Four Types of *Streptococcus mutans* Based on Their Genetic, Antigenic and Biochemical Characteristics

By A. L. COYKENDALL

Veterans Administration Hospital, Newington, Connecticut 06111, U.S.A.

(Received 10 January 1974; revised 11 March 1974)

SUMMARY

Eighteen cariogenic streptococcal strains identified as members of *Streptococcus mutans* (Clarke, 1924) were compared on the basis of biochemical tests, mannitol-1-phosphate dehydrogenases, DNA base compositions and DNA base sequence homologies. Some slight biochemical differences were found which correlated with the large differences in DNA base composition and sequence heterology which exist among these strains. All strains could be assigned to one of four groups based on these biochemical and genetic differences. Furthermore, these four groups correlated with four serological groups described by Bratthall (1970). It is proposed to divide *S. mutans* into four subspecies: *S. mutans* subsp. *mutans*, the type subspecies, has a guanine-cytosine (G-C) of 36 to 38 mol % and belongs to Bratthall serological group *c*; *S. mutans* subsp. *rattus* subsp. nov. has 41 to 43 mol % G-C, belongs to Bratthall group *b*, and can be distinguished from the other subspecies by its production of ammonia from arginine; *S. mutans* subsp. *cricetus* subsp. nov. has 42 to 44 mol % G-C, belongs to Bratthall group *a*, and can be distinguished by its lack of growth in air; *S. mutans* subsp. *sobrinus* subsp. nov. has 44 to 46 mol % G-C, belongs to Bratthall group *d*, and can be distinguished by its failure to ferment raffinose.

INTRODUCTION

Streptococcus mutans was originally described by Clarke (1924), who believed that it caused dental caries. It was the subject of a few later reports (Maclean, 1927; Abercrombie & Scott, 1928; Appleton, 1933), but interest in the species dwindled. Then, in the past decade, several unspiciated 'viridans' streptococci were shown to cause dental caries in gnotobiotic rats (Fitzgerald, Jordan & Stanley, 1960; Gibbons, Berman, Knoettner & Kapsimalis, 1966) and conventional hamsters (Fitzgerald & Keyes, 1960; Krasse, 1966; Zinner, Jablon, Aran & Saslaw, 1965). Carlsson (1967) noted the similarity between Clarke's description of *S. mutans* and certain of these cariogenic 'viridans' strains (Krasse, 1966). Subsequently, Guggenheim (1968) and Edwardsson (1968) showed that most of the cariogenic streptococci conformed to the description of *S. mutans*. Since several of these cariogenic strains were of human origin (Zinner *et al.* 1965; Gibbons *et al.* 1966; Krasse, 1966; Edwardsson, 1968; Guggenheim, 1968), and the presence of *S. mutans* on human teeth can be correlated with tooth decay (Krasse, Jordan, Svensson & Trell, 1968; de Stoppe-laar, Van Houte & Backer Dirks, 1969; Englander & Jordan, 1972; Hoerman, Keene, Shklair & Burmeister, 1972; Shklair, Keene & Simonson, 1972), Clarke's species is now considered a major etiologic factor in human caries.

The fermentation of several sugars, including mannitol and sorbitol, plus production of glucans from sucrose, distinguish *S. mutans* from other streptococci (Carlsson, 1967; Guggenheim, 1968). Several traits appear to enhance the bacterium's odontolytic potential. The glucans (Guggenheim & Schroeder, 1967; Wood, 1967) may function as adhesives

binding the streptococci to tooth surfaces (Gibbons & Banghart, 1967) and promote formation of aggregates (Gibbons & Fitzgerald, 1968). They store an intracellular iodophilic polysaccharide (Van Houte, de Moor & Jansen, 1970; de Stoppelaar, 1971) which probably can be catabolized in the absence of exogenous carbon sources (Gibbons, 1964; M. L. Freedman & A. L. Coykendall, unpublished). Finally, the organism can produce a pH of 4.0 in sucrose medium (Edwardsson, 1968).

Strains of *S. mutans* are phenotypically quite homogeneous, as demonstrated by numerical taxonomy (Carlsson, 1968; Drucker & Melville, 1971).

Despite the phenotypic homogeneity of the species, recent investigations have revealed a remarkable degree of genetic and antigenic heterogeneity. Wide variations have been found in DNA base contents among *S. mutans* strains (Coykendall, 1970; Dunny, Hausner & Clewell, 1972), and DNA-DNA reassociation experiments have demonstrated four distinct genetic groups within the species (Coykendall, 1971; Coykendall, Daily, Kramer & Beath, 1971). Intergroup reassociations were low, never greater than 55 %, and intergroup hybrids were thermolabile (Coykendall, 1971). Bratthall (1970) has described five serological groups within *S. mutans*: Bratthall *a*, *b*, *c*, *d* and Lancefield E. Strains which react with Lancefield E antiserum cross-react with *S. mutans* group *c* antiserum (Bratthall, 1972). The genetic groups correlate with the serological groups. Recently, enzymatic differences have been found among *S. mutans* strains which correlate with the antigenic and genetic groups (Brown & Patterson, 1972; Brown & Wittenberger, 1972). Differences have also been reported in the glucans produced by strains of different genetic groups (Newbrun, 1972). Some morphological differences have also been noted (Coykendall *et al.* 1971).

This communication reports the genetic position of several additional strains including four which react with Lancefield group E antiserum [Bratthall's (1970) fifth serological group], presents physiological patterns of the four groups which help to delineate them, and proposes that *S. mutans* be divided into four subspecies based on genetic and antigenic characters.

METHODS

Bacteria. The strains used in this study are listed in Table 1 with their antigenic and genetic group designations as determined in this or previous work. All were confirmed to be members of *Streptococcus mutans*, on the basis of phenotypic characters (Edwardsson, 1968; Guggenheim, 1968). Stock cultures were maintained in Todd-Hewitt broth (BBL) with calcium carbonate and 1 % additional glucose. Cultures were periodically examined for typical growth on Mitis-salivarius agar (Difco) and for purity by streaking on blood agar or Trypticase Soy agar (BBL) and by examination of Gram-stained smears. Usually, cultures were grown anaerobically.

Biochemical tests. Sugar fermentations were tested in Cystine Trypticase agar (CTA; BBL) with 1 % carbohydrate. The medium was autoclaved at 118 °C. In some tests filter-sterilized sugars were added after the medium was autoclaved; the results did not differ from those using autoclaved sugars. Inulin was dissolved in hot water and filter-sterilized. The production of ammonia from L-arginine-HCl was determined by the method of Niven, Smiley & Sherman (1942). The inoculum was one drop of an 18 h culture grown anaerobically in Todd-Hewitt broth and the CTA was stabbed several times with a sterile loop. A tube of CTA with no sugar was also inoculated.

The effect of various atmospheres on growth was tested by streaking a loop of culture on to each of three Trypticase Soy agar plates and incubating one each in air, a candle jar, and an anaerobic jar (90 % H₂, 10 % CO₂; GasPak, BBL).

Table 1. *Strains of S. mutans used in this study*

Strain	Genetic Group*	Antigenic Group†	Guanine plus cytosine (mol %)		Source	Remarks
			From Tm‡	From CsCl§		
NCTC10449	I	c	37.9	37.1	Human carious lesion	Cariogenic for hamsters, Edwardsson (1968). Derived from NCTC10449
GS5	I	c	37.7	36.9	Human carious lesion	Cariogenic for gnotobiotic rats, Gibbons <i>et al.</i> (1966)
Ingbritt	I	c	37.1	37.5	Human buccal plaque	Cariogenic for hamsters, Krasse (1966)
FA1	II	b	42.2	41.3	Rat mouth	Cariogenic for gnotobiotic rats, Fitzgerald <i>et al.</i> (1960). Believed identical to ATCC19645
BHT	II	b	43.4	41.7	Human carious lesion	Cariogenic for hamsters, Zinner & Jablon (1968)
SL1	III	d	45.1	44.9	Human plaque	McCabe, Keyes & Howell (1967)
K1R	III	d	45.2	45.3	Human plaque	Cariogenic for gerbils, Fitzgerald & Fitzgerald (1965)
OMZ176	III	d	N.D.	44.7	Human smooth-surface plaque	Cariogenic for rats, Guggenheim (1968)
B13	III	d	N.D.	N.D.	Human plaque	Edwardsson (1968) (see also Bratthall, 1970)
HS6	IV	N.D.	42.7	N.D.	Plaque from hamster carious lesion	Cariogenic for hamsters, Fitzgerald & Keyes (1960). Believed identical to ATCC19642
E49	IV	a	43.7	41.8		Streptomycin resistant HS6
AHT	IV	a	43.7	41.3	Human carious lesion	Cariogenic for hamsters, Zinner <i>et al.</i> (1965)
OMZ61	IV	a	42.7	N.D.	Rat tooth	Cariogenic for rats, Guggenheim, König & Mühlemann (1965)
LM7	I	E	N.D.	36.6	Human plaque	Among strains studied by Edwardsson (1968). See Bratthall (1970)
B2	I	E	N.D.	36.9		
P4	I	E	N.D.	N.D.		
AT10	I	E	N.D.	N.D.		
SD1	I		37.6	N.D.	Rat mouth	Isolated by H. V. Jordan (personal communication)

* From this work or previous results (Coykendall, 1971).

† From Bratthall (1970, 1972).

‡ From thermal denaturation (Coykendall, 1970; and this work).

§ From CsCl density (Dunny *et al.* 1972, and personal communication).

|| Denotes results of the present work.

N.D., Not done.

Rate of glycolysis. The rate of change in pH of a weak buffer-washed bacterial suspension after the addition of a fermentable carbohydrate was used as a measure of the rate of glycolysis of that substrate. The experiments were designed to compare the rate of glycolysis of glucose with those of fructose and sucrose by strains of *S. mutans*.

Bacteria were grown to early stationary phase (determined turbidimetrically) in Todd-Hewitt broth (0.2 % glucose), harvested, washed with 50 mM-KCl + 1 mM-phosphate buffer (pH 6.9), stored at 4 °C overnight, suspended in fresh buffer, and a pH electrode was immersed in the suspension while it was stirred at 37 °C. After temperature equilibration the

suspension was brought to 1 mM-glucose, -fructose, or -sucrose; volumes were adjusted finally to 25 ml with an absorbance of about 0.30 at 600 nm in a Bausch & Lomb Spectronic 20 spectrophotometer. After addition of the sugar, the pH was recorded until it stabilized. The pH did not decrease in the absence of sugar.

Mannitol-1-phosphate dehydrogenase patterns. Brown & Patterson (1972) have shown that the electrophoretic pattern of mannitol-1-phosphate dehydrogenase is specific for each genetic group of *S. mutans*. The patterns for the serological group E strains and strain SD1 were determined using the methods described by Brown & Patterson (1972). Strains NCTC10449 and Ingbritt were assayed simultaneously for comparison.

Deoxyribonucleic acid. Deoxyribonucleic acids were extracted as previously described (Coykendall, 1970). Radioactive DNA from strain GS5 was obtained from D. B. Clewell. Base contents, as mol % guanine plus cytosine, were determined by CsCl density (Schildkraut, Marmur & Doty, 1962) or by thermal denaturation (Marmur & Doty, 1962). Base-sequence homologies between strains were assessed by DNA-DNA reassociation on membrane filters (Denhardt, 1966) as detailed previously (Coykendall, 1971), except that thymidine-methyl[³H] was used instead of [¹⁴C]thymidine for labelling DNA and labelled GS5 DNA was sheared by sonication for 12 s at low power (Sonicator Model W185, Heat Systems-Ultrasonics, Inc.). All reassociations were carried out at 67 °C in 3×SSC (0.45 M-NaCl+0.045 M-sodium citrate).

Serology. To confirm the presence of serological group E antigens, HCl extracts (Lancefield, 1933) were prepared from cultures of alleged serological group E strains and several control strains grown overnight in Todd-Hewitt broth. The extracts were tested against commercial Lancefield group E antisera (Difco Laboratories, and Wellcome Reagents Ltd) in capillary tubes. A precipitate at the interface of antigen and antiserum was considered a positive reaction.

RESULTS

All strains fermented mannitol and inulin, and all but B13 fermented sorbitol. Strains which reacted with Bratthall group *d* antiserum (SL1, K1R, OMZ176 and B13) did not ferment raffinose or melibiose. The Lancefield group E strains (LM7, B2, P4, AT10) and *Streptococcus mutans* strain SD1 fermented raffinose very slowly. A positive colour change (to yellow) was observed only after 3 to 12 days. Slow growth with acid production could be observed along the agar stab and was a consistent observation. Melibiose was not fermented by these five strains. Strains FA1 and BHT, which are very closely related to each other (Coykendall, 1971) and comprise Bratthall's (1970) group *b*, were the only strains which produced ammonia from arginine. All strains grew best in the presence of CO₂. Growth, judged by colony size, was as good in a candle jar as in the strictly anaerobic (H₂+CO₂) atmosphere. In air, growth was slower, and strains HS6, E49 and OMZ61 did not grow at all. Strain AHT grew feebly, producing very small colonies. Thus, genetic group IV strains appear to have a requirement for CO₂ or an intolerance of oxygen.

Mannitol-1-phosphate dehydrogenase. The electrophoretic patterns of mannitol-1-phosphate dehydrogenases are shown in Fig. 1. The pattern from strain SD1 was identical to that of B2 and is not shown. Similarly, P4 and AT10 patterns, which were identical to that of LM7, are not shown. There are two bands common to all strains. The Lancefield group E strains show some isozyme bands of lower mobility. Overall, the group E patterns resemble the patterns of genetic group I strains (NCTC10449 and Ingbritt) when contrasted to the markedly different patterns produced by strains of other genetic groups (Brown & Patterson, 1972).

Fig. 1. Electrophoretic patterns of mannitol-1-phosphate dehydrogenases from extracts of *Streptococcus mutans* strains. Extracts were applied to 7.5 % polyacrylamide gels, pH 9.3. (1) NCTC10449; (2) strain Ingbritt; (3) strain LM7; (4) strain B2; (5) mixture of extracts from NCTC10449 and Ingbritt; (6) mixture of extracts from LM7 and B2; (7) mixture of extracts from NCTC10449 and LM7; (8) mixture of extracts from NCTC10449 and B2.

Rates of glycolysis. Washed, resting, glucose-grown bacteria metabolized glucose at a rate faster than sucrose, an observation reported previously by Tanzer, Brown & Meyers (1972). However, the rate of glycolysis of fructose relative to the other sugars varied with the genetic group. In three genetic groups, the rate of fructose metabolism paralleled that of glucose (FA1 and BHT, group II; HS6, E49, OMZ61 and AHT, group IV; SL1 and B13, group III; Bratthall serological groups *b*, *a* and *d*, respectively). In the other strains tested, the rate of fructose metabolism paralleled the rate of sucrose metabolism (Ingbritt, GS5, NCTC10449, group I; SD1, LM7, B2: Bratthall serological group *c* and Lancefield group E, respectively) (Fig. 2). If strains were grown in sucrose rather than glucose, all three sugars were metabolized at the same rate except in the case of GS5. Growth in sucrose did not induce rapid sucrose fermentation in this strain.

DNA base content and hybridization. The base contents of strains not previously reported are incorporated in Table 1. Strains HS6, OMZ61 and AHT have DNA base contents close to that of strain E49; this was expected because of their serological similarity (Bratthall, 1970). Radioactive OMZ61 DNA reassociated extensively with DNA of the other three strains (Table 2). Strain B13 DNA reassociated only 43 % with OMZ61 DNA which, on the basis of previous experiments (Coykendall, 1971), was about the value expected for DNA reassociation between genetic group III and group IV strains.

In a separate experiment, strain OMZ176 DNA was 81.5 % homologous with strains K1R and SL1 (group III, Bratthall serological group *d*).

The base contents of the serological group E strains and *S. mutans* strain SD1 were similar to those of genetic group I (Bratthall serological group *c*) (Table 1). Labelled LM7 DNA hybridized extensively with DNA from these five strains and also with DNA from NCTC10449 (Table 3). In a reciprocal experiment, labelled GS5 DNA reassociated extensively with DNA

Fig. 2. Decrease of pH in washed, resting, glucose-grown bacterial suspensions in 50 mM-KCl-1 mM-phosphate buffer (pH 6.9) after addition of carbohydrate to 1 mM. The pattern shown by *S. mutans* NCTC10449 (a) is typical of all strains in genetic group I. The pattern shown by *S. mutans* strain HS6 (b) is typical of strains in genetic groups II to IV. GLU, Glucose; FRU, fructose; SUC, sucrose.

Table 2. *Extent of reassociation of DNA of genetic group IV strains with radioactive OMZ61 DNA at 67 °C in 3 × SSC*

Immobilized DNA strands	Radioactive OMZ61 DNA bound	
	% of total [³ H]DNA available	% relative to OMZ61
OMZ61	51.6	100
AHT	51.4	99.4
HS6	50.3	97.2
E49	50.8	98.3
B13	23.5	45.5
<i>Escherichia coli</i>	1.5	2.9

from serological group E strains and, as expected, with DNA from NCTC10449. These experiments indicate that the Lancefield serological group E strains and SD1 are genetically indistinguishable from genetic group I strains.

Serology. Extracts from the strains reported by Bratthall (1970) to react with Lancefield group E antiserum gave a positive precipitin test with both Difco and Wellcome anti-E sera. Strain SD1, which is biochemically similar to serological group E strains, was not positive for the E antigen nor were the controls (NCTC10449, HS6, SL1).

Table 3. *Reassociation between DNA from Lancefield group E and genetic group I S. mutans strains at 67 °C in 3 × SSC*

Immobilized DNA strands	Radioactive LM7 DNA bound		Radioactive GS5 DNA bound	
	% of total [³ H]DNA available	% relative to LM7-LM7	% of total [³ H]DNA available	% relative to GS5-GS5
LM7	42.6	100.0	40.6	76.0
B2	37.1	87.2	51.0	94.9
P4	38.0	89.3	53.8	100.2
At10	38.6	90.6	51.6	96.1
SD1	40.8	95.9	67.0	123.0
10449	36.7	86.2	53.5	99.7
GS5	Not done	—	53.7	100.0
<i>Escherichia coli</i>	1.1	2.6	2.6	4.9

DISCUSSION

The results presented here support previous evidence that *Streptococcus mutans* strains can be assigned to one of four genetic groups and that these groups correlate with four of Bratthall's five serologic groups.

Members of the fifth serologic group react with Lancefield group E antiserum. Although biochemically different from other serological group E streptococci [*S. infrequens* (Deibel, Yao, Jacobs & Niven, 1964; de Moor & Thal, 1968) and *S. uberis* (Cullen, 1967)], these *Streptococcus mutans* strains of serological group E resemble *S. uberis* strains of serological group E in their inability to evoke group E antiserum when injected into rabbits (Bratthall, 1970; Cullen, 1967). They resemble *S. mutans* genetic group I strains in their slow glycolysis of fructose and in some mannitol-1-phosphate dehydrogenase isozymes. Genetically, the serological group E strains are so closely related to the genetic group I strains as to be indistinguishable by the DNA reassociation techniques used here. Furthermore, these strains cross-react with antiserum to genetic group I strains (Bratthall serological group *c*) (Bratthall, 1972), and Hardie & Bowden (1971) have shown that serological group E and genetic group I strains have the same carbohydrates in their cell walls. It is reasonable to consider the serological group E strains as variants of genetic group I. It is interesting to note that strain SD1, which resembles the serological group E strains, did not react with commercial group E antisera.

Differences in biochemical properties among *S. mutans* strains such as reaction to raffinose and arginine have been observed by others (Edwardsson, 1968; Guggenheim, 1968). However, these differences are seen to correlate with the genetic groups. Thus the members of genetic group III (Bratthall serological group *d*) can be identified by their failure to ferment raffinose and those of group II can be identified by the production of ammonia from arginine. Strains of genetic groups I (Bratthall serological group *c*) and IV (Bratthall serological group *a*) are biochemically very similar but can be separated by the reluctance of genetic group IV strains to grow in air.

The difference in the rate of metabolism of fructose relative to glucose and sucrose in genetic group I strains compared with all other strains does not constitute a rapid means of identification and is more of academic interest. The dissimilation of sucrose in *S. mutans* is complex (Tanzer, 1972). It is known that sucrose is metabolized more rapidly if cells are grown in sucrose (Tanzer, Brown & Meyers, 1972). Thus the fate of sucrose is determined, at least in part, by an inducible system, probably invertase (Gibbons, 1972; Tanzer, Brown &

McInerney, 1973). The experiments with fructose reported here demonstrate that in genetic group I strains, rapid fructose metabolism is, like the metabolism of sucrose, not constitutive (as it is in other strains) but is induced by growth in sucrose. The failure of sucrose-grown GS5 to ferment sucrose rapidly may be due to the low level of invertase observed when this strain is grown on sucrose (Kuramitsu, 1973). Other *S. mutans* strains have increased levels of invertase after growth in sucrose (Tanzer, Brown & McInerney, 1973).

Organisms phenotypically homogeneous but genetically heterogeneous present a taxonomic dilemma. If genetic relatedness completely determined taxonomy *Escherichia coli* would be considered a member of the genus *Shigella* (Brenner, Fanning, Miklos & Steigerwalt, 1973) and *Streptococcus mutans* could be divided into four species. Such classifications would be unacceptable to many microbiologists and clinicians. Without DNA hybridization techniques there would be little argument that *S. mutans* should remain a single species. Despite the presence of different antigens and small biochemical differences, *S. mutans* strains show great overall phenotypic similarity. However, we know that significant genetic disparities also exist within *S. mutans*. Should the four genetic groups be considered four separate species? This may be reasonable on genetic grounds. On other grounds it would present difficulties. Tests such as fermentation of raffinose and the production of ammonia from arginine serve to identify genetic groups among the strains in this study but may not be completely predictive for all future isolates. Thus, laboratories lacking specific *S. mutans* antisera and equipment for DNA analysis could not reliably separate four 'mutans-like' species. Furthermore, *S. mutans* strains of all genetic groups have been found in human mouths, and since there is no evidence that one is more cariogenic for humans than another it would be clinically unimportant to consider these organisms as four separate species. Yet, for those who are probing the metabolism, genetics, pathogenic mechanisms and epidemiology of *S. mutans* the existence of four 'genotypes' assumes more importance, for one cannot extrapolate to the whole species the results of experiments on a member of only one genetic group.

In order to give some taxonomic status to the four types of *Streptococcus mutans*, it is suggested that the genetic groups be considered subspecies. The biochemical features of these subspecies are based only on the strains included in this study. The number of strains is small, and the phenotypic differences among the subspecies are small. Thus the proposed subspecies should be considered genotypes, but not necessarily phenotypes. The binomial *Streptococcus mutans* will continue to denote a streptococcus which ferments mannitol, usually ferments sorbitol and raffinose, produces glucan from sucrose, and is not an enterococcus.

1. *Streptococcus mutans* subsp. *mutans* (Clarke, 1924). Gram-positive cocci in pairs and chains. Mannitol, sorbitol, raffinose and inulin fermented. Glucan produced from sucrose. Grow in air but CO₂ required for optimum growth. Ammonia not produced from arginine. DNA base composition 36 to 38 mol % guanine plus cytosine. Strains in this subspecies react with Bratthall group *c* antiserum. Some strains also react with Lancefield group E antiserum.

Clarke's original strains no longer exist, but NCTC10449 was deposited by W. Sims as a 'representative strain of *S. mutans*' (Edwardsson, 1968) and has been shown to resemble Clarke's original description (Edwardsson, 1968; Guggenheim, 1968). Therefore, NCTC10449 is designated the neotype strain of the species.

2. *S. mutans* subsp. *rattus* subsp. nov. (rat'tus). M.L. n. from E. rat. Originally isolated from a rat (genus *Rattus*). Gram-positive cocci in pairs and chains. Mannitol, sorbitol, raffinose and inulin fermented. Glucan produced from sucrose. Grow in air but CO₂

Fig. 3. Approximate intergroup homologies. With one exception (LM7 = 76%), strains *within* a group were at least 85% homologous, with many approaching 100%. For exact values see Tables 2 and 3, and Coykendall (1971).

Table 4. Summary of the four subspecies of *Streptococcus mutans* and their characteristics

Subspecies	Genetic group*	Serologic group†	DNA base content (% G-C)‡	Wall carbohydrates§	Other distinguishing features
<i>S. mutans</i> subsp. <i>mutans</i>	I	<i>c</i>	36-38	Glucose Rhamnose	Slow glycolysis of fructose when grown on glucose; some strains Lancefield E
<i>S. mutans</i> subsp. <i>rattus</i>	II	<i>b</i>	41-43	Galactose Rhamnose	Produces ammonia from arginine
<i>S. mutans</i> subsp. <i>sobrinus</i>	III	<i>d</i>	44-46	Glucose Galactose Rhamnose	Does not ferment raffinose
<i>S. mutans</i> subsp. <i>cricetus</i>	IV	<i>a</i>	42-44	Glucose Galactose Rhamnose	Does not grow in air

* Coykendall (1971).

† Bratthall (1972).

‡ Coykendall (1970) and Dunny *et al.* (1972).

§ Hardie & Bowden, in Bratthall (1972).

required for optimum growth. Ammonia produced from arginine. DNA base composition 41 to 43 mol % guanine plus cytosine. Strains in this subspecies react with Bratthall group *b* antiserum. Strain FA1 (ATCC19645) is designated the type strain of the subspecies.

3. *S. mutans* subsp. *sobrinus* subsp. nov. (so-bri-nus). L. masc. n. *sobrinus* male cousin on the mother's side. This subspecies is the least related to *S. mutans* subsp. *mutans*. Gram-positive cocci in pairs and chains. Mannitol, inulin, and usually sorbitol fermented. Raffinose not fermented. Glucan produced from sucrose. Ammonia not produced from arginine. Grow in air but CO₂ required for optimum growth. DNA base composition 44 to 46 mol % guanine plus cytosine. Strains in this subspecies react with Bratthall *d* antiserum. Strain SL1 is designated the type strain of the subspecies.

4. *S. mutans* subsp. *cricetus* subsp. nov. (cri-ce-tus). M.L. From Czech *Kreck* hamster. Originally isolated from a hamster (genus *Cricetus*, or *Mesocricetus*). Gram-positive cocci

in pairs and chains. Mannitol, sorbitol, raffinose and inulin fermented. Glucan produced from sucrose. CO₂ or reduced oxygen tension required for growth. Ammonia not produced from arginine. DNA base composition 42 to 44 mol % guanine plus cytosine. Strains in this subspecies react with Bratthall group *a* antiserum. Strain HS6 (ATCC19642) is designated the type strain of the subspecies.

The characteristics of the four subspecies are summarized in Table 4. Intergroup homologies are summarized in Fig. 3. It should be borne in mind that intergroup hybrids have been shown to contain some nonspecific base pairings (Coykendall, 1971). Thus the true degree of intergroup relatedness is probably lower.

Part of this work was done at the Naval Dental Research Institute. I wish to thank the staff of that Institute, and especially S. Shelton, for their support. I thank G. Dunny and D. Clewell (University of Michigan, Ann Arbor) and Frank Tyeryar (Naval Medical Research Institute, Bethesda) for providing data on the G-C contents of some strains. I am indebted to A. Brown for electrophoresis of mannitol-1-phosphate dehydrogenases. I thank D. Bratthall for many helpful discussions.

REFERENCES

- ABERCROMBIE, G. F. & SCOTT, W. M. (1928). A case of infective endocarditis due to *Streptococcus mutans*. *Lancet* **ii**, 697-699.
- APPLETON, J. L. T., JUN. (1933). *Bacterial Infection*. Philadelphia: Lea & Febiger.
- BRATTHALL, D. (1970). Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans*. *Odontologisk revy* **21**, 143-152.
- BRATTHALL, D. (1972). Immuno-fluorescent identification of *Streptococcus mutans*. *Odontologisk revy* **23**, 181-196.
- BRENNER, D. J., FANNING, G. R., MIKLOS, G. V. & STEIGERWALT, A. G. (1973). Polynucleotide sequence relatedness among *Shigella* species. *International Journal of Systematic Bacteriology* **23**, 1-7.
- BROWN, A. T. & PATTERSON, C. E. (1972). Heterogeneity of *Streptococcus mutans* strains based on their mannitol-1-phosphate dehydrogenases: criterion for rapid classification. *Infection and Immunity* **6**, 422-424.
- BROWN, A. T. & WITTENBERGER, C. L. (1972). Fructose-1,6-diphosphate-dependent lactate dehydrogenase from a cariogenic streptococcus: purification and regulatory properties. *Journal of Bacteriology* **110**, 604-615.
- CARLSSON, J. (1967). Presence of various types of non-hemolytic streptococci in dental plaque and in other sites of the oral cavity in man. *Odontologisk revy* **18**, 55-74.
- CARLSSON, J. (1968). A numerical taxonomic study of human oral streptococci. *Odontologisk revy* **19**, 137-160.
- CLARKE, J. K. (1924). On the bacterial factor in the aetiology of dental caries. *British Journal of Experimental Pathology* **5**, 141-147.
- COYKENDALL, A. L. (1970). Base composition of deoxyribonucleic acid isolated from cariogenic streptococci. *Archives of Oral Biology* **15**, 365-368.
- COYKENDALL, A. L. (1971). Genetic heterogeneity in *Streptococcus mutans*. *Journal of Bacteriology* **106**, 192-196.
- COYKENDALL, A. L., DAILY, O. P., KRAMER, M. J. & BEATH, M. E. (1971). DNA-DNA hybridization studies of *Streptococcus mutans*. *Journal of Dental Research* **50**, 1131-1139.
- CULLEN, G. A. (1967). Classification of *Streptococcus uberis* with biochemical tests. *Research in Veterinary Science* **8**, 83-88.
- DEIBEL, R. H., YAO, J., JACOBS, N. J. & NIVEN, C. F. (1964). Group E streptococci. I. Physiological characterization of strains isolated from swine cervical abscesses. *Journal of Infectious Diseases* **114**, 327-332.
- DENHARDT, D. T. (1966). A membrane-filter technique for the detection of complementary DNA. *Biochemical and Biophysical Research Communications* **23**, 641-646.
- DRUCKER, D. B. & MELVILLE, T. H. (1971). The classification of some oral streptococci of human or rat origin. *Archives of Oral Biology* **16**, 845-853.

- DUNNY, G. M., HAUSNER, T. & CLEWELL, D. B. (1972). Buoyant densities of DNA from various strains of *Streptococcus mutans*. *Archives of Oral Biology* **17**, 1001-1003.
- EDWARDSSON, S. (1968). Characteristics of caries-inducing human streptococci resembling *Streptococcus mutans*. *Archives of Oral Biology* **13**, 637-646.
- ENGLANDER, H. R. & JORDAN, H. V. (1972). Relation between *Streptococcus mutans* and smooth surface caries in the deciduous dentition. *Journal of Dental Research* **51**, 1505.
- FITZGERALD, D. B. & FITZGERALD, R. J. (1965). Induction of dental caries in gerbils. *Archives of Oral Biology* **11**, 139-140.
- FITZGERALD, R. J., JORDAN, H. V. & STANLEY, H. R. (1960). Experimental caries and gingival pathologic changes in the gnotobiotic rat. *Journal of Dental Research* **39**, 923-935.
- FITZGERALD, R. J. & KEYES, P. H. (1960). Demonstration of the etiologic role of streptococci in experimental caries in the hamster. *Journal of the American Dental Association* **61**, 9-19.
- GIBBONS, R. J. (1964). Metabolism of intracellular polysaccharide by *Streptococcus mitis* and its relation to inducible enzyme formation. *Journal of Bacteriology* **87**, 1512-1520.
- GIBBONS, R. J. (1972). Presence of an invertase-like enzyme and a sucrose permeation system in strains of *Streptococcus mutans*. *Caries Research* **6**, 122-131.
- GIBBONS, R. J. & BANGHART, S. B. (1967). Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. *Archives of Oral Biology* **12**, 11-24.
- GIBBONS, R. J., BERMAN, K. S., KNOETTNER, P. & KAPSIMALIS, B. (1966). Dental caries and alveolar bone loss in gnotobiotic rats infected with capsule forming streptococci of human origin. *Archives of Oral Biology* **11**, 549-560.
- GIBBONS, R. J. & FITZGERALD, R. J. (1968). Dextran-induced agglutination of *Streptococcus mutans* and its potential role in the formation of dental plaques. *Journal of Bacteriology* **98**, 341-346.
- GUGGENHEIM, B. (1968). Streptococci of dental plaques. *Caries Research* **2**, 147-163.
- GUGGENHEIM, B., KÖNIG, K. G. & MÜHLEMANN, H. R. (1965). Modifications of the oral flora and their influence on dental caries in the rat. I. The effects of inoculating 4 labelled strains of streptococci. *Helvetica odontologica acta* **9**, 121-129.
- GUGGENHEIM, B. & SCHROEDER, H. E. (1967). Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. *Helvetica odontologica acta* **11**, 131-152.
- HARDIE, J. M. & BOWDEN, G. H. (1971). Carbohydrate components of the cell walls of *Streptococcus mutans* and the possible value in serological grouping. *Caries Research* **6**, 80-81.
- HOERMAN, K. C., KEENE, H. J., SHKLAIR, I. L. & BURMEISTER, J. A. (1972). The association of *Streptococcus mutans* with early carious lesions in human teeth. *Journal of the American Dental Association* **85**, 1349-1352.
- KRASSE, B. (1966). Human streptococci and experimental caries in hamsters. *Archives of Oral Biology* **11**, 429-436.
- KRASSE, B., JORDAN, H. V., SVENSSON, I. & TRELL, L. (1968). The occurrence of certain 'caries-inducing' streptococci in human dental plaque material. *Archives of Oral Biology* **13**, 911-918.
- KURAMITSU, H. K. (1973). Characterization of invertase activity from cariogenic *Streptococcus mutans*. *Journal of Bacteriology* **115**, 1003-1010.
- LANCEFIELD, R. C. (1933). A serological differentiation of human and other groups of hemolytic streptococci. *Journal of Experimental Medicine* **57**, 571-595.
- MACLEAN, I. H. (1927). The bacteriology of dental caries. *Proceedings of the Royal Society of Medicine* **20**, 873-878.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109-118.
- MCCABE, R. M., KEYES, P. H. & HOWELL, A., JUN. (1967). An *in vitro* method for assessing the plaque forming ability of oral bacteria. *Archives of Oral Biology* **12**, 1653-1656.
- DE MOOR, C. E. & THAL, E. (1968). Beta haemolytic streptococci of the Lancefield groups E, P and U: *Streptococcus infrequens*. *Antonie van Leeuwenhoek* **34**, 377-387.
- NEWBRUN, E. (1972). Extracellular polysaccharides synthesized by glucosyl-transferases of oral streptococci. *Caries Research* **6**, 132-147.
- NIVEN, C. F., SMILEY, K. L. & SHERMAN, J. M. (1942). The hydrolysis of arginine by streptococci. *Journal of Bacteriology* **43**, 651-660.
- SCHILDKRAUT, C., MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *Journal of Molecular Biology* **4**, 430-443.

- SHKLAI, I. L., KEENE, H. J. & SIMONSON, L. G. (1972). Distribution and frequency of *Streptococcus mutans* in caries-active individuals. *Journal of Dental Research* **51**, 882.
- DE STOPPELAAR, J. D. (1971). *Streptococcus mutans*, *Streptococcus sanguis* and dental caries. Thesis, University of Utrecht.
- DE STOPPELAAR, J. D., VAN HOUTE, J. & BACKER DIRKS, O. (1969). Relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. *Caries Research* **3**, 190-199.
- TANZER, J. M. (1972). Studies on the fate of the glucosyl moiety of sucrose metabolized by *Streptococcus mutans*. *Journal of Dental Research* **51**, 415-423.
- TANZER, J. M., BROWN, A. T. & MCINERNEY, M. F. (1973). Identification, preliminary characterization and evidence for regulation of invertase in *Streptococcus mutans*. *Journal of Bacteriology* **116**, 192-202.
- TANZER, J. M., BROWN, A. T. & MEYERS, K. I. (1972). Sucrose dissimilation by *Streptococcus mutans* independent of glycosyl and fructosyl transferase. *Program and Abstracts of Papers*, Fiftieth General Session International Association for Dental Research, p. 106.
- VAN HOUTE, J., DE MOOR, C. E. & JANSEN, H. M. (1970). Synthesis of iodophilic polysaccharide by human oral streptococci. *Archives of Oral Biology* **15**, 263-266.
- WOOD, J. M. (1967). A dextransucrase activity from streptococcus FA-1. *Archives of Oral Biology* **12**, 1659-1660.
- ZINNER, D. D. & JABLON, J. M. (1968). Human streptococcal strains in experimental caries. In *Art and Science of Dental Caries Research*, pp. 87-109. Edited by R. S. Harris. New York: Academic Press.
- ZINNER, D. D., JABLON, J. M., ARAN, A. D. & SASLAW, M. S. (1965). Experimental caries induced in animals by streptococci of human origin. *Proceedings of the Society for Experimental Biology and Medicine* **118**, 766-770.