INFECTION AND IMMUNITY, Jan. 1980, p. 25-31
0019-9567/80/01-0025/07$02.00/0

Vol. 27, No. 1

Virulence of *Streptococcus mutans*: Revertants of Mutant C4

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Mutant C4, a poor plaque-forming mutant of *Streptococcus mutans* 6715-HSR, was employed to obtain isolates resembling the parent strain (a plaque former). Seventeen presumptive revertants, as identified by colonial morphology, were isolated from mutant C4 after enrichment cycles in a sucrose-glass beads medium. These isolates displayed properties which resembled the parent in ability to produce plaque, patterns of fermentation, and resistance to streptomycin. In a detailed study, five selected isolates were found to be similar to the parent-type 6715-HSR with respect to content of the serotype antigen, sucrose- or dextran-induced cell aggregation, glucosyltransferase and adherence activities, and cariogenicity. Thus, in selection for revertants to parental colonial morphology, the pleiotropic changes in plaque formation, adherence, glycosyltransferase activity, and virulence demonstrated by C4 all concomitantly reverted to their parental phenotypes.

It is believed that the role of *Streptococcus mutans* as the principal etiological agent of dental caries in rodents (6, 7, 9, 17, 31), monkeys (1), and humans (12, 20, 21) is due to its ability to produce glucan from sucrose. This enables these bacteria to adhere to the smooth surfaces of the tooth, with subsequent colonization and plaque formation (11, 30). Gibbons and Nygaard (11) have suggested that the adherence of *S. mutans* to smooth surfaces is dependent on its unique water-insoluble glucan. The glucosyltransferase (GT) of this organism is capable of synthesizing both water-insoluble and water-soluble glucans.

Mutants of *S. mutans* apparently altered in their ability to synthesize water-insoluble or water-soluble glucan have been reported (4, 8, 18, 23, 24, 27). In this laboratory, *S. mutans* 6715-HSR was mutagenized with nitrosoguanidine to obtain a mutant, C4. The mutant strain, C4, was identical to the parent 6715-HSR strain in fermentation pattern, including the ability to ferment sorbitol and mannitol. However, unlike strain 6715-HSR, C4 showed low GT activity for synthesis of insoluble glucan, a decrease in adherence and plaque formation, and diminished cariogenicity (23). Furthermore, although 6715-HSR formed rough, hard colonies on mitis salivarius (MS; Difco) agar medium, C4 grew as smooth, soft colonies (23).

Because C4 demonstrated these pleiotropic changes of phenotype differing from that of 6715, we wondered whether isolates selected for reversion of a single phenotypic trait, colonial morphology, would also show reversion to wild-type levels of adherence, water-insoluble glucan synthesis, plaque formation, and cariogenicity. We considered that the isolation and characterization of such revertants would help elucidate the mechanism of *S. mutans* virulence.

MATERIALS AND METHODS

Bacterial strains and growth media. *S. mutans* 6715 was kindly provided by Robert Fitzgerald; it was made more resistant to streptomycin (10 mg/ml) and designated 6715-HSR, for high streptomycin resistance. The parent of C4 was 6715-HSR, though that strain designation was not used in reference 23. The *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine)-treated mutant C4 which is defective in its ability to synthesize water-insoluble glucan has been previously described (23). The colonial morphology of strain 6715-HSR and the revertants of C4, whose isolation is described in this paper, are pulvinate, rough, and hard on MS agar plates according to a cultural method described by Jordan, et al. (15). Mutant C4, on the other hand, grows as convex, smooth, and soft colonies. The five revertant strains employed are C4R1, C4R2, C4R3, C4R4, and C4R5.

Strains were maintained in a brain heart infusion agar (BHI, Difco) supplemented with $CaCO_3$. The stock cultures were transferred monthly. Partially defined medium (13) with 0.5% glucose (PD-glucose), 0.5% sucrose (PD-sucrose), and MS agar were employed in this study. Cultures were always grown at 37°C, under an atmosphere of 95% N_2–5% CO_2.

Design of revertant selection protocol. Two

preliminary experiments were designed to test the feasibility of isolation of C4 revertants through enrichment for adherence to glass surfaces. The first measured the relative efficiency with which bacterial strains 6715-HSR or C4 colonize and grow on glass surfaces; the second ascertained whether 6715-HSR could efficiently compete with mutant C4 for colonization and growth on glass surfaces.

(i) Efficiency of colonization and growth on glass surfaces by 6715-HSR and mutant C4. Bacterial strain 6715-HSR and its mutant, C4, were individually incubated in BHI for 20 h. The culture broths were diluted with BHI to concentrations of 6.6×10^3 colony-forming units (CFU)/ml for 6715-HSR and 5.9×10^7 CFU/ml for C4. One-tenth milliliter of the diluted broth culture was then used to inoculate each of two 125-ml Erlenmeyer flasks containing 9.9 ml of BHI supplemented with 5% sucrose, 0.2% yeast extract (Difco), and 17 g of glass beads (3-mm diameter).

After 24 h of incubation, the medium was removed aseptically by aspiration, and the glass beads were gently washed 10 times with 9 ml of BHI. The flask was gently rotated two times after each wash. The culture medium and the washed materials were pooled, and the CFU were determined. These were considered "nonadherent" CFU. The washed glass beads were then shaken with 9 ml of BHI with a Vortex Genie (Scientific Inc.) at maximum speed for 1 min. After waiting for foam to subside, we transferred the cell suspensions to a test tube and then sonicated (Sonifier model W140) them in an ice bath for 15 s at a setting of 10. This sample was used to enumerate "adherent" CFU. Enumeration of CFU was by plate dilution on MS agar, with an incubation for 24 h at 37°C followed by 24 h at room temperature.

(ii) Efficiency of growth and colonization on glass surfaces in mixed culture. The method used for this experiment was like the relative efficiency experiment described above, except that both 6715-HSR and C4 were simultaneously inoculated into a single flask, containing 9.9 ml of BHI supplemented with yeast extract, sucrose, and glass beads. Initial cell concentrations, per flask (10 ml), were 6.6×10^2 CFU for 6715-HSR and 5.9×10^6 CFU for C4. After incubation for 24 h, the culture medium was removed and the beads were washed, shaken in a Vortex Genie, and sonicated, as described above. The sonicated cell suspension was used to enumerate CFU concentrations and also as an inoculum for another enrichment cycle. This enrichment cycle of the adherent fraction was performed serially, five times. CFU were enumerated as described above.

Isolation of revertants. Mutant C4 was grown in BHI for 20 h and then diluted to 5.9×10^6 CFU per ml. One-tenth milliliter of culture was used to inoculate a 125-ml Erlenmeyer flask containing 9.9 ml of BHI supplemented with sucrose, yeast extract, and glass beads, as described above. Five cycles for enrichment of the adherent fraction were carried out as described for the mixed-culture experiment. After each cycle, the adherent cell suspension obtained after the sonication step was enumerated by dilution on MS agar plates, and the number of colonies showing morphologies like 6715-HSR or mutant C4 were distinguished.

Fermentation and antibiotic resistance. Fermentation tests were performed as described by Shklair and Keene (29), and the streptomycin sensitivity test (10 mg/ml) was carried out in MS agar plates.

Plaque formation. Plaque formation on glass surfaces was measured in preweighed culture tubes (10 by 100 mm). Bacterial strains were grown in PD-sucrose medium. After 24 h of growth, the medium and nonadhering cells were removed and discarded, and fresh medium was added. The medium was changed daily. On day 5 each tube was washed three times with water, dried, and weighed.

Assay of GT activity. The GT preparation was obtained and assayed by a method described previously (13), but the procedures used to collect glucan were modified as follows. Water-insoluble glucan was collected on a 2.4-cm glass microfiber filter (Whatman, GF/C); the filter was washed nine times with 0.5 ml of water, dried, and counted for radioactivity. To the filtrate collected above, an equal volume of ethanol was added, and the resulting precipitate (water-soluble glucan) was collected on another glass microfiber filter. This filter was washed nine times with 0.5 ml of 50% ethanol, dried, and counted for radioactivity. One unit of GT enzyme is that amount of enzyme which catalyzes the incorporation of 1 μmol of glucose moiety of sucrose into water-insoluble or water-soluble glucan, under conditions previously described (13). Specific activity is the number of micromoles of glucose incorporated into glucan per milligram of protein per hour.

Adhesive glucan. Adhesive glucan produced by GT was measured by a modified method of Kuramitsu (19). The 0.5-ml reaction mixture described previously (13) was prepared in a 1-dram screw-cap vial (Fisher Scientific Co.). The vial was incubated at 37°C at an angle of 25° from the horizontal. After incubation, the vial was washed three times with 4 ml of water by aspiration, dried, and counted for radioactivity.

Adherence and aggregation assays. The assay for adherence of radioactive heat-killed cells was a modification (13) of a method described by Schachtele et al. (28). Dextran T 2000 (Pharmacia)- or sucrose-induced aggregation of cells was assayed by the procedure described by Gibbons and Fitzgerald (10).

Test for virulence. The virulence of bacterial strains was tested in a gnotobiotic system by monoinfecting 20-day-old rats with appropriate strains of *S. mutans* (24). Rats were sacrificed at 45 days of age. After sacrifice, individual mandibles were stained with murexide, and proximal molar surfaces were scored for caries by using the Keyes procedure (16). The scores from each group of rats were statistically analyzed by computing means, standard deviations, and standard errors. Differences among means were evaluated by an analysis of variance and by mean comparisons using the Duncan test (5).

Double immunodiffusion analysis. Rantz-Randall preparations (26) of *S. mutans* strains and by a purified preparation of serotype *g* antigen were tested against antiserum prepared by using whole cells of 6715-HSR. The purified serotype *g* antigen was a generous gift of R. Linzer.

Preparation of hyperimmune antisera. Cells of strain 6715-HSR were obtained after growth in PD-glucose medium. The cells were washed three times

with 0.1 M potassium phosphate-buffered saline solution (pH 7.0) and suspended in 0.5% Formalin-saline solution, and the cell suspension was washed with the potassium phosphate-buffered saline three times to remove Formalin. The cells were resuspended in the same buffer to give an absorbance of 0.45 at 660 nm (ca. 5×10^8 CFU/ml). Hyperimmune 6715-HSR serum was prepared in New Zealand white rabbits by intravenous administration of whole cells (2.5×10^8 CFU) daily for 3 consecutive days. This schedule was repeated for 2 additional weeks with two and three times the original dosage, respectively (week 2, 5×10^8 CFU; week 3, 7.5×10^8 CFU). The animals were bled 7 days after the last injection. This procedure yielded antiserum with bacterial agglutination titers of 5,120.

Preparation of radioactive cells. Bacterial strains were inoculated in 50 ml of PD-glucose medium supplemented with 100 mCi of [4,5-^{3}H(N)]-L-leucine containing 1.1 mg of L-leucine. After 20 h of growth, cells were harvested and washed three times with water by centrifugation and suspended in water to an absorbance of 1.0 (660 nm). The cell suspension was placed in a boiling-water bath for 20 min. After cooling, the cells were washed three times with 0.05 M potassium phosphate buffer (pH 6.8) by centrifugation. The cells were resuspended in potassium phosphate buffer containing 0.2% sodium merthiolate to give an absorbance of 1.0 at 660 nm. The radioactivity of such cell suspensions ranged from 0.98×10^5 dpm/ml to 1.64×10^5 dpm/ml.

Miscellaneous. Radioactivity was assayed by using Bray scintillation fluid (2) in a Packard Tricarb model 3375. Protein was determined by the method of Lowry et al. (22) by using bovine serum albumin as the standard.

RESULTS

Isolation of revertants. We wished to enrich for parent-type revertants of C4 by their preferential ability to adhere to glass surfaces in sucrose cultures of C4. The distinctive colonial forms on MS agar would enable one to select and isolate the revertants. Accordingly, two preliminary experiments were performed to gauge the efficacy of this approach, and the results are presented in Tables 1 and 2.

Table 1 shows the ability of a low number (CFU) of 6715-HSR in flask 1, or a high number (CFU) of mutant C4 in flask 2, to adhere and to grow as plaque on glass-bead surfaces in a sucrose-containing medium. At zero time, the inoculum size of strain 6715-HSR was 6.6×10^2 CFU/flask. After 24 h of incubation and removal of medium and washings, 7.08×10^8 CFU were found in the medium and 5.8×10^7 CFU were associated with the glass beads, indicating that ca. 7.7% of the total number of CFU was attached to glass beads. On the other hand, ca. 0.3% of the cells of mutant C4 was associated with glass beads. These results show that strain 6715-HSR adhered more efficiently on glass surfaces than did mutant C4 in a sucrose medium.

TABLE 1. *Efficiency of parent strain 6715-HSR and mutant C4 to colonize and grow on glass surfaces*

Sample	CFU/flask		
	0 h	24 h	
		Nonadherent[a]	Adherent[b]
6715-HSR (flask 1)	6.6×10^2	7.1×10^8	5.8×10^7
C4 (flask 2)	5.9×10^6	1.3×10^8	3.5×10^5

[a] Cells in culture medium and washings (see text).
[b] Cells attached to glass beads that were not washed off, but were removed by shaking and sonication (see text).

TABLE 2. *Efficiency of colonization and growth on glass surfaces in mixed culture*

Cycle of enrichment	CFU associated with beads		Ratio (6715-HSR/C4)
	6715-HSR	C4	
0	6.6×10^2	5.9×10^6	1/8,030
1	3.0×10^4	1.9×10^8	1/6,333
2	2.2×10^4	1.5×10^7	1/682
3	7.8×10^6	1.2×10^9	1/154
4	2.0×10^7	2.4×10^9	1/120
5	8.1×10^7	1.8×10^9	1/22

Table 2 shows the efficiency of growth and colonization on glass surfaces when strain 6715-HSR and mutant C4 were placed together in a sucrose-containing medium with glass beads. When strain 6715-HSR and mutant C4 were grown together with an initial ratio of 1:8,030, progressive enrichment for strain 6715-HSR occurs through the 5th cycle. The high numbers of mutant C4 recovered from glass beads (Table 2) may be due to their adherence to glass surfaces via the insoluble glucan produced by strain 6715-HSR. The growth rates of mutant C4 and strain 6715-HSR, as determined by the rate of acid they produced during exponential phase of growth in PD-sucrose medium, were identical: 0.029 meq of acid produced per 10 ml of culture per h. The higher CFU found with strain 6715-HSR (Table 1) may be due to its greater survival.

The results of these preliminary experiments suggested that under similar conditions it would be possible to enrich for spontaneous parent-type revertants in sucrose cultures of mutant C4. Accordingly, the experiment presented in Table 3 was performed. Presumed revertants, that is, colonies resembling 6715-HSR, were detected on MS agar plates prepared after four and five enrichment cycles. Seventeen colonies with parental colonial morphology were picked at random, purified by single colony isolation, and characterized further.

Fermentation reaction, antibiotic resistance and plaque formation. The 17 presumptive phenotypic revertants, mutant C4 and strain 6715-HSR were subjected to various biochemical analyses, including a test for resistance to streptomycin and a test for their ability to produce plaque. The results shown in Table 4 indicate that all 17 presumptive revertants were resistant to streptomycin and behaved like strain 6715-HSR with respect to the tests performed. The fermentation patterns obtained with all the strains indicated that they belonged to the serotype *g* class, according to the classification of Shklair and Keene (29). All 17 phenotypic revertants produced plaque in amounts similar to that found with strain 6715-HSR.

Five of the 17 revertants were selected randomly and designated C4R1, C4R2, C4R3, C4R4, and C4R5. These strains were further tested with comparison to mutant C4 and strain 6715-HSR. A GT preparation from each bacterium was examined for its ability to synthesize water-soluble, water-insoluble, and adhesive glucans. Each strain was also tested for its ability to adhere to glass surfaces and to aggregate. Cell extracts and a serotype *g* antigen preparation were subjected to immunodiffusion to determine their serotype specificity. Finally, these cultures were tested for virulence in gnotobiotic rats.

GT activity. GT preparations obtained from a 70% ammonium sulfate treatment of supernatant fluids (D70ASP) from cultures of mutant C4, revertants C4R1, C4R2, C4R3, C4R4, and C4R5, and parent strain 6715-HSR were assayed for their water-soluble, water-insoluble, and adhesive glucan synthesis activities. The results presented in Table 5 indicate that D70ASP from C4 showed the lowest activity for adhesive and water-insoluble glucan synthesis and the highest activity for water-soluble glucan synthesis. All the revertants showed a high activity for water-insoluble and for adhesive glucan synthesis, with activities ranging from 15.3 to 26.8 μmol/mg per h and 40.7 to 52.8 μmol/mg per 5 h, respectively. These activities were comparable to those found for the 6715-HSR strain. The water-soluble glucan synthesis activity was low for both the revertants and for strain 6715-HSR.

Cellular aggregation. The results presented in Table 6 show that both dextran and sucrose induced cells of mutant C4, the five revertants, and strain 6715-HSR to aggregate to the same extent by 2 h.

Adherence. When the various heat-treated radioactive cells were tested for adherence, strain 6715-HSR and revertants C4R1, C4R2, C4R3, C4R4, and C4R5 were all found to adhere well to glass surfaces (Table 6). The relative number of added cells which adhered to glass surfaces ranged from 50 to 62%. On the other hand, only 6% of added mutant C4 cells adhered to glass surfaces.

Immunodiffusion analysis. An experiment was performed to determine the serotype of these organisms, and the results are presented in Fig. 1. Rantz-Randall (26) extract preparations of revertants C4R1, C4R2, C4R3, C4R4, and C4R5, mutant C4, parent strain 6715-HSR, and a purified preparation of serotype *g* antigen (wells 1 to 7, respectively) were tested against an antiserum to 6715-HSR (well 8). The gel diffusion test showed that all the cell extract preparations and the purified serotype *g* antigen produced a reaction of identity with the antiserum to whole cells of 6715-HSR. These results suggest that C4R1, C4R2, C4R3, C4R4, C4R5,

TABLE 3. *Isolation of phenotypic revertants of mutant C4 by adherence enrichment*

Cycle of enrichment	CFU associated with beads[a]	
	Convex, soft (C4)	Pulvinate, hard (6715-HSR)
0	5.9×10^5	
1	1.2×10^8	$<1.0 \times 10^4$
2	1.9×10^7	$<1.0 \times 10^4$
3	1.1×10^8	$<1.0 \times 10^4$
4	2.7×10^8	4.0×10^4
5	7.8×10^7	1.5×10^5

[a] Colonies in MS agar (see text). The terms used to describe the colonial morphology are those recommended in the *Manual of Microbial Methods* (25).

TABLE 4. *Fermentation reactions,[a] antibiotic resistance,[a] and plaque production[b]*

Strain	Mannitol	Sorbitol	Raffinose	Melibiose	NH_3 from arginine	Mannitol plus bacitracin	Streptomycin (10 mg/ml)	Plaque (mg)
Mutant C4	+	+	−	−	−	+	+	0.7 ± 0.5
17 revertants[c]	+	+	−	−	−	+	+	16.8 ± 6.2
6715-HSR	+	+	−	−	−	+	+	18.7 ± 2.7

[a] +, Growth and acid production; −, no growth.
[b] ± Standard error from three experiments, each performed in triplicate.
[c] Same results were obtained with the biochemical tests.

C4, and 6715-HSR all have the identical serospecific *g* antigen.

Virulence of presumptive revertants. Table 7 shows the caries scores obtained in gnotobiotic rats infected with revertants C4R1 and C4R2, mutant C4, and parent strain 6715-HSR. The scores of various surfaces of teeth of rats infected with C4 were significantly lower than those obtained with 6715-HSR and the two revertants tested. Furthermore, the scores obtained with 6715-HSR and the two revertants indicate that the level of virulence activities of these three strains were similar.

DISCUSSION

The early observation that *S. mutans* produced glucan from sucrose suggested that this polysaccharide, which is found in plaque, is important in the formation of plaque and caries (3, 12, 18, 30). It was also shown that the cariogenic streptococci were unique in their ability to produce plaque in a sucrose medium, whereas the noncariogenic streptococci were unable to make plaque (14). Although *S. mutans* produces both water-soluble and water-insoluble types of glucan, it was believed that the latter was responsible in plaque formation (3, 11). In a previous report (23), C4, a mutant of parent strain 6715-HSR, was described as being defective in its ability to synthesize water-insoluble glucan but enhanced in its ability to synthesize water-soluble glucan. Moreover, unlike its parent, C4 colonizes poorly on smooth surfaces and produces little plaque. Furthermore, C4 was found to be less cariogenic than its parent, and this decrease

TABLE 5. *GT activity*

Source of D70ASP	Synthesis of indicated glucan:[a]		
	Water soluble	Water insoluble (μmol/mg per h)	Adhesive (μmol/mg per 5 h)
C4	30.2 ± 3.1	0.4 ± 0.3	0.0 ± 0.0
Five revertants[b]	1.5 ± 0.4	15.8 ± 3.0	43.7 ± 1.1
6715-HSR	1.2 ± 0.6	15.7 ± 3.5	41.6 ± 2.9

[a] Mean ± standard error of three separate experiments performed in triplicate.
[b] C4R1, C4R2, C4R3, C4R4, and C4R5.

TABLE 6. *Adherence and aggregation*

Strain	Adherence activity[a]	Aggregation[b]	
		Dextran	Sucrose
Mutant C4	6.0 ± 1.2	4+	3+
Five revertants[c]	55.8 ± 2.2	4+	3+
6715-HSR	57.0 ± 3.1	4+	3+

[a] Mean ± standard error of three separate experiments performed in triplicate (see text).
[b] Results were scored as 0 (no aggregation) to 4+ (maximum aggregation).
[c] C4R1, C4R2, C4R3, C4R4, and C4R5.

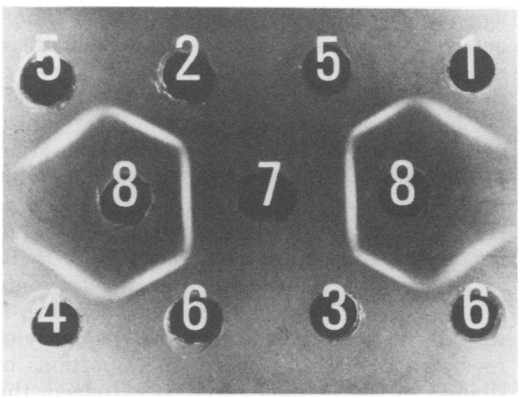

FIG. 1. *Double immunodiffusion analysis. Rantz-Randall extracts of revertants C4R1, C4R2, C4R3, C4R4, and C4R5, mutant C4, parent strain 6715-HSR, and a purified preparation of serotype g antigen added to wells 1 to 7, respectively, were tested against an antiserum to whole cells of 6715-HSR (wells 8). The amount of extracts and the serotype g antigen preparation added per well was about 6 μg, as carbohydrate equivalent.*

TABLE 7. *Virulence of bacterial strains in gnotobiotic rats*

Strain	Rats per group	Mean caries score[a]					
		Buccal		Sulcal		Proximal	
		E	Dx	Dm	Dx	Ds	Dx
C4	8	11.0 ± 0.5	6.2 ± 0.6	10.6 ± 0.6	6.2 ± 0.9	1.8 ± 0.6	0.9 ± 0.5
C4R1	10	18.4 ± 0.8	12.5 ± 0.5	16.4 ± 0.3	15.1 ± 0.3	8.0 ± 0.0	8.0 ± 0.0
C4R2	8	17.6 ± 0.8	11.0 ± 0.7	15.4 ± 0.6	12.9 ± 0.7	7.9 ± 0.0	7.3 ± 0.3
6715-HSR	10	18.1 ± 0.7	12.3 ± 0.3	15.2 ± 0.2	14.0 ± 0.3	7.9 ± 0.1	7.9 ± 0.1

[a] Evaluated by the Keyes procedure (16). Values represent the caries score ± standard error. E, Slight penetration into enamel; Ds, slight penetration into dentin; Dm, moderate penetration into dentin; Dx, extensive penetration into dentin. Values of buccal and sulcal surfaces of the group infected with 6715-HSR are not significantly different ($P < 0.05$) from those obtained from rats infected with either C4R1 or C4R2.

in cariogenic activity was believed to be the direct consequence of inability to produce water-insoluble glucan and plaque.

In an early report, de Stoppelaar and co-workers stated that a non-plaque-producing mutant C67-25 could be mutated back to a parent type (4). In our hands a method involving the cycling of cultures of nonadhering mutant C4 in a sucrose medium containing glass beads resulted in obtaining a number of colonies whose morphology and certain biochemical properties resembled the parent. Bacteriological studies of C4-infected gnotobiotic rats to test for reversions also indicated the occurrence of parent-type revertants. These results will be presented elsewhere.

A comparative study using parent strain 6715-HSR, mutant C4, and revertants from C4 indicates that these revertants possessed features, colonial morphology, GT activity, adherence, agglutination, and cariogenicity, that are characteristic of 6715-HSR. It is clear from these results that the concomitant loss of the properties of cariogenicity, ability to synthesize water-insoluble glucan, plaque formation, and adherence activities which accompanied the mutation of 6715-HSR to mutant C4 was restored for parent-type revertants of mutant C4. This suggests that the pleiotropic change in the C4 phenotype, in comparison with that of 6715-HSR, was the result of a single mutation affecting only the *GTF* gene. Because of the daily cycling, it was not possible to determine the frequency of reversion due to the unknown proportions of siblings of the original revertants. Indeed, the revertants studied may all be siblings.

Our results are tempered to the fact that nitrosoguanidine was employed to obtain mutant C4 (23). A definitive answer to the question of whether mutant C4 is the result of single or multiple genetic lesions must await the establishment of a genetic exchange system in *S. mutans*.

ACKNOWLEDGMENTS

We acknowledge the many helpful suggestions made by Jeff Hansen and Tom Brown in the preparation of this manuscript.

This investigation was supported in part by Public Health Service grants DE 62491, DE 04217, and DE 02670 from the National Institute of Dental Research.

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