Proposal to Elevate the Subspecies of *Streptococcus mutans* to Species Status, Based on Their Molecular Composition

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It is proposed to confer species rank to the genetically distinct streptococci that have been considered subspecies of *Streptococcus mutans* Clarke: *S. mutans* subsp. *rattus* Coykendall, *S. mutans* subsp. *cricketus* Coykendall, and *S. mutans* subsp. *sobrinus* Coykendall. *S. mutans* is defined to exclude phenotypically similar bacteria that have deoxyribonucleic acid (DNA) guanine plus cytosine contents appreciably different from 36 to 38 mol% and/or that do not demonstrate DNA base-sequence homology with the type strain. Streptococci that resemble *S. mutans* but that are quite disparate in molecular constitution are here regarded as comprising four new species: *S. rattus* (Coykendall) comb. nov., *S. cricketus* (Coykendall) comb. nov., *S. sobrinus* (Coykendall) comb. nov., and *S. ferus* sp. nov. NCTC 10449 is here designated as the neotype strain of *S. mutans*. The type strains of *S. rattus*, *S. cricketus*, *S. sobrinus*, and *S. ferus* are FA1 (= ATCC 19645), HS6 (= ATCC 19642), SL1, and 8S1, respectively. Simple biochemical tests serve to identify most strains of all five species. Serological procedures are capable of differentiating most human isolates.

Deoxyribonucleic acid (DNA) hybridization (32) and the analysis of enzymes by serological and electrophoretic methods have provided new approaches to bacterial taxonomy and fostered a molecular concept of species (27, 34). Thus, one may now define a species as a bacterium that has a unique overall sequence of nucleotide bases in its chromosome and a complement of enzymes characteristic of that species. Put simply, we expect members of a species to not only "look alike" and "act alike," but also to be constructed of, and controlled by, molecules that are alike. Thus, members of a species should be molecularly homologous. Bacteria that are molecularly disparate should not be called by the same species name.

Some organisms reported to resemble *Streptococcus mutans* Clarke 1924 (8) are phenotypically quite similar (6, 16, 17, 30) but are molecularly heterogeneous. Analysis of their DNA base-sequence similarities has shown the existence of four genetic groups (11), which we call "genospecies," a term introduced by Ravin (31). DNA from strains within each genospecies hybridized almost completely, but DNA from strains of different genospecies hybridized poorly (10, 11). Furthermore, intergroup hybrid duplexes were unstable (10). These findings indicate a degree of molecular disparity not expected within a single species. Indeed, every molecule or subcellular component isolated from *S. mutans* and studied comparatively has shown differences that correlate with the DNA base-sequence differences. Brown has shown

differences in lactate dehydrogenases among three of the genospecies in respect to the enzyme's kinetic response to pyruvate (5). He also demonstrated electrophoretic differences in mannitol- and sorbitol-1-phosphate dehydrogenases (4). Using serological methods, London found that aldolases of "*S. mutans*" were heterogeneous and concluded that "*S. mutans*" included "divergent groups" of organisms (26). Heterogeneity has also been observed in polysaccharide-synthesizing enzymes (7, 28) and in invertase (J. M. Tanzer et al., J. Dent. Res., vol 54, special issue A; Abstr. 53rd Gen. Session Inter. Assoc. Dent. Res., 1975). *S. mutans* genospecies correlate well with the serological groups described by Bratthall (2). Additional serogroups found by Perch et al. (30) seem to represent variants within the genospecies (12). Differences have been observed in cell wall carbohydrates among some genospecies (24), and some morphological differences have been reported (13).

It is clear that "*S. mutans*" is actually a group of streptococci, but, because of the overall biochemical similarity of all *S. mutans* strains, it was proposed to conserve the nomen species "*Streptococcus mutans*" and to assign subspecies rank to the genospecies (11). However, new information on the evolutionary distance between members of the different genospecies (26) and the discovery of a fifth genospecies (14) make it increasingly difficult to consider all these streptococci as "*Streptococcus mutans*." Furthermore, additional biochemical differ-

ences between the present subspecies have been observed (30, 33) which augment those originally described (11) and which appear quite useful for differentiating the genospecies. Shklair and Keene (33) have found bacitracin susceptibility particularly useful for differentiating the two genospecies that are biochemically most similar (i.e., *S. mutans* subsp. *mutans* and *S. mutans* subsp. *cricketus*). A clear taxonomic definition of these organisms is now appropriate. Therefore, it is proposed to elevate each subspecies of *S. mutans* to the rank of species.

***Streptococcus mutans* Clarke 1924 (8)**

Gram-positive cocci occurring in pairs and chains. Cells measure 0.5 μm in diameter. Mannitol, sorbitol, and raffinose are fermented. An adhesive glucan is produced from sucrose. Growth occurs in air but is enhanced in atmospheres of reduced oxygen content. Ammonia is not produced from arginine. Colonies on agar containing sucrose are ca. 1 mm in diameter, rough, and heaped, and often have beads or puddles of liquid (containing soluble glucan) around the colonies. The DNA guanine plus cytosine (G+C) content is 36 to 38 mol%.

Neotype strain. Edwardsson (16) reported that Clarke's strains no longer exist but that W. Sims had deposited a "representative strain of *S. mutans*" in the National Collection of Type Cultures under the number NCTC 10449. The characteristics of this strain resemble those in Clarke's description of *S. mutans* (Table 1). Thus, NCTC 10449 is here designated the neotype strain of the species. (Although this strain was designated as the neotype strain by the author in 1974 [11], according to Rule 18e of

the Bacteriological Code, neotypes must be proposed in the *International Journal of Systematic Bacteriology*. Consequently, the proposal made here is the first valid proposal of a neotype strain for *S. mutans*.) Edwardsson (16) described NCTC 10449 as having "characteristics identical with those of strain Ingbritt," which fermented mannitol, sorbitol, sucrose, raffinose, lactose, inulin, mannose, melibiose, salicin, and trehalose, but not glycerol, starch, arabinose, melezitose, or xylose. It did not hydrolyze arginine. It would not tolerate 10 or 45°C, pH 9.6, or 6.5% NaCl. The final pH in glucose broth was below 4.3. On horse blood agar, the strain was not hemolytic. It acidified and curdled litmus milk. The G+C content of NCTC 10449 DNA is 37.9 mol% by thermal denaturation (9) and 37.1 mol% by buoyant density in CsCl (15). Strain NCTC 10449 DNA is homologous with strain Ingbritt DNA (10). It was isolated from a decayed tooth (16).

Additional comments. Electron micrographs of *S. mutans* strains NCTC 10449 (13) and GS5 (25) show coccal cells, 0.5 μm in diameter, and occasional rod forms as described by Clarke (8). The walls show an inner electron-dense layer and a more lucent outer layer (12). Most members of this species react with the Bratthall *c* antiserum (2). Some strains react with the Lancefield E antiserum (11). Occasional strains react strongly with neither, and they were placed in a separate serotype, *f* (33). Strains of all these serotypes are biochemically similar except that some group E strains do not ferment melibiose (11, 30). Strains of all three serotypes share extensive DNA base sequences with strain NCTC 10449 (11, 12). *S. mutans* cells produce intracellular polysaccharide that

TABLE 1. Comparison of *S. mutans* as described by Clarke (8) with the neotype strain NCTC 10449 as described by Edwardsson (16)

Determination	<i>S. mutans</i> (Clarke)	NCTC 10449
Morphology	Noncapsulated coccus; chains of medium length; 0.75 μm diameter	Gram-positive coccus; short-to-medium chains; size not stated
Colony morphology	On glucose agar: small, grey-white; become rough, heaped after 3-4 days; 1 mm diameter; hard to pick; "run along in front of needle"	On blood agar: white, circular or irregular; 0.5-1 mm diameter; "rather hard and adherent"
Temperature tolerance	No growth at 22°C	No growth at 10°C
Acid but no gas produced from:		
Glucose	+	+
Lactose	+	+
Raffinose	+	+
Mannitol (mannite)	+	+
Inulin	+	+
Salicin	+	+
Dulcitol (dulcitol)	~	-
pH in glucose broth	4.2	4.0-4.3

can be catabolized when exogenous carbon sources are absent (21). The habitat of *S. mutans* is the surface of the human tooth (3, 17, 30).

***Streptococcus rattus* (Coykendall) comb. nov.**

(Basionym: *S. mutans* subsp. *rattus* Coykendall [11].) (M. L. n. *Rat' tus* generic name of the rat [referring to the source of the original isolate].) Gram-positive cocci, 0.5 μ m in diameter, occurring in pairs and chains. Mannitol, sorbitol, raffinose, and inulin are fermented. An adhesive glucan is produced from sucrose. Growth occurs in air but is enhanced in atmospheres of reduced oxygen content. Ammonia is produced from arginine. Colonies on agar containing sucrose are ca. 1 mm in diameter, rough, and heaped, and may have beads or puddles of liquid (containing glucan) around the colonies. The DNA base content is 41 to 43 mol% G+C.

Type strain. Strain FA1 (ATCC 19645) is the type strain (11). This strain was described by Fitzgerald et al. (19) as a microaerophilic, α -hemolytic streptococcus isolated from laboratory rats. Colonies on Trypticase-soy agar were gray-white and opaque, with a raised center. On media containing sucrose, colonies were firm and almost "rubbery." Mannitol, sorbitol, raffinose, sucrose, lactose, maltose, and inulin, but not glycerol, melezitose, rhamnose, or xylose, were fermented. Starch was not hydrolyzed. A pH of 4.2 to 4.4 was produced from carbohydrates. This strain was intolerant of 40% bile and 6.5% NaCl and would grow at 45°C but not at 10°C. Strain FA1 produces ammonia from arginine (11). Its DNA has 42.2 mol% G+C by thermal denaturation (9) and 41.3 mol% G+C by buoyant density in CsCl (15).

Additional comments. Cells of *S. rattus* strains FA1 (13) and BHT (1) are slightly elongated cocci 0.5 μ m in diameter. These cells have an outer electron-dense cell wall layer in addition to the two layers seen in *S. mutans*. Strains of *S. rattus* react with the Bratthall *b* antiserum (2). Like *S. mutans*, *S. rattus* can store, and then catabolize, intracellular polysaccharide (21). The species is not as common in humans as is *S. mutans* (3, 30), although it may be more common in certain populations (3). The DNA from a human strain, BHT, was homologous with that of strain FA1 (10). Although *S. rattus* was originally isolated from a laboratory rat, it is possible that the animal was infected via humans. *S. rattus* was not found in wild rats (14).

***Streptococcus cricetus* (Coykendall) comb. nov.**

(Basionym: *S. mutans* subsp. *cricetus* Coykendall [11].) (M. L. n. *Cri' ce-tus* hamster [referring to the source of the original isolate].) Gram-positive cocci, 0.5 μ m in diameter, occurring in pairs and chains. Mannitol, sorbitol, raffinose, and inulin are fermented. Adhesive glucan is produced from sucrose. An atmosphere of reduced oxygen content is required for growth. Ammonia is not produced from arginine. Colonies on agar containing sucrose are ca. 1 mm in diameter, rough, heaped, often glossy, and often surrounded by liquid containing soluble glucan. The DNA base composition is 42 to 44 mol% G+C.

Type strain. Strain HS6 (ATCC 19642) is the type strain (11). This strain was isolated from a decayed hamster tooth and was described by Fitzgerald and Keyes (20). It produced acid but not gas from fructose, glucose, inulin, lactose, mannitol, sorbitol, mannose, melibiose, raffinose, salicin, sucrose, and trehalose. It did not ferment arabinose, glycerol, inositol, melezitose, rhamnose, or xylose. Esculin was hydrolyzed, but starch, hippurate, and arginine were not. Litmus milk was reduced, acidified, and coagulated. The final pH in glucose or sucrose broth was 4.1 to 4.3. Colonies on blood agar were smooth and round, 2 to 3 mm in diameter, with a slightly raised center. The G+C content of this strain is 42.7 mol% (11).

Additional comments. Electron micrographs of *S. cricetus* OMZ61 (23) indicate that the morphology of the species is similar to that of *S. mutans*. Most strains of *S. cricetus* react with the Bratthall *a* antiserum (2), but those isolated from mouths of wild rats that ate sugar cane did not have the *a* antigen (12). The DNAs of *S. cricetus* strains E49, AHT, and OMZ61 (11), and of the strains from the cane-eating rats (12), were homologous with HS6 DNA. *S. cricetus* cells produce and catabolize intracellular polysaccharide (21). Human strains of *S. cricetus* are susceptible to bacitracin (2 U/ml) (33). As with *S. rattus*, the animal from which the species was first isolated may have been infected through contact with humans. *S. cricetus* is found in the human mouth but is not as common in man as *S. mutans* (3, 30).

***Streptococcus sorbrinus* (Coykendall) comb. nov.**

(Basionym: *S. mutans* subsp. *sobrinus* Coykendall [11].) (L. masc. n. *so-bri' nus* male cousin on mother's side [referring to the "distant relationship" between this species and *S. mutans*].) Gram-positive cocci, 0.5 μ m in diameter,

TABLE 2. Characteristics of streptococcal species resembling *S. mutans*

Species	DNA base content (mol% G+C) ^a	Serological reaction ^b	Cell wall carbohydrates ^c	Biochemical characteristics useful for identification
<i>S. mutans</i>	36-38	<i>c, e, f</i>	Glucose, rhamnose	Produces ammonia from arginine; grows at 45°C Susceptible to bacitracin ^d
<i>S. rattus</i>	41-43	<i>b</i>	Galactose, rhamnose	
<i>S. cricetus</i>	42-44	<i>a</i>	Glucose, galactose, rhamnose	Fails to ferment raffinose; produces H ₂ O ₂ ^e Fails to ferment raffinose; susceptible to bacitracin
<i>S. sobrinus</i>	44-46	<i>d, g</i>	Glucose, galactose, rhamnose	
<i>S. ferus</i>	43-45	<i>c</i>	Not known	

^a Coykendall (9) and Dunny et al. (15).

^b Bratthall (2), Perch et al. (30), and Coykendall et al. (12).

^c Hardie and Bowden (24).

^d Shklair and Keene (33).

^e Perch et al. (30).

occurring in pairs and chains, which are often long. Mannitol and sorbitol are fermented; raffinose and melibiose are not. An adhesive glucan is produced from sucrose. Growth is enhanced in an atmosphere of reduced oxygen content. Ammonia is not produced from arginine. Colonies on agar containing sucrose are ca. 1 mm in diameter, rough, and heaped, and often have droplets of liquid (containing glucan) at the top or the border. Hydrogen peroxide is produced. The DNA base content is 44 to 46 mol% G+C.

Type strain. Strain SL1 is the type strain (11). It was isolated from a human mouth and was first mentioned by Fitzgerald and Jordan (18) but was not described. Strain SL1 produces acid from mannitol and sorbitol, but not from raffinose or melibiose; it does not split arginine (11). Perch et al. (30) reported that strain SL1 was alpha hemolytic on horse blood agar, produced H₂O₂, and would not grow at 45°C. Its base content was 45.1 mol% G+C by thermal denaturation (9) and 44.9 mol% G+C by density in CsCl (15).

Additional comments. Electron micrographs of *S. sobrinus* strains K1R (13), 6715 (28), and OMZ174 (22) show cocci with an outer dendritic layer, or "fuzz," which has not been observed in *S. mutans*, *S. rattus*, or *S. cricetus*. Most *S. sobrinus* strains react with the Bratthall *d* antiserum (2). Some strains did not give a strong *d* reaction and were put into a separate serotype, designated *g* by Perch et al. (30). Strain SL1 did not react with either *d* or *g* antibody. *S. sobrinus* strains SL1, K1R, and OMZ176 (11) and 14H, O1, and 1C (12) all share extensive common DNA base sequences. Some strains do not ferment sorbitol (11, 30), and strain 14H fermented neither sorbitol nor mannitol (12). *S. sobrinus* cells appear incapable of synthesizing

or catabolizing significant amounts of intracellular polysaccharide (21). The habitat of *S. sobrinus* is the surface of the human tooth (3, 30).

Streptococcus ferus sp. nov.

(L. adj. *fer'us* wild [referring to the wild rats from which the organism was isolated].) Gram-positive cocci, 0.5 µm in diameter, occurring in pairs and chains. Mannitol and sorbitol are fermented; raffinose is not. An adhesive glucan is produced from sucrose. It is intolerant of 45°C or 6.5% NaCl. Ammonia is not produced from arginine. Colonies on agar containing sucrose are ca. 1 mm in diameter, raised, and somewhat adherent, with no liquid on or around the colony. The final pH in 1% glucose broth is 4.2 to 4.5. The DNA base content is 43 to 45 mol% G+C (12).

Type strain. The type strain is 8S1 (14), which conforms to the above description.

Additional comments. *S. ferus* was isolated in Florida from the mouths of wild rats that ate sugar cane (14) and from rats living in a landfill dump in Hartford, Conn. (12). Strains from both locales were homologous by DNA hybridization (12). Strains of *S. ferus* react with the Bratthall *c* antiserum, although they are not related (by DNA homology) to *S. mutans* (12). *S. ferus* strains do not grow on agar containing bacitracin (0.2 U/ml). The cells produce and catabolize intracellular polysaccharide (21). *S. ferus* has not been isolated from humans.

The characteristics of these five species are summarized in Table 2.

ACKNOWLEDGMENTS

I thank D. B. Clewell, R. J. Gibbons, and M. Mandel for their advice.

This work was supported by the Medical Research Service of the Veterans Administration.

REPRINT REQUESTS

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