INFECTION AND IMMUNITY, Sept. 1976, p. 667-670

Vol. 14, No. 3
Printed in U.S.A.

Serological and Genetic Examination of Some Nontypical *Streptococcus mutans* Strains

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Received for publication 23 March 1976

Thirty-four strains of *Streptococcus mutans* whose antigenic or genetic positions were unclear or unknown with respect to the serological scheme of Bratthall (1970) and Perch et al. (1974), or the genetic (deoxyribonucleic acid base sequence homology) scheme of Coykendall (1974), were analyzed to clarify their relationship to previously well-characterized strains. Strain OMZ175 of the "new" serotype *f* was genetically homologous with strains of *S. mutans* subsp. *mutans*. Strains of the "new" serotype *g* were homologous with serotype *d* strains (*S. mutans* subsp. *sobrinus*). Strains isolated from wild rats constituted a new genetic group but carried the *c* antigen. Thus, strains within a "genospecies" (subspecies) of *S. mutans* may not always carry a unique or characteristic antigen. We suggest that the existence of multiple serotypes within subspecies represents antigenic variation and adaptations to hosts.

Despite considerable phenotypic similarity (6, 12, 13, 15), the species called *Streptococcus mutans* is actually a group of at least four bacteria (9). Each one is genetically distinct; that is, strains of one do not share appreciable common deoxyribonucleic acid (DNA) base sequences with strains of any other (8, 9). Thus, we consider these four entities "genotypes" or "genospecies" and have proposed that the four genospecies be considered subspecies of *S. mutans* (9). The genotypes correlate with the serotypes described by Bratthall (2) except that strains reacting with Lancefield group E serum (called serotype *e*) are genetically indistinguishable from those of Bratthall serotype *c* (9). Recently, two more serotypes, *f* and *g*, were described (18). Also, a new genotype was found in the mouths of wild rats (10). This report describes genetic and antigenic studies on some of these newer isolates. The results indicate that the *f* and *g* serotypes are genetically homologous with two previously described genotypes and that strains from wild rats carry the *c* antigen. The variability in antigens within a genospecies may represent evolutionary adaptations to the host.

MATERIALS AND METHODS

Bacterial strains. The strains used in these studies are listed in Table 1. Strains isolated from wild rats living in an urban (Hartford, Conn.) landfill dump were obtained and characterized in a manner similar to that used for isolation of *S. mutans* from sugar cane-eating rats (10; R. A. Dvarskas and A. L. Coykendall, Prog. and Abstr., Meet. Am. Assoc. Dent. Res., p. 127, 1975). Strains isolated from Tokelauan Islanders (16) were kindly supplied by E. Guy. They were of interest because of the isolated location of the Tokelauans and because some of the strains fermented neither mannitol nor sorbitol, two sugars nearly always fermented by *S. mutans* strains, although in other characteristics these strains resembled *S. mutans* (16). The FA1 substrains were reisolated and described by Bratthall and Gibbons (4).

Isolation and characterization of DNA. DNA was isolated from cells exposed to penicillin during logarithmic growth in Todd-Hewitt broth (BBL). The harvested, penicillin-treated cells were lysed by treatment with lysozyme [10 mg in 25 ml of 0.15 M tris(hydroxymethyl)aminomethane buffer, pH 8.0] followed by sodium lauryl sulfate treatment (7). DNA was purified by deproteinization with phenol and chloroform, ribonuclease, and precipitation with ethanol. To produce labeled DNA, 500 ml of the same medium was supplemented with 1 mCi of [*methyl*-^{3}H]thymidine. Guanine plus cytosine contents were determined by thermal denaturation in SSC (standard saline citrate: 0.15 M NaCl-0.015 M sodium citrate) (17). DNA base sequence similarities were assessed by hybrid reassociation on membrane filters (8, 11) incubated at 67°C in 3× SSC (triple-strength SSC: 0.45 M NaCl-0.045 M sodium citrate). Usually Schleicher and Schuell filters (B6, 25 mm; Scheicher & Schuell, Keene, N.H.) were used, but in some experiments Millipore HAWP-type membrane filters (Millipore Corp., Bedford, Mass.) were used and compared with the Schleicher and Schuell filters. A 50-μg portion of each strain's DNA was denatured and applied to each of three filters and then incubated with 1.0 or 0.5 μg (5,000

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

Strains	Origin
4S1, 8S1, 11S1, 11S3, 5T1	Isolated in Florida from wild rats that ate sugar cane (10). These strains were not genetically homologous with any previously studied *S. mutans* genotypes. Referred to as *S. mutans* subsp. *ferus*.
HA2, HF4, HG3, HG5, HL1, HL2, HL3, HL4, HM1, HH3, HD3, HD7, HJ3, HJ4, HK2, HO1	Isolated in Hartford from wild rats in a landfill dump. These strains are biochemically and colonially similar to the Florida strains described above.
1S7, 3S1, 10S2, 11S2	Isolated in Florida from wild rats that ate sugar cane. These strains are genetically indistinguishable from *S. mutans* subsp. *cricetus* (10).
$FA1_1$, $FA1_{20}$	Laboratory strains of *S. mutans* subsp. *rattus*. React with Bratthall *b* antiserum. Reisolated from infected gnotobiotic rats (4). Originally isolated by Fitzgerald et al. (14).
$FA1_{13}$, $FA1_{17}$	Similar to $FA1_1$ and $FA1_{20}$, but have "lost" the *b* antigen (4).
14H, A1, O1, 1C	Isolated by Guy from Tokelauan natives (16).
CMZ175	Isolated by Guggenheim (15) from dental plaque. Placed in serotype *f* by Perch et al. (18).
10449, FA1, HS6, SL1	Well-characterized strains of the four *S. mutans* subspecies (9).

to 10,000 cpm) of sheared denatured ^{3}H-labeled DNA from an index strain. Hybrid reassociation (labeled DNA from the index strain, which united with unlabeled DNA from any other strain) compared to homologous reassociation (labeled index DNA united with unlabeled index DNA) gives a measure of DNA base sequence homology. When a hybrid reassociation approaches 80% of the homologous reassociation, we consider the two strains to have similar genetic molecules. When DNA from two strains hybridize more than 80%, we consider the two organisms genetically homologous.

Serological tests. Autoclaved antigen extracts were prepared (19) from strains cultured in Trypticase soy broth (BBL). The extracts were tested by gel diffusion techniques against antisera prepared from heat-killed cells of *S. mutans* strains AHT (serotype *a*), FA1 (*b*), JC2 (*c*), B13 (*d*), and OMZ65 (*g*), using methods described earlier (1, 2). In addition, a commercial Lancefield group E antiserum (Difco) was used.

RESULTS

Strains isolated from wild rats. The *S. mutans* strains isolated from Hartford rats were genetically homologous with each other and also with representative strains isolated in Florida (8S1, 4S1; Table 2). The average guanine plus cytosine contents of these strains were all between 42.7 and 45.0 mol%. Serological examination of representative strains (5T1, 8S1, 11S1, and 11S3 from Florida rats; HD3, HL4, and HM1 from Hartford rats) showed reactions of identity with the *c* antigen of strain JC2. These extracts also reacted with the anti-AHT serum, but no relation with the *a* antigen was observed in the gel diffusion tests. It has been shown previously that these rat strains do not share many DNA base sequences with other *S. mutans* genotypes (10). Their guanine plus cytosine contents alone rule out close relationship to human serotype *c* strains (36 to 38% versus 43 to 45%). Thus, these "wild-rat strains" represent a fifth genospecies within the *S. mutans* group of streptococci. We assign the subspecific epithet *ferus* (wild) to this organism.

***S. mutans* subsp. *cricetus* strains from wild rats.** Some of the Florida rats harbored organisms genetically related to strains considered *S. mutans* subsp. *cricetus* (10). *S. mutans* subsp. *cricetus* strains generally react with the Bratthall *a* antiserum (2). However, the four *S. mutans* subsp. *cricetus* strains from Florida that were examined (1S7, 3S1, 10S2, 11S2) lacked the *a* antigen but gave reactions of partial identity with the *b* antigen of the reference strain FA1. In immunoelectrophoresis, this "*b*" antigen was different compared to the *b* antigen in that only one arc was formed.

Strains isolated from Tokelauans. Three of the strains isolated from Tokelauan natives (O1, 1C, and 14H) were genetically homologous with each other and with *S. mutans* subsp. *sobrinus* strain SL1 (Table 3). Strains 1C and 14H had an antigen that gave a reaction of identity with the *g* antigen of strain OMZ65 (18). As with other *g* strains (2, 5), there was a reaction of partial identity with serotype *d* antiserum. (Strain O1 was not done.) Like SL1 and other *S. mutans* subsp. *sobrinus* strains, they did not ferment raffinose. The remaining strain (A1) was homologous with *S. mutans* subsp. *mutans* strain 10449 and reacted with antiserum *c*. Strains 14H and A1 failed to ferment mannitol or sorbitol.

Genetic homology of antigenic variants of *S. mutans* strain FA1. To confirm that anti-

TABLE 2. *DNA base sequence homologies among the S. mutans strains investigated*

Expt	*S. mutans* subsp. epithet	% Homology		Serotype (if known)
1. Wild rat strains, with a Hartford isolate as the index strain	*ferus* HG5	100		
	ferus HA2	71		
	ferus HD3	108		*c*
	ferus HD7	103		
	ferus HF4	96		
	ferus HG3	94		
	ferus HH3	86		
	ferus HJ3	105		
	ferus HJ4	94		
	ferus HK2	96		
	ferus HL1	94		
	ferus HL2	123		
	ferus HL3	116		
	ferus HL4	107		*c*
	ferus HO1	104		
	ferus 8S1	96		*c*
	cricetus 1S7	39		*b'*
	cricetus HS6	34		*a*
	Escherichia coli	5		
2. Wild rat strains, with a Florida isolate as the index strain	*ferus* 8S1	100		*c*
	ferus 4S1	88		
	ferus HA2	91		
	ferus HL4	85		*c*
	ferus HO1	91		
	E. coli	4		
3. Tokelauan strains compared with other *S. mutans* subspecies	*sobrinus* 14H	100		*g*
	sobrinus O1	91		
	sobrinus 1C	97		*g*
	mutans A1	4		
	sobrinus SL1	96		
	cricetus HS6	23		*a*
	rattus FA1	7		*b*
	E. coli	2		
4. Strains A1 and OMZ175 compared with *S. mutans* 10449	*mutans* 10449	100[a]	100[b]	*c*
	mutans A1	96	88	*c*
	mutans OMZ175	90	89	*f*
	E. coli	3	4	
5. Strains lacking the *b* antigen compared with *b*-positive parent	*rattus* $FA1_1$	100[a]	100[b]	*b*
	rattus $FA1_{13}$	101	97	No *b* antigen
	rattus $FA1_{17}$	91	90	No *b* antigen
	rattus $FA1_{20}$	98	101	*b*

[a] Using Schleicher & Schuell filters.
[b] Using Millipore filters.

gens could be changed or lost without appreciable loss in overall genetic homology, four substrains of *S. mutans* subsp. *rattus* strain FA1 were compared. Three substrains were reisolated from gnotobiotic rats that had been infected with strain FA1 (serotype *b*). Reisolates $FA1_{13}$ and $FA1_{17}$ had lost the *b* antigen (i.e., it was undetectable); $FA1_{20}$ had retained the *b* antigen (4). Hybridization experiments showed that these antigenic variants were still genetically indistinguishable from the parent strain (Table 2).

***S. mutans* strain OMZ175.** Strain OMZ175 is a representative of the proposed new serotype *f* (18). Using gel diffusion techniques, an antiserum to OMZ175 does not reveal any antigen corresponding to the type antigens of other *S. mutans* strains (5). However, two other antigens, common to several other streptococcal species, can be shown. Using immunofluorescence, an unabsorbed antiserum against type *c* strains reacts with OMZ175 (3). DNA from strain OMZ175 had 38.6% guanine plus cytosine and was homologous with *S. mutans* subsp. *mutans* strain 10449 (serotype *c*).

DISCUSSION

Most streptococcal strains considered to be *S. mutans* join one of Bratthall's five serological groups. Of 70 strains in the original study, only

TABLE 3. *Summary of serological types found within subspecies of S. mutans*

S. mutans subsp.	Serological type	Representative strains
mutans	*c*	JC2, 10449
	e	LM7, B2
	f	OMZ175[a]
rattus	*b*	FA1, BHT
cricetus	*a*	HS6, OMZ61
	b'	1S7, 10S2
sobrinus	*d*	B13, OMZ176
	g	K1,[a] 14H
	SL1	SL1[a]
ferus	*c*	8S1, 11S1, HG5

[a] As determined by Perch et al. (18); all others from Bratthall (2).

one (OMZ175) could not be typed, and one other (OMZ65) had an aberrant serotype *d* reaction (2). Perch et al. (18) tested 210 strains and found 29 that did not join any of the Bratthall serotypes. Ten of the 29, including OMZ175, were assigned to a new serotype, *f*. These strains were biochemically similar to serotype *c* strains. Our results with strain OMZ175 indicate that the *f* strains of Perch et al. are antigenic variants within the genospecies which includes serotypes *c* and *e*, i.e., *S. mutans* subsp. *mutans* (9). Of the remaining 19, 10, including K1 and OMZ65, were assigned to a seventh serotype, *g*, and were biochemically similar to serotype *d*. Strain SL1 was considered a separate serotype, "SL1." We believe that the *g* strains (and SL1) are serological variants of *S. mutans* subsp. *sobrinus*, because strains K1R, SL1, and OMZ176 are genetically and biochemically homologous (9). Three Tokelauan strains also join this group, since their DNAs were homologous with SL1 DNA and since they gave a *g* serological reaction plus the *d* cross-reaction. Thus, the strains within a genospecies can present slightly different serological and biochemical reactions, but the overall genetic homology of strains within a genospecies is clearly evident. Table 3 summarizes the serological types found within the genospecies (subspecies) of *S. mutans*.

The antigen of *S. mutans* may be changing constantly and rapidly. If a change is beneficial, then the altered cells would have a selective advantage. In a matter of weeks, antigenic patterns were seen to change in *S. mutans* strain FA1 (*b*) reisolated from gnotobiotic rats (4). However, these FA1 cells, which no longer showed the characteristic *b* antigen precipitin arc, did not become dominant in the rats, indicating that the *b* antigen may be of some benefit to this organism or that this antigen and the host have reached an accommodation. Strains of *S. mutans* subsp. *cricetus* usually have the *a* antigen, but those isolated from Florida rats had one related to the *b* antigen and lacked the *a* antigen. This particular antigen may be the most accomodated in this particular host. Thus, antigenic changes may represent an adaptive mechanism, operating through mutation and selection, which, as pointed out by Bratthall and Gibbons (4), has implications for efforts at immunoprophylaxis of caries.

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