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Streptococcus downei sp. nov. for Strains Previously Described as *Streptococcus mutans* Serotype h

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Strains of streptococci originally isolated from the dental plaque of monkeys (*Macaca fascicularis*) and designated as *Streptococcus mutans* serotype h were compared with the other species of the mutans streptococcus group. Despite the close resemblance noted previously between these strains and *Streptococcus sobrinus*, closer examination revealed several important differences. Strains of serotype h ferment mannitol but not sorbitol, melibiose, inulin, or raffinose, do not produce hydrogen peroxide, and are unable to grow in the presence of bacitracin at 2 units per ml. They exhibit a distinct polypeptide pattern by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and possess several antigens absent from *S. sobrinus* as revealed by Western blotting (immunoblotting). Virtually identical polar lipid patterns were observed by two-dimensional thin-layer chromatography for both serotype h strains and *S. sobrinus*, although on the basis of long-chain fatty acid analysis by capillary gas-liquid chromatography, the former could be distinguished by the presence of a peak tentatively identified as cyclopropane acid (*cis*-9,10-methyleneoctadecanoate ($\Delta C_{19:0}$). Deoxyribonucleic acid (DNA)-DNA hybridization studies by both the S1 nuclease and renaturation rate methods showed that serotype h strains differ from *S. mutans*, *S. sobrinus*, *Streptococcus cricetus*, *Streptococcus rattus*, *Streptococcus ferus*, and *Streptococcus macacae*. On the basis of these data, we believe that *S. mutans* serotype h strains represent a distinct species for which the name *Streptococcus downei* is proposed. The DNA base composition is 41 to 42 moles percent guanine plus cytosine. The type strain is strain MFe28 (NCTC 11391^T), which is cariogenic in monoassociated germfree rats.

Previous investigations on the streptococcal population of the dental plaque of monkeys (*Macaca fascicularis*) resulted in the isolation of a new serotype (h) within the mutans streptococci (2). These strains were cariogenic for germfree rats fed a high-sucrose diet and appeared to be implicated in monkey caries. Serological methods demonstrated the major polysaccharide antigen to be antigenically distinct from, although closely resembling, the specific antigens of serotypes a (*Streptococcus cricetus*) and d and g (*Streptococcus sobrinus*). A more recent study showed the purified antigen to be composed of galactose, glucose, and rhamnose (18). Whole-cell polypeptide patterns of the serotype h strains obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were also very similar to the patterns obtained for serotypes d and g (2). Despite the close similarity observed between the serotype h strains and *S. sobrinus*, the biochemical characteristics of these isolates together with data from the chemotaxonomic and immunological studies and deoxyribonucleic acid (DNA)-DNA hybridization experiments described here demonstrate that these make up a new species within the so-called *Streptococcus mutans* group (10).

MATERIALS AND METHODS

Strains. The three strains selected for detailed study were randomly chosen from several hundred isolates of serotype h (identified by biochemical and serological tests) from monkey plaque. The strains used are listed in Table 1.

Methods of characterization. The phenotypic reactions of strains were studied by using media and methods described previously (2).

SDS-PAGE of whole-cell extracts. Protein patterns of *Streptococcus downei* and other mutans streptococci were obtained by polyacrylamide slab gel electrophoresis (PAGE) of whole-cell extracts in the presence of SDS, using the discontinuous buffer system of Laemmli (14) as described previously (24).

Analysis of long-chain fatty acids. Fatty acids were extracted from lyophilized cells by the whole-cell acid methanolysis and thin-layer procedure described by Minnikin et al. (15). Fatty acid methyl esters were analyzed on a Carlo Erba Fractovap 2150 flame ionization gas chromatograph with a 25-m fused silica column (SE54; Carlo Erba) operated isothermally at 180°C. The injection port and detector temperatures were 250°C, and a sample split of 30:1 was used. Helium was used as the carrier gas (flow rate, 1.0 ml/min). Individual esters were identified by comparison of their retention times with those of standard fatty acid esters (bacterial acid methyl ester mixture 4-5436; Supelco, Inc., Bellefonte, Pa.). The presence of unsaturated esters was confirmed by hydrogenation of one-half of the sample with H_2 gas in the presence of a palladium catalyst on charcoal (BDH, Poole, England).

Polar lipid patterns. Lipids were extracted from lyophilized cells by a modification (5, 16) of the procedure of Bligh and Dyer (3) and separated by two-dimensional thin-layer chromatography on Merck Kieselgel$_{60}$ plates (aluminum backed). Chromatograms were developed in the first dimension with chloroform-methanol-water (65:25:4 by volume) and in the second dimension with chloroform-acetic acid-methanol-water (80:15:12:4 by volume). Polar lipids were revealed by spraying chromatograms with 10% (wt/vol) dodecamolybdo-phosphoric acid in ethanol followed by charring at 130°C for approximately 10 min. Parallel chromatograms were developed with specific spray reagents for lipid phosphate (8), free amino groups (ninhydrin in water-saturated butanol), sugars (13), and α-glycols (23).

Immunological methods. Antiserum against heat-killed bacteria was raised by giving rabbits intravenous injections

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TABLE 1. DNA-DNA relatedness between strains MFe28^T, B1314, *S. sobrinus*, and *S. cricetus* by the S-1 nuclease method[a]

Unlabeled DNA from:	Serotype[b]	mol% G+C	% homology with ^{3}H-labeled DNA from:		
			S. sobrinus SL-1^T	*S. cricetus* HS-6^T	MFe28^T
Monkey plaque strains					
MFe28^T (NCTC 11391^T)	h	41.3	38	21	100
B1314	h	41.9	46	27	100
S. sobrinus					
SL-1^T (ATCC 33478^T)		42.8	100	18	53
K1-R (NCTC 10921)	d	43.9	79	NT[c]	NT
OMZ65 (NCTC 11061)	g	43.3	102	16	NT
TH21	g	43.4	119	NT	NT
S. cricetus					
HS6^T (ATCC 19642^T)	a	42.1	24	100	15
AHT (NCTC 10919)	a	42.6	33	120	NT
3720	a	42.1	28	123	NT

[a] Labeled DNA from *S. mutans* serotype c NCTC 10449^T (ATCC 25175^T), *Streptococcus rattus* serotype b FA-1^T (NCTC 10920^T, ATCC 19645^T), *Streptococcus ferus* serotype c 8S1^T (ATCC 33477^T), and *Streptococcus macacae* serotype c 25-1^T (NCTC 11558^T) showed less than 10% homology with strains MFe28^T and B1314.

[b] Serotypes a, b, c, and d are described by Bratthall (4), serotype g is described by Perch et al. (19), and serotype h is described by Beighton et al. (2).

[c] NT, Not tested.

as described by Bratthall (4). For detection of antigens by Western blotting (immunoblotting), the bacteria from 20-ml cultures grown at 37°C for 16 h in Todd-Hewitt broth (Oxoid Ltd., London, England) were sedimented by centrifugation and suspended in 75 μl of water plus 75 μl of SDS-sample buffer (Laemmli). After 1.5 h at room temperature, insoluble bacterial residue was removed by centrifugation at 10,000 × *g* for 3 min and the supernatant was subjected to SDS-PAGE as described above with 9% acrylamide. Conditions for transfer to nitrocellulose and subsequent treatment with antisera were as described before (20) with the exception that the blocking stage utilized 5% skim milk powder in 0.05 M Tris hydrochloride (pH 7.5).

DNA analyses. DNA was extracted and purified from approximately 2 to 3 g (wet weight) of bacterial cells by the method of Garvie (9). For base composition determinations, the DNA was finally dialyzed exhaustively in standard 1× SSC (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0) and the mole percent guanine-plus-cytosine (mol% G+C) content was determined from the thermal melting point of the sample as described previously (1).

DNA-DNA hybridizations were done by the S1 nuclease method of Shah et al. (22) based on the method of Crosa et al. (6) and also spectrophotometrically from renaturation rates (7, 12) with a Gilford model 240 spectrophotometer at 260 nm linked to a Gilford model 2527 thermoprogrammer.

RESULTS AND DISCUSSION

SDS-PAGE of whole-cell extracts. The protein profiles obtained for monkey dental plaque strains MFe28^T and B1314 together with those of *S. sobrinus* SL-1^T and K1-R are shown in Fig. 1. Despite the close overall resemblance between the profiles for these strains as previously reported (2), several differences in both band positions and staining intensities can be seen throughout the patterns.

Long-chain fatty acid analysis and polar lipid patterns. The determination of cellular long-chain fatty acids by capillary gas-liquid chromatography of acid methanolysates showed that in the monkey stains between 3 and 4% of the total acid had the relative retention time of the cyclopropane acid (*cis*-9,10-methyleneoctadecanoic acid; $\Delta C_{19:0}$) that resisted hydrogenation with H_2 gas in the presence of a palladium catalyst on charcoal. This served to distinguish the monkey strains from *S. sobrinus*, which was otherwise similar with major amounts of hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$), octadecenoic ($C_{18:1}$), and eicosenoic ($C_{20:1}$) acids.

The polar lipid patterns of strains MFe28^T and B1314 were virtually identical to those obtained for strains of *S. sobrinus* (SL-1^T and K1-R) (Fig. 2). In addition to major amounts of diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG), which were identified from their chromatographic

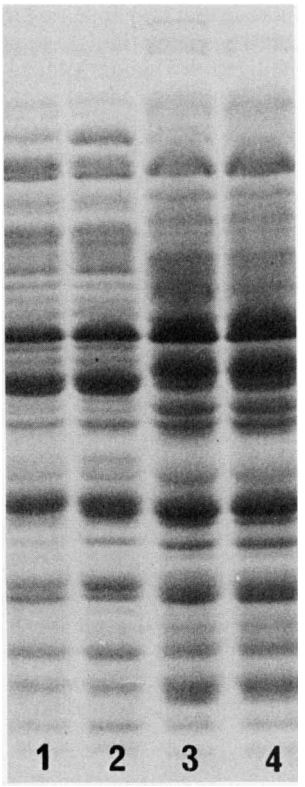

FIG. 1. SDS-PAGE polypeptide patterns of monkey plaque strains and *S. sobrinus*. Lanes 1 and 2, *S. sobrinus* SL-1^T and K1-R, respectively; lanes 3 and 4, strains MFe28^T and B1314, respectively.

behavior and reactions to the specific spray reagents, the patterns were characterized by the presence of major amounts of a phospholipid (P) and by an unidentified glycolipid which also reacted with ninhydrin (Ga). Components G1 (a and b) and G2 which corresponded to mono- and diglycosylglyceride, respectively, were also present in all patterns.

Antigenic relationships. We previously reported that the major polysaccharide antigen of MFe28^T was composed of glucose and galactose as in *S. sobrinus* (2). These results have been confirmed by Okahashi et al. (18). We also demonstrated the partial antigenic identity of glucosyltransferase and wall-associated protein antigen B from MFe28^T and *S. sobrinus* strains (2). The latter antigen corresponds to that referred to by others as SpaA and PAg (11, 17). Western blotting experiments confirmed the extensive antigenic cross-reactivity of MFe28^T and *S. sobrinus*. Antisera raised against either intact MFe28^T or *S. sobrinus* revealed that each was capable of recognizing a large number of antigens of the other species (Fig. 3). Many of the antigens recognized appear to have direct counterparts in each species, but it is also clear that antigens of distinctive mobility also occur in each.

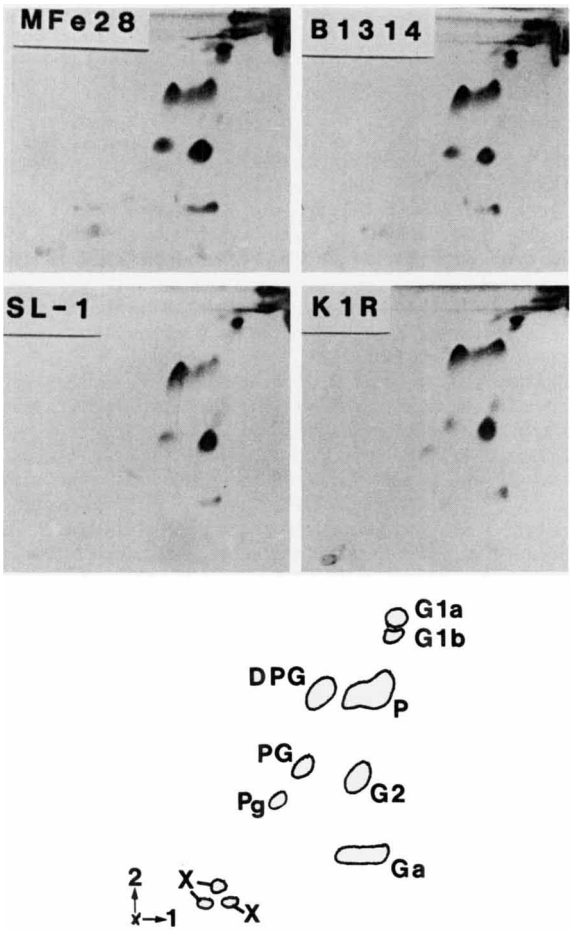

FIG. 2. Polar lipid patterns of *S. downei* MFe28^T and B1314 and *S. sobrinus* SL-1^T and K1-R. 1, First dimension (chloroform-methanol-water, 65:25:4, vol/vol/vol); 2, second dimension (chloroform-acetic acid-methanol-water, 80:15:12:4, vol/vol/vol/vol). G1a, G1b, Monoglycosyldiglyceride; G2, diglycosyldiglyceride; Ga, unknown glycolipid also reacting with ninhydrin; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; P, phospholipid; Pg, phosphoglycolipid; X, unknown polar lipids.

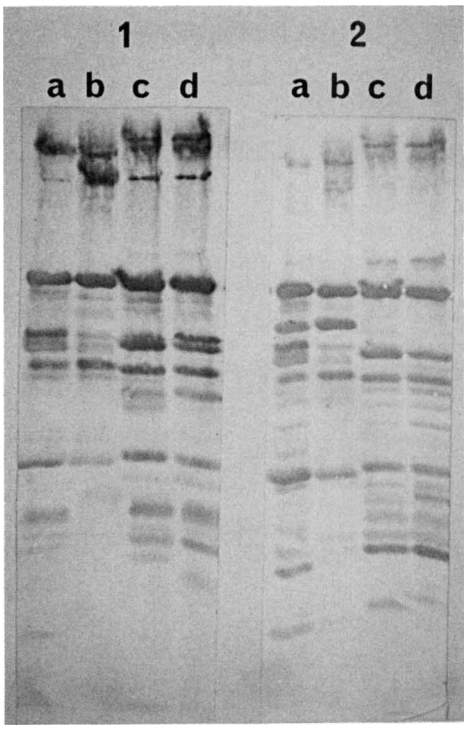

FIG. 3. Western blot analyses of whole-cell extracts of *S. sobrinus* SL-1^T (lane a) and K1-R (lane b) and strains MFe28^T (lane c) and B1314 (lane d) with antiserum raised against *S. sobrinus* (panel 1) and antiserum raised against MFe28^T (panel 2).

DNA analyses. The G+C contents of strains MFe28^T and B1314 are 41.3 and 41.9 mol%, respectively, which is slightly lower than that obtained for strains of *S. sobrinus* (Table 1).

Strains MFe28^T and B1314 formed a single DNA homology group by both the S1 nuclease and spectrophotometric methods (Tables 1 and 2). Recently, it has been reported that a 3.3-kilobase fragment of chromosomal DNA of MFe28^T reveals differences between MFe28^T and *S. sobrinus* strains in Southern blotting hybridization experiments (21). Despite the relatively high degree of DNA homology observed between the monkey plaque strains and strains of *S. sobrinus*, compared with representatives of the other species within the mutans streptococci, the results show that the monkey strains and *S. sobrinus* are sufficiently dissimilar to warrant separate species status, for which we propose the name *Streptococcus downei*.

Description of *Streptococcus downei* sp. nov. *Streptococcus downei* (dowṅ.e.i. Lat. derivative of Downe, the village in Kent where the type strain was isolated) cells are gram-positive, catalase-negative cocci that grow in chains. They form small, dark blue, crinkled colonies up to 1 mm in diameter with an erose edge slightly pitting the agar but easily dislodged although difficult to disperse when grown on mitis salivarius agar. Growth on 5% sucrose agar (TYC, Lab M) produces large, white, conical colonies 2 to 3 mm in diameter with an erose edge surrounded by a distinctive white halo. Acid is produced from glucose, sucrose, fructose, galactose, mannose, mannitol, lactose, maltose, salicin, trehalose, and inulin but not from adonitol, melezitose, melibiose, sorbose, cellobiose, glycogen, soluble starch, inositol, xylitol, sorbitol, glycerol, arabinose, or raffinose. Starch, esculin, and hippurate are not hydrolyzed. Ammonia is not produced from arginine. Strains do not grow at 45°C,

TABLE 2. DNA-DNA relatedness between monkey plaque strains and *S. mutans* (NCTC 10449^T), *S. sobrinus* (SL-1^T), and *S. cricetus* (HS-6^T) based on renaturation rates

Strain	Degree of binding (%) to[a]:					
	MFe28^T	B1314	B1315	SL-1^T	HS-6^T	NCTC 10449^T
Monkey plaque						
MFe28^T	100					
B1314	94 ± 7	100				
B1315	108	109 ± 4	100			
S. sobrinus SL-1^T	50 ± 4	40 ± 2	50 ± 3	100		
S. cricetus HS-6^T	31 ± 9	25 ± 2	33 ± 2	35 ± 1	100	
S. mutans NCTC 10449^T	22 ± 1	32 ± 2	21 ± 0	23 ± 7	24 ± 1	100

[a] Values averaged from two determinations and normalized to the homologous reaction.

TABLE 3. Differential phenotypic characteristics of *S. downei* and other mutans streptococci[a]

Species	mol% G+C of DNA	Serotype(s)	Acid produced from:				Bacitracin resistance	H_2O_2 production
			Inulin	Raffinose	Melibiose	Sorbitol		
S. downei	41–42	h	+	−	−	−	−	−
S. sobrinus	43–46	d, g	−	+	−	±	+	+
S. cricetus	42–44	a	+	+	+	+	−	−
S. rattus	41–43	b	+	+	+	+	+	−
S. mutans	36–38	c	+	+	+	+	+	−
S. macacae	35–36	c	−	+	−	+	−	−
S. ferus	43–45	c	+	−	−	+	−	−

[a] Data obtained from references 1 and 2.

at pH 9.6, or in the presence of 6.5% NaCl. Variable growth is obtained on 10 and 40% bile agar. Hydrogen peroxide and intracellular polysaccharides are not produced. Acetyl methyl carbinol is produced from glucose. Strains possess a serologically distinct polysaccharide antigen designated h. Cellular long-chain fatty acid composition consists of major (>10% of total fatty acids present) amounts of hexadecanoic (16:0, palmitic), octadecanoic (18:0, stearic), octadecenoic (18:1, vaccenic), and eicosenoic (20:1) acids together with minor amounts of tetradecanoic (14:0, myristic), hexadecenoic (16:1, palmitoleic), octadecenoic (18:1, oleic), and eicosanoic (20:0, arachidic) and cyclopropane acid (*cis*-9,10-methyleneoctadecanoic acid). It has been isolated from the dental plaque of monkeys (*M. fascicularis*). It is cariogenic when monoassociated with germfree rats. The G+C content of the DNA is 41 to 42 mol%. The type strain is MFe28 (= NCTC 11391).

Biochemical characteristics. Phenotypic characteristics for distinguishing strains of *S. downei* and other species within the mutans streptococci are shown in Table 3.

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