) similarities to *S. uberis* and *S. infrequens* (serogroup E beta-hemolytic streptococci), it is easily differentiated from these streptococci by differences in serological, hemolytic, and physiological characteristics. Each of these are distinct, clearly definable species.

Some strains of *S. mutans* share physiological characteristics of the group D streptococci. The tolerance to 40% bile, the bile-esculin reaction, and 6.5% NaCl tolerance are characteristics of *S. faecalis* and its varieties, *S. faecium* and *S. durans*. The majority of these strains grow at 10 C, tolerate 0.1% methylene blue milk, and yield serological group D reactions (16). These characteristics along with other physiological differences easily differentiate (by spectrum analysis) these organisms from *S. mutans*. The group D species, *S. bovis*, shares a number of physiological characteristics with *S. mutans*. *S.*

bovis is differentiated from *S. mutans* by obtaining serological group D reactions from extracts of *S. bovis* strains. Extracellular polysaccharide formation by *S. bovis* in 5% sucrose broth is different from that of *S. mutans*. *S. bovis* increases the viscosity of the broth but does not gel or form the gelatinous deposits in the broth. Glucan formation by *S. bovis* on 5% sucrose agar is usually, but not always, different from that of *S. mutans*. *S. bovis* occasionally produces adherent, highly refractile colonies on 5% sucrose agar (typical of *S. mutans*) but more often produces the nonadherent, opaque gum-drop colonies. Typical strains of *S. bovis* always hydrolyze starch, whereas *S. mutans* strains do not. An occasional *S. mutans* strain will hydrolyze starch very weakly, unlike *S. bovis* which hydrolyzes starch very strongly. The colonial morphology on blood agar also helps in recognizing *S. mutans*. It grows poorly; a thin film of growth is barely visible after 24 h at 35 C. *S. bovis* grows very readily, like the enterococci; lush growth is observed at 24 h at 35 C. When examined by spectrum analysis, these organisms can be differentiated.

It is not surprising to find that *S. mutans* is antigenically heterogeneous. Bratthall's studies (3, 4, 5, 6) have shown five different serotypes of *S. mutans*, one of which is homologous to serogroup E streptococci. In view of our own studies, some strains of *S. mutans* may be antigenically related to streptococci other than group E streptococci (group K and F). The deoxyribonucleic acid (DNA) homology studies of Coykendall (13, 14) also support the fact that the taxon *S. mutans* includes a heterogeneous collection of strains. Both Bratthall and Coykendall feel that their data support each other. Coykendall reported that there was more DNA homology between strains of the same Bratthall serotype and less homology between strains of different Bratthall serotypes; however, he has reported on only nine strains and has not included type "e" in his studies.

Recently, Brown and Patterson (8) demonstrated a heterogeneity among *S. mutans* strains by electrophoretic mobility of mannitol-1-phosphate dehydrogenases from a variety of *S. mutans* strains. Bleiweis et al. (2) reported that the cell wall composition (polysaccharide, teichoic acid, and peptidoglycan) among certain strains of *S. mutans* was not homologous. All these data by different investigators point out the heterogeneity of this collection of streptococci termed *S. mutans*.

Chemical definition of the type antigens of *S. mutans* has been attempted primarily by two groups of investigators. Mukasa and Slade (23,

24) believe that the antigens of *S. mutans* types "a" and "b" are polysaccharide in nature, but Bleiweis and co-investigators (2, 25) believe that the antigenic determinants for types "a" and "b" *S. mutans* are teichoic acids. Recently, Burgess and Edwards (9) demonstrated that different extraction procedures yielded different chemical compositions of the type "b" antigen. Their results tended to support the results of Bleiweis and his co-workers rather than the results of Mukasa and Slade. These results may indicate that there is more than one specific antigen for each type, but these discrepancies should be resolved before accepting serological methods for classification and differentiation of *S. mutans*. The problem of clearly defining serotypes "c", "d," and "e" has not been completely resolved. The chemical nature of the antigens has not been elucidated, and troublesome cross-reactions between types "c" and "e" and between types "a" and "d" remain to be solved (6, 21).

Investigators should be aware that these type antigens of *S. mutans* may not be species-specific, i.e., other streptococci may possess these antigens. Bratthall (3) warned that the "type specific" antisera for *S. mutans* had been tested for specificity only with a battery of organisms considered most likely to possess common antigens to *S. mutans*. The true specificity of the antisera will be demonstrated when the sera are used for epidemiological studies involving "wild" strains of streptococci that have been shown to be *S. mutans* by physiological reactions. Grenier et al. (21) reported a significant discrepancy between serological (fluorescent antibody) identification and cultural isolation of *S. mutans*. *S. mutans* was culturally isolated in 60% of the samples that gave positive fluorescent-antibody reactions for *S. mutans*. There are several possible explanations offered for this discrepancy, and nonspecific reactions is one of the possible reasons.

This taxon is as well defined (physiologically) as many of the species of streptococci and is better defined than any of the viridans streptococci. I do not think there is any value at the present in dividing *S. mutans* into additional species. Recognizing the different antigenic compositions of strains should be sufficient for epidemiological studies. Investigators should use caution in the use of terminology when referring to the typing of *S. mutans*. It is unfortunate that the term "group" has been used by some investigators when referring to the different type antigens of *S. mutans*. Since the letter system (A, B, C) has been widely adopted to refer to the Lancefield system of

identification, it will become confusing to have another system of naming antigens with the term "group" and letters used for identifying antigens among the streptococci. Typing species of streptococci is clear and convenient when arabic or roman numerals are used to designate subtypes.

The antibiotic susceptibilities of *S. mutans* isolated from documented cases of subacute bacterial endocarditis are quite uniform (C. Thornsberry, C. N. Baker, and R. R. Facklam, Prog. Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 13th, Washington, D.C., Abstr. 209, 1973). The organism is highly susceptible to penicillin and patients are cured of *S. mutans* subacute bacterial endocarditis quite successfully with penicillin therapy (unpublished data). Thus, there is no medical reason to subdivide the species. It is felt that *S. mutans* is a clearly definable species and can be recognized by its physiological characteristics. The failure of Lancefield extracts of *S. mutans* to react with streptococcal group D antisera, the formation of characteristic gelatinous deposits in 5% sucrose broth, and acid formation in mannitol broth are major criteria for recognizing *S. mutans*.

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