Streptococcus macacae sp. nov. from Dental Plaque of Monkeys (*Macaca fascicularis*)

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We propose the name *Streptococcus macacae* sp. nov. for gram-positive, catalase-negative streptococcal strains that were isolated from the dental plaque of monkeys (*Macaca fascicularis*). This organism is distinct from other oral streptococci in that it produces acid from mannitol and raffinose but not from inulin or dextrin. It is not able to grow in the presence of bacitracin and does not produce hydrogen peroxide or hydrolyze arginine, but esculin is hydrolyzed, and dextran is produced from sucrose. *Streptococcus macacae* sp. nov. possesses the serotype *c* antigen described by Bratthall, as do *Streptococcus mutans* and *Streptococcus ferus*; however, the protein profiles of whole-cell extracts subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and differences in deoxyribonucleic acid composition demonstrate that the new isolates are distinct from the two other species that possess the serotype *c* antigen and from other *mutans* streptococci. The guanine-plus-cytosine content of *S. macacae* is 35 to 36 mol%. The type strain is strain 25-1 (- NCTC 11558).

During the course of studies into the ecology of streptococci in the dental plaque of monkeys (*Macaca fascicularis*), we isolated organisms that differ from any previously reported species. These streptococci are *mutans* streptococci in that they produce acid from mannitol, form dextran from sucrose, and have the teeth as their major oral habitat (5). The biochemical and genetic differences between the new isolates and previously described *mutans* streptococcal species lead us to propose that the new strains represent a distinct species.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Methods of characterization. The phenotypic reactions of strains were studied by using media and methods which we have described previously (1). Additionally, we incorporated into our conventional identification system a test for acid production from dextrin which Kral and Daneo-Moore (12) found, using a miniaturized system, enabled differentiation of *Streptococcus ferus* from *Streptococcus mutans*. We found that *S. ferus* strains HD3 and 8SI^T (T = type strain) gave the same results in our test system as previously reported (12).

Determination of DNA base composition. To determine deoxyribonucleic acid (DNA) base composition, approximately 2 g (wet weight) of bacteria were lysed and treated by the method of Garvie (9) up to the step involving incubation with ribonuclease (final concentration, 50 µg/ml; type XIV; Sigma Chemical Co.), and incubation was continued for an additional 60 min. The solution was deproteinized by shaking with an equal volume of chloroform-octanol (24:1) and then centrifuged at 5,000 rpm for 10 min, and the upper aqueous layer was passed through a column (1 by 10 cm) of Sepharose 4B that was irrigated with standard saline citrate (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0) at a flow rate of 0.1 ml/min (10). Fractions (2 ml) were collected, and the fraction corresponding to the first peak eluted from the column (determined by absorbance readings at 260 nm) was used to determine the guanine-plus-cytosine (G+C)

content of the DNA. The thermal melting point of each DNA sample was determined in triplicate with a Gilford model 240 spectrophotometer at 260 nm by using a Gilford model 2527 thermoprogrammer with a heating rate of 1°C/min. DNAs isolated from *S. mutans* strain NCTC 10449^T and *Streptococcus rattus* strain FA-1^T were used for reference.

Intraoral distribution. Plaque samples from the developmental grooves of premolar teeth and tongue swabbings were taken from eight monkeys and treated as previously described (1) in order to isolate streptococci.

Serological procedures. Antisera were raised by giving rabbits multiple injections of heat-killed bacteria as described by Bratthall (2). Immunodiffusion tests were performed by using glass slides coated with 1% (wt/vol) agarose in 0.5 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5). The sample wells were 4 mm in diameter and 4 mm apart and had a volume of 20 µl. Serotyping was performed by using the bacteria collected by centrifugation from 20 ml of a Todd-Hewitt broth (Oxoid Ltd.) culture as antigen (1).

Polysaccharide antigen extraction. Polysaccharides were prepared by hot phenol extraction of lyophilized cells (21). For purification of antigen, the polysaccharide preparation was applied to a column of diethylaminoethyl-Trisacryl M (LKB products Ltd.) in 0.5 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5). The column was eluted with a gradient of increasing concentrations of NaCl. The antigen contents of the eluted fractions were monitored by immunodiffusion.

The sugar contents of the polysaccharides were determined by hydrolyzing samples in 3 M HCl for 3 h at 105°C, removing the acid by evaporation, and subjecting the hydrolysates to thin-layer chromatography (16).

SDS-PAGE of cell extracts. The polyacrylamide gel electrophoresis (PAGE) patterns of sodium dodecyl sulfate (SDS) extracts of the new isolates were compared with the patterns of the other *mutans* streptococci by using previously described methods (1, 19).

RESULTS AND DISCUSSION

The 10 monkey isolates examined were gram-positive cocci that grew in chains and were isolated primarily from

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TABLE 1. Strains used

Species	Serotype ^a	Strain(s) ^b
<i>S. mutans</i>	<i>c</i>	Ingbritt, NCTC 10449 ^T
	<i>e</i>	P4
	<i>f</i>	151
<i>Streptococcus sobrinus</i>	<i>d</i>	B13
	<i>g</i>	K1
	<i>h</i>	MFe28 (= NCTC 11391)
<i>Streptococcus cricetus</i>	<i>a</i>	NCTC 10919 ^T
<i>S. rattus</i>	<i>b</i>	NCTC 10920 ^T
<i>S. ferus</i>	<i>c</i>	HD3, 8SI ^T
<i>S. macacae</i>	<i>c</i>	25-1 ^T (= NCTC 11558 ^T), B1107, 653, H1569, B1435, H1670, 2638a, 2583d, 2632, 2532a, H1727

^a Serotypes *a*, *b*, *c*, *d*, and *e* are described by Bratthall (3), serotypes *f* and *g* are described by Perch et al. (17), and serotype *h* is described by Beighton et al. (1).

^b With two exceptions, the *S. mutans*, *S. sobrinus*, *S. cricetus*, and *S. rattus* strains were obtained from J. M. Hardie, London Hospital Medical College, London, United Kingdom; *S. mutans* strain Ingbritt was obtained from G. Colman, and *S. sobrinus* strain MFe28 was isolated in our laboratory. The *S. ferus* strains were obtained from A. L. Coykendall, and all of the *S. macacae* strains were isolated in our laboratory.

dentition rather than tongues. They produced acid from mannitol and dextrin from sucrose but were unable to form H₂O₂ or grow in the presence of 6.5% NaCl. These are characteristics of *mutans* streptococci (5). These isolates were similar to two previously described species, *S. mutans* and *S. ferus*, in that they possessed the serotype *c* antigen described by Bratthall (3); the composition of the antigen also resembled the composition of the antigen of *S. mutans* (15, 22). However, an examination of the biochemical and physiological characteristics of the new isolates by using the scheme of Kral and Daneo-Moore (12) showed that they differed from *S. mutans* in their inability to grow in the presence of bacitracin. They differed from *S. ferus* in that they produced acid from raffinose but failed to produce acid from dextrin or starch. Other differences were also apparent as the new isolates failed to produce acid from inulin, whereas both *S. mutans* and *S. ferus* did so, and only *S. ferus* produced acid from glycogen.

Data obtained with SDS-PAGE extracts of the *mutans*

FIG. 1. Immunodiffusion. *S. mutans* serotype *c*-specific antiserum raised against *S. mutans* strain Ingbritt (well A) was tested against pelleted whole cells of *S. mutans* strain Ingbritt (well 1), *S. ferus* strain 8SI (well 2), and *S. macacae* strain 25-1^T (well 3).

streptococci (1, 19) have supported the proposal of Coykendall (5) for the establishment of distinct species within this heterogeneous group of oral streptococci. The protein profiles obtained with SDS-PAGE of the serotype *c*-bearing strains have demonstrated the distinctness of *S. ferus* and *S. mutans*, and this supports our proposal that the new monkey isolates represent a distinct species.

The composition of the DNAs from the monkey isolates indicated a mean G+C content of 35 to 36 mol%, whereas the G+C content of *S. mutans* is 36 to 38 mol% and that of *S. ferus* is 42 to 44 mol%. Although we have not performed DNA homology studies, the differences in the DNA compositions indicate the separateness of the three groups of streptococci that possess the serotype *c* polysaccharide antigen.

We conclude that the monkey strains are different from other *mutans* streptococci and represent a new species, for which we propose the name *Streptococcus macacae* sp. nov. (*ma.cacae*.M.L.fem.n.*Macaca* genus name of macaque; M.L. gen.n.*macacae* of macaque). The type strain is strain 25-1 (= NCTC 11558).

Morphology and growth characteristics of *S. macacae*. *S. macacae* cells are gram-positive cocci which grow in chains

TABLE 2. Differential characteristics of *S. macacae* and other *mutans* streptococci

Characteristic	<i>S. macacae</i>	<i>S. mutans</i>	<i>S. ferus</i>	<i>S. sobrinus</i>	<i>S. cricetus</i>	<i>S. rattus</i>
G+C content of DNA (mol%) ^a	35-36	36-38	43-45	44-46	42-44	41-43
Serotype(s) ^b	<i>c</i>	<i>c</i> , <i>e</i> , <i>f</i>	<i>c</i>	<i>d</i> , <i>g</i> , <i>h</i>	<i>a</i>	<i>b</i>
Cell wall carbohydrates ^c	Glucose, rhamnose	Glucose, rhamnose	Not known	Glucose, galactose, rhamnose	Glucose, galactose, rhamnose	Galactose, rhamnose
Acid production from: ^d						
Dextrin	—	—	+	—	—	—
Glycogen	—	—	+	ND ^e	ND	ND
Inulin	—	+	+	— ^f	+	+
Raffinose	+	+	—	—	+	+
Starch	—	—	+	—	—	—
Resistance to bacitracin ^d	—	+	—	+	—	+
H ₂ O ₂ production ^d	—	—	—	+	—	—
Arginine hydrolysis ^d	—	—	—	—	—	+

^a Data from references 4 and 7.

^b Data from references 1, 3, and 17.

^c Data from reference 11.

^d Data from reference 12.

^e ND, Not determined.

^f Serotype *h* strains produce acid from inulin, are not resistant to bacitracin, and do not produce H₂O₂ (1).

TABLE 3. *S. macacae* occurrence as a percentage of the total anaerobic colony count in the dental plaque and on the tongues of monkeys fed a sucrose-rich diet^a

Monkey no.	% of total count in plaque	% of total count on tongue
1	2.7	ND ^b
2	28.4	6.6
3	5.3	ND
4	8.2	ND
5	32.6	ND
6	24.4	ND
7	22.1	ND
8	10.3	ND

^a See reference 1.

^b ND, Not detected (detection level, 0.05%).

in Todd-Hewitt broth and on sucrose-containing solid medium. The strains grow poorly in air, but growth is stimulated by the presence of CO₂; hence, growth occurs in candle jars and in anaerobe jars fitted with cold Deoxy catalyst (Oxoid) in an atmosphere initially consisting of 90% (vol/vol) H₂ and 10% (vol/vol) CO₂.

On sucrose-containing medium the colonies are easily removed but coherent. They are transparent and 1 to 2 mm in diameter after 3 days of incubation. As with other mutans streptococci (8), a less frequent colony form is produced, which is vivid white, less than 1 mm in diameter, erose, crumbly, and easily removed from the medium with a loop. The transparent forms always give rise to the two colony types, but the vivid white forms retain their original colonial morphology despite repeated streaking onto fresh medium. Strains produce greening of horse blood agar plates whether they are grown anaerobically or in candle jars.

Biochemical characteristics. The strains examined all give similar results in the tests which we performed. Acid is produced from *N*-acetyl glucosamine, amygdalin, cellobiose, fructose, glucose, galactose, maltose, sucrose, and trehalose; sorbitol is fermented slowly. The terminal pH in sucrose broth varies from 4.6 to 4.9. No acid is produced

from adonitol, arabinose, glycerol, inositol, melezitose, melibiose, ribose, sorbose, xylitol, or xylose. Additional characteristics are shown in Table 2. Dextran is produced from sucrose, and catalase is not produced; H₂O₂ is not produced. Growth occurs in media containing 10 and 40% (wt/vol) bile but not in the presence of 6.5% (wt/vol) NaCl, at 45°C, or at pH 9.6. Esculin is hydrolyzed; starch and blue dextran are weakly hydrolyzed, and arginine is not hydrolyzed.

Serotype c antigen. The strains of *S. mutans*, *S. macacae*, and *S. ferus* all contain an antigen which forms a precipitin line of identity when a bacterial pellet one of the strains is tested in an immunodiffusion assay with typing sera raised against *S. mutans* (three different sera) or strain 25-1^T (Fig. 1). No other precipitin lines are observed with a wide range of antiserum concentrations and varying amounts of bacterial pellet. This antigen can be extracted from the bacteria by the Lancefield (14), Rantz-Randall (18), and hot phenol-water (21) procedures and has been purified by ion-exchange chromatography on diethylaminoethyl-Trisacryl. The serotype c antigen elutes at approximately 0.1 M NaCl. Glucose and rhamnose are the major sugars detected after hydrolysis of the antigen, as previously found for *S. mutans* (15, 22), although a trace of galactose also is found.

DNA composition. The G+C contents of three of the new isolates (strains H1569, H1762, and 25-1^T) are 35 and 36 mol%, respectively. The values found for *S. mutans* NCTC 10449^T and *S. rattus* Fitzgerald FA1^T are 37 and 42 mol%, respectively; these values are almost identical to the values found by other workers (5).

Intraoral distribution. The strains of *S. macacae* were isolated primarily from dental plaque samples, indicating that tooth surfaces are the preferred habitat of these organisms (Table 3).

SDS-PAGE of cell extracts. The SDS-PAGE profiles of the 11 strains of *S. macacae* were identical and were distinct from the profiles of the other *Streptococcus* species examined. Figure 2 shows only the difference between the three streptococcal species bearing the serotype c polysaccharide antigen, as these species may be confused if serotyping is used to identify isolates. *S. macacae*, *S. mutans*, and *S.*

FIG. 2. SDS-PAGE patterns of strains of *S. mutans* serotype c, *S. macacae*, and *S. ferus*. Lane 1, Molecular weight standards; lanes 2 through 5, *S. mutans* serotype c strain Ingbritt, strain NCTC 10449^T, and two fresh monkey isolates, respectively; lanes 6 through 9, *S. macacae* strains 25-1^T, B1107, 653, and H1569, respectively; lanes 10 and 11, *S. ferus* strains HD3 and 8S1^T, respectively.

ferus each has a characteristic protein profile that is distinctly different from the profiles of all other mutans streptococci.

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