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Virulence of *Streptococcus mutans*: Characterization of a Serotype *g* Antigen-Defective Mutant and Its Revertants

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A mutant of *Streptococcus mutans* 6715 HSR, which is defective in serotype antigen and designated C307, was shown to exhibit full virulence on buccal, sulcal, and proximal surfaces similar to that of 6715 HSR. In addition, this bacterium caused significant decay on the lingual surfaces. Its colonial morphology and certain biochemical activities which may be related to caries production were distinct from those of 6715 HSR. This mutant adsorbed to saliva-treated hydroxyapatite beads in greater amounts and aggregated in the presence of either sucrose or dextran in excess of that seen with the parent strain. The abilities of C307 to grow and to produce acid from sucrose and to adhere to glass surfaces were similar to that of 6715 HSR. Although revertants of C307 exhibited biological activities and a content of serotype *g* antigen similar to that of 6715 HSR, the virulence pattern was still unlike the parent strain. These results suggest that the serotype *g* antigen is not required for the adherence of cells to smooth surfaces or for caries formation and that the loss of this antigen may alter the surface of cells causing enhanced ability of the cells to aggregate and to adsorb to saliva-treated hydroxyapatite beads.

Bacteria belonging to the *Streptococcus mutans* group have been implicated as the principal etiological agents of dental caries in humans (17, 18) and in experimental animals (33). It is believed that the factors important in the virulence of *S. mutans* are related to its ability to metabolize sucrose for the production of acid (31) synthesis of glucan (6) and the possession of certain cell surface components as specific recognition sites for the process of plaque formation (5, 8, 19-22). In a previous study (10), experiments were performed which documented that mutant C307, a derivative of *S. mutans* strain 6715 HSR, contained less than 1% of the amount of serotype *g* antigen found in its parent, *S. mutans* 6715. In this paper, the biochemical and virulence features of *S. mutans* 6715 and mutant C307 and its revertants are presented. The results obtained indicate that loss of the *g* antigen causes a profound change in the ability of C307 to bind glucan, to aggregate, to adsorb to saliva-treated hydroxyapatite (HA) beads, and to produce dental caries in gnotobiotic rats.

MATERIALS AND METHODS

Bacteria and culture media. *S. mutans* strain 6715, kindly provided by Robert Fitzgerald, was resistant to a low level of streptomycin. This strain was made resistant to a high level of streptomycin (10 mg/ml) and is henceforth designated 6715 high streptomycin resistance (HSR). Strain C4 is a mutant of 6715 HSR which is defective in the synthesis of water-insoluble glucan (22); strain C307 (10) is a mutant defective in serotype *g* antigen. Soon after its initial isolation, mutant C307 was found to adhere less well than its parent strain, *S. mutans* 6715 (25). However, this property of C307 has changed since then, and it is now similar to that of 6715 HSR. From C307-infected gnotobiotic rats, several colonies resembling those of the parental type were isolated. For this study, three presumptive revertants, C307R1, C307R2 and C307R3, were employed. All bacteria were maintained in brain heart infusion agar (BHI, Difco Laboratories, Detroit, Mich.) supplemented with solid $CaCO_3$, and stock cultures were transferred monthly. A partially defined medium (12) containing 0.5% glucose (PD-glucose), 0.5% sucrose (PD-sucrose) and mitis salivarius agar (MS, Difco) were employed in this study. Cultures were grown at 37°C in an atmosphere of 95% N_2-5% CO_2.

Determination of cell-associated and extracellular GTF activities of cultures of *S. mutans*. Strain 6715 HSR, C307, C307R1, C307R2, and C307R3 were grown in 500 ml of PD-glucose medium. After 20 h of growth, the cells were removed and washed three times with water by centrifugation. The cells were suspended in 0.05 M potassium phosphate buffer, pH 6.8, to an absorbance of 1.0 at 540 nm. This suspension was centrifuged and suspended in 10% of the original volume to give a suspension equivalent to an absorbance value of 10 (approximately 3×10^{10} colony-forming units per ml). The supernatant fluid, obtained after the removal of cells by centrifugation, was treated

with ammonium sulfate (70% saturation), and the precipitate obtained was dissolved and dialyzed against 0.05 M potassium phosphate buffer, pH 6.8. This preparation, designated D70ASP, was used as the glucosyltransferase (GTF) preparation.

Cell-associated GTF activity or extracellular GTF activity was determined by incubating either cells or D70ASP with 0.05 M sodium acetate buffer (pH 5.5), 0.1 μCi [glucose-^{14}C(*U*)]sucrose, and 0.01 M sucrose in a total volume of 0.5 ml for 1 h at 37°C. The water-soluble and -insoluble activities of D70ASP were obtained with a glass microfiber filter (Whatman GF/C) as previously described (11). Total cell-associated GTF activity was determined by adding 3 volumes of absolute ethanol to each reaction mixture and collecting the precipitates on a glass microfiber filter. The filter was washed nine times with 75% ethanol and dried, and its radioactivity was counted. One unit of GTF enzyme is that amount of enzyme which catalyzes the incorporation of 1 μmol of the glucose moiety of sucrose into water-soluble or -insoluble glucan under the conditions described.

Adhesive glucan. Adhesive glucan was measured by the deposition of radioactive glucan onto glass surfaces (15) after incubation of [glucose-^{14}C(*U*)]sucrose (New England Nuclear Corp., Boston, Mass.) with a preparation of D70ASP. The procedure used was that described for the GTF assay except that the reaction mixture was prepared in 1-dram (ca. 3.7 ml) screw-cap vials (Fisher Scientific Co., Pittsburgh, Pa.). The vials were incubated at 37°C at an angle of 25° from the horizontal for 5 h, at which time the vials were washed three times with 2 ml of 0.05 M potassium phosphate buffer, pH 6.8. The vials were dried and counted for radioactivity in a scintillation counter.

In vitro plaque formation. The ability of *S. mutans* to produce plaque was ascertained by two methods: (a) the dry weight of adherent material (plaque), and (b) the amount of bacterial deoxyribonucleic acid in plaque which accumulated on glass surfaces after 1 day of growth in PD-sucrose medium. In the first method, a culture was grown in 5% sucrose in PD-medium in a preweighed test tube for 24 h, at which time the medium was carefully removed and discarded. The contents of the tube were washed three times with water, dried, and weighed. In the second method, the adherent material was dispersed in 0.1 N NaOH by a Vortex mixer, centrifuged, washed once with 0.1 N NaOH, three times with water, and once with 5% trichloroacetic acid, and finally suspended in 5% trichloroacetic acid. After incubation at 5°C for 1 to 24 h, the cells were washed twice with 5% trichloroacetic acid and once with 0.2% trichloroacetic acid. To the cell pellet, 0.5 ml of 1 N perchloric acid was added, and the suspension was incubated at 70°C for 30 min. The tube was cooled, and the material was assayed for deoxyribonucleic acid by a method previously described by Burton (1). Purified deoxyribonucleic acid from salmon eggs, which was used as the standard, was a generous gift of Deepak Bastia.

Aggregation of cells. Dextran T2000 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and sucrose were used to induce the aggregation of cells of various strains of *S. mutans* by a procedure described by Gibbons and Fitzgerald (7); the activity was ascertained by measuring the aggregation time as defined by McCabe and Smith (20).

Adherence assay. The adherence assay was performed as suggested by Mukasa and Slade (23). Varying amounts of a suspension of L-[4,5-^{3}H; N]leucine-labeled cells (optical density, 1.0 at 540 nm) containing 73,000 to 88,000 dpm/ml, were added to 4-ml screw-cap vials (Fisher Scientific Co.) containing 0.07 units of GTF, 5 μmol of potassium phosphate (pH 6.8), 0.2 mg of sodium merthiolate, and 25 μmol of sucrose. The total reaction volume was 0.5 ml. The vials were incubated and processed for counting of radioactivity as previously described (12).

Adsorption of cells to saliva-treated HA beads. Adsorption of cells to saliva-treated HA beads (BDH Biochemicals Ltd., Poole, England) was performed by the method of Clark et al. (2) with modifications. Cells of parent-type 6715, mutant C307, and revertants C307R1, C307R2, and C307R3 were grown for 18 h in Trypticase broth (BBL Microbiology Systems, Cockeysville, Md.) containing 0.2% glucose, 0.01% sucrose, and 10 μCi of [*methyl*-^{3}H]thymidine (ICN, Cleveland, Ohio) per ml of medium. Cells were harvested and prepared as described (2) except that only fresh cells were used. Cell suspensions were adjusted to give an absorbance value of 0.11 at 540 nm, which corresponded to a cell density of 4×10^8 to 7×10^8 cells per ml, depending on the strain, by direct microscopic count. Cells were diluted to approximately 4.5×10^7 cells per ml before use. Tubes containing saliva-treated and untreated HA beads were prepared and incubated with 1.6 ml of various concentrations of cells. After a 90-min incubation period, the supernatant fluids from sample (saliva-treated HA beads) tubes were removed and discarded, and the beads were washed 3 times with 1 ml of 1 mM potassium phosphate buffer, pH 6.0, containing a buffered salts solution composed of 50 mM KCl, 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$. The beads were transferred to scintillation vials with two 1-ml washes of buffered salts solution to give a final volume of 2 ml. For the standard tubes, the entire contents (cell suspension plus untreated HA beads) were transferred to a scintillation vial and adjusted to 2 ml with buffered salts solution. Aquasol (8 ml) (New England Nuclear Corp.) was added to each vial, and the radioactivity of the vials was counted in a scintillation spectrometer. Percent adsorption was calculated by dividing the disintegrations per minute in the sample tubes by the disintegrations per minute in the standard tubes.

Preparation of [^{14}C]glucan. [^{14}C]glucan was prepared by incubating a D70ASP preparation from C4 (35 units of GTF activity with respect to water-soluble glucan synthesis) with 5 μCi (500 μmol) of [^{14}C]sucrose and 250 μmol of sodium acetate (pH 5.5) in a total volume of 5 ml at 37°C. After 18 h, the reaction mixture was filtered by a glass microfiber filter. To the filtrate, 3 volumes of absolute ethanol was added, and the precipitate was collected by centrifugation. The precipitate was dissolved in water, and the glucan was precipitated again with ethanol. The glucan solution was added to a column of Biogel A-1.5 M (3 by 45 cm; Bio-Rad Laboratories, Richmond, Calif.) which had been previously calibrated with Pharmacia dextran T10, T110, and T2000. The material was eluted with

0.2 M NaCl, and a radioactive peak fraction corresponding to the peak fractions obtained with dextran T2000 was collected and dialyzed to remove the NaCl. The dialyzed preparation contained 73,830 dpm/ml and 1 mg of carbohydrate per ml as determined by the phenol-sulfuric acid assay (4) with dextran T2000 as the standard.

Adsorption of [^{14}C]glucan by cells. To determine the ability of resting cells to bind [^{14}C]glucan (water-soluble), each strain was grown in PD-glucose medium, and the cells were washed and suspended to give an absorbance value equivalent to 10.0 at 540 nm. Reaction mixtures were prepared and contained 3×10^9 colony-forming units, 25 μmol of sodium acetate buffer (pH 5.5), and varying amounts of [^{14}C]water-soluble glucan ranging from 25 to 200 μg (1,950 to 14,800 dpm) in a total volume of 0.6 ml. The reaction mixtures were incubated at 37°C for 30 min and at 5°C for 18 h as suggested by Kuramitsu and Ingersoll (16); the cells were then collected on glass microfiber disks, washed nine times with water, and air dried, and the filters were counted for radioactivity.

In vivo virulence. To determine the virulence of the strains of *S. mutans* studied, the gnotobiotic rat system described previously was employed (21). The rats were infected with a test strain at 20 days of age and sacrificed at 45 days of age. Individual mandibles were removed and stained with murexide, lingual surfaces were scored for caries activity, and molars were hemisectioned. Buccal, sulcal, and proximal molar surfaces were subsequently scored for caries by the Keyes procedure (13).

Growth and acid production. The rate of growth and acid production were determined during the logarithmic phase of growth of *S. mutans* strains in PD-sucrose medium. Acid was measured titrimetrically.

Quantitative analysis of the amount of cell surface serotype g antigen. The methods employed for the preparation of immunoglobulin G (IgG) anti-serotype *g* antibodies and for the quantitative determination of the amount of cell surface serotype *g* antigen on revertants of C307 were as described previously (10). IgG anti-serotype *g* antibody (100 μg of IgG in 0.5 ml volume) was admixed with increasing amounts of either *S. mutans* 6715 HSR, C307, C307R1, C307R2, or C307R3 lyophilized cells. The reaction mixtures were incubated for 2 h at room temperature; this was followed by overnight incubation at 4°C. The supernatant fluids were removed after centrifugation (12,000 × *g*, 20 min), and the amount of unabsorbed anti-serotype *g* antibody was determined by radial immunodiffusion with monospecific goat antiserum to rabbit gamma globulins (10).

Protein determination. Cellular protein was extracted by heating a cell suspension to 100°C in 1 N KOH for 1 h. The protein content of the KOH extract and of other materials was determined by a dye-binding assay (Bio-Rad Laboratories).

RESULTS

In a previous report (10), C307 was shown to be defective in the serotype *g* antigen which is present in the parent strain 6715 HSR. In this report, a detailed study was carried out that compared the following characteristics of strains 6715 HSR, C307, C307R1, C307R2, and C307R3: colonial morphology, ability to produce plaque, GTF activity, aggregation activity, adherence activity, content of cell-associated GTF, ability to bind soluble glucan, ability to adsorb to saliva-treated HA beads, and cariogenicity.

In vivo virulence pattern. When C307 was tested for virulence in monoassociated gnotobiotic rats and the caries pattern was obtained and compared with that obtained from rats infected with 6715 HSR (Table 1 and Fig. 1), C307 was found to be more virulent in terms of its effect on lingual surfaces. The caries incidence found on buccal, sulcal, and proximal surfaces of molar teeth from both groups of rats was similar. The phenotypic revertants of C307 (based on colonial morphological features) showed reduced caries activity on all surfaces of molar teeth except proximal ones. The scores also showed that the lingual surfaces of rats infected with revertants showed caries, but these scores were lower than for those obtained from rats infected with C307.

Colonial morphology and diagnostic tests. In Table 2, the results obtained for colonial morphology indicate that C307 grew on mitis salivarius plates with a morphology dis-

TABLE 1. *Virulence pattern of 6715 HSR, C307, C307R1, C307R2, and C307R3 in 45-day-old monoassociated rats*

Strain	No. of animals	Mean caries score[a] on the following surfaces:							
		Buccal		Lingual		Sulcal		Proximal	
		E	Ds	E	Ds	Ds	Dx	E	Ds
C307	32	20.0 ± 0.4	13.6 ± 0.5	14.7 ± 0.7	5.9 ± 0.6	18.1 ± 0.3	12.8 ± 0.4	6.6 ± 0.2	4.0 ± 0.1
6715 HSR	29	22.4 ± 0.6	14.4 ± 0.8	0.0[b]	0.0[b]	21.4 ± 0.5	14.6 ± 0.5	7.7 ± 0.2	5.1 ± 0.4
C307R1	12	17.3[c] ± 0.8	7.8[c] ± 0.4	11.1[c] ± 1.6	2.1[c] ± 0.6	15.3[c] ± 0.4	8.6[c] ± 0.5	7.1 ± 0.3	3.8 ± 0.2
C307R2	12	15.9[c] ± 0.7	8.4[c] ± 0.6	8.6[c] ± 1.4	0.7[c] ± 0.2	15.1[c] ± 0.4	8.6[c] ± 0.6	6.7 ± 0.4	3.7 ± 0.4
C307R3	12	15.8[c] ± 1.2	8.3[c] ± 0.6	6.9[c] ± 1.3	0.8[c] ± 0.2	15.6[c] ± 0.4	10.3[c] ± 0.6	7.5 ± 0.2	4.3 ± 0.2

[a] Values represent the mean ± standard error of 12 to 32 rats per group. E, Slight penetration into enamel; Ds, slight penetration into dentin; Dx, extensive penetration into dentin.

[b] Differences between C307- and 6715 HSR-infected rats are significant at $P < 0.001$.

[c] Differences between C307- and revertant-infected rats are significant at $P < 0.01$.

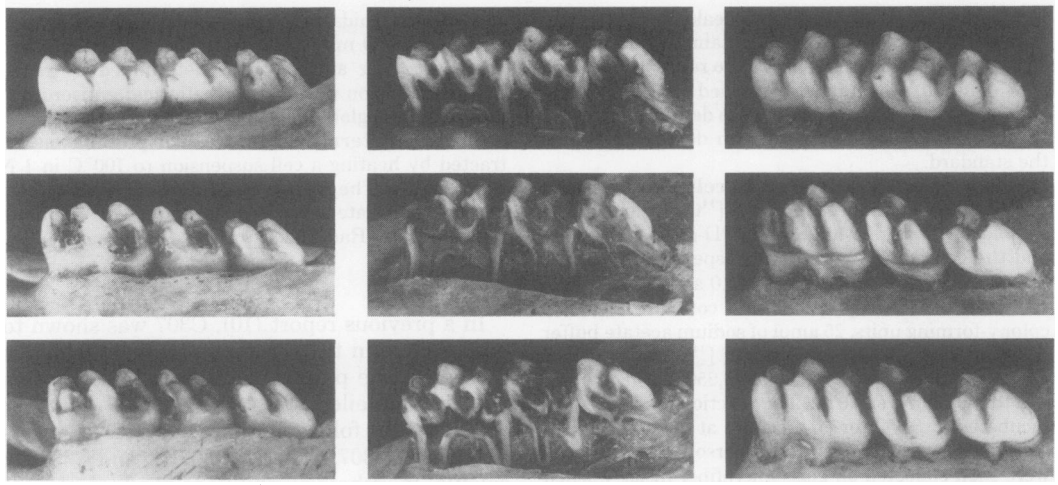

FIG. 1. *Buccal (left), sulcal (center), and lingual (right) surfaces of right mandibles from 45-day-old gnotobiotic rats uninfected (top), infected with C307 (middle), and infected with 6715 HSR (bottom).*

TABLE 2. *Colonial morphology and diagnostic tests*

Strain	Colonial morphology[a]	Diagnostic tests[b]
6715 HSR	Rough, hard, pulvinate	Typical for 6715
C307	Rough, firm, pulvinate	Same as 6715 HSR
C307R1	Rough, hard, pulvinate	Same as 6715 HSR
C307R2	Rough, hard, pulvinate	Same as 6715 HSR
C307R3	Rough, hard, pulvinate	Same as 6715 HSR

[a] The morphology of the colonies was determined after incubation of mitis salivarius agar plates at 37°C, under an atmosphere of 95% N_2–5% CO_2 for 24 h and then at room temperature for an additional 24 h. The terms used to describe the colonial morphology are those recommended in the *Manual of Microbial Methods* (27).

[b] Gram-positive streptococcus; gamma hemolysis on blood agar, no growth at 10°C or 45°C; no growth in 4% NaCl, 6.5% NaCl, pH 9.6 broth, 0.1% methylene blue broth, or 40% bile agar; growth in 10% bile agar and 1% streptomycin; no ammonia from arginine; did not hydrolyze sodium hippurate, gelatin, esculin; fermented lactose, mannitol, sorbitol, sucrose, galactose, and milk; did not ferment raffinose, melibiose, salicin, inulin, or starch.

tinct from its parent strain. The revertants obtained from C307, on the other hand, grew like the parent strain, 6715 HSR. The series of tests comprising a routine diagnostic test of all these strains gave results which were typical of 6715 HSR.

Plaque formation. The ability of these strains to produce plaque in vitro was tested in PD-sucrose medium (Table 3). Although C307 produced slightly less plaque than 6715 HSR,

TABLE 3. *Plaque formation by 6715 HSR, C307, C307R1, C307R2, and C307R3*[a]

Strain	Dry wt (mg)	DNA (μg)
6715 HSR	9.59 ± 1.82 (29)	105.33 ± 11.27 (9)
C307	7.08 ± 2.06 (12)	94.75 ± 19.97 (8)
C307R1	8.56 ± 0.50 (3)	102.67 ± 16.43 (3)
C307R2	8.51 ± 1.15 (3)	98.67 ± 8.22 (3)
C307R3	7.83 ± 1.00 (3)	102.00 ± 13.20 (3)

[a] The weight of plaque in each tube was initially measured and then the bacterial deoxyribonucleic acid (DNA) content of the plaque was determined as described in the text. The results are presented as the means ± standard deviations. The numbers within parentheses are the number of trials.

this result and those obtained with the revertants were considered to be similar to those obtained with 6715 HSR.

GTF activity. A comparison of glucan synthesis by the various strains was made. The results shown in Table 4 indicate that the water-soluble glucan activity of C307 was lower than its parent strain. On the other hand, C307R1, C307R2, and C307R3 showed activities similar to 6715 HSR with respect to synthesis of water-soluble glucan. The activities found for synthesis of water-insoluble glucan by all these strains were considered to be similar. The activities for adhesive glucan synthesis paralleled those for the water-insoluble GTF activities.

Aggregation activity. Table 5 shows the results obtained for aggregation activity of the cells studied. C307 exhibited an enhanced ability to aggregate in the presence of sucrose or dextran when compared with other strains tested. The revertants of C307 aggregated similarly to 6715 HSR.

Adherence of cells to glass surfaces. Various amounts of radioactive, heat-killed cells of 6715 HSR, C307, C307R1, and C307R2 were tested for their ability to adhere to glass surfaces in the presence of sucrose plus a GTF preparation from 6715 HSR, C307, C307R1, or C307R2. The results (Fig. 2) suggest that each type of cell appears to adhere with similar efficiency. Since all results were similar, the data obtained with only the GTF preparation from 6715 HSR are presented.

Adsorption of water-soluble glucan to cells. The ability of resting cells of 6715 HSR, C307, C307R1, and C307R2 to bind ^{14}C-labeled water-soluble glucan was determined. The results (Fig. 3) indicate that resting cells of C307 were less efficient in binding glucan than those of either the parent strain 6715 HSR or the C307R1 and C307R2 strains.

Adsorption of cells to saliva-treated HA beads. The efficiency with which cells of 6715 HSR, C307, C307R1, C307R2, and C307R3 adsorb to saliva-treated HA beads was examined. Cells of C307 were most efficient in adsorbing to saliva-treated HA beads (Fig. 4). Although the activities shown in Fig. 4 are in the decreasing order of 6715 HSR, C307R3, C307R2, and C307R1, the activities of all these strains, with the exception of C307, appeared to be similar.

TABLE 4. *GTF activity of 6715 HSR C307, C307R1, C307R2, and C307R3*

Strain	GTF activity		
	Water-insoluble (μmol/mg of protein per h)	Water-soluble (μmol/mg of protein per h)	Adhesive (μmol/mg of protein per h)
6715 HSR	24.20 ± 3.10[a]	0.74 ± 0.06	61.10 ± 1.40
C307	20.90 ± 2.00	0.44 ± 0.20	51.00 ± 2.10
C307R1	26.4 ± 2.40	0.53 ± 0.06	70.5 ± 1.63
C307R2	24.70 ± 3.30	0.54 ± 0.04	64.8 ± 1.90
C307R3	22.0 ± 2.30	0.64 ± 0.14	63.1 ± 1.30

[a] The values shown are the means of triplicate samples ± standard deviation.

TABLE 5. *Aggregation of S. mutans 6715 HSR, C307, C307R1, C307R2, and C307R3*[a]

Strain	Time(s) of reaction	
	Sucrose induced	Dextran T2000 induced
6715 HSR	80	5
C307	10	1
C307R1	85	5
C307R2	80	5
C307R3	90	5

[a] Cells were grown for 18 h in PD-glucose medium and washed twice with saline, and 0.1 ml (1×10^9 cells) of cells was added to tubes containing 0.3 ml of 0.2 M glycine-sodium hydroxide buffer, pH 8.6. The reaction was initiated by the addition of either 0.2 ml of 4% sucrose or 0.2 ml of 2% dextran T2000.

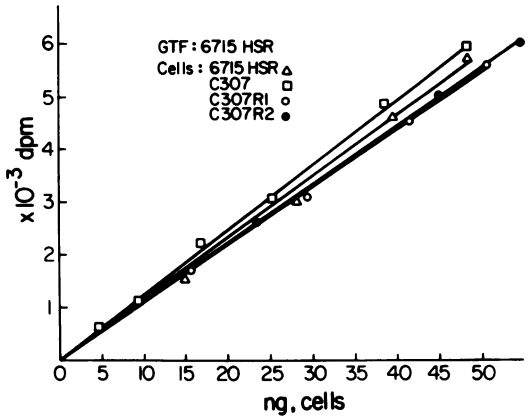

FIG. 2. *The ability of heat-killed cells of 6715 HSR, C307, C307R1 and C307R2 to adhere to glass surfaces in the presence of sucrose plus a GTF preparation from 6715 HSR.*

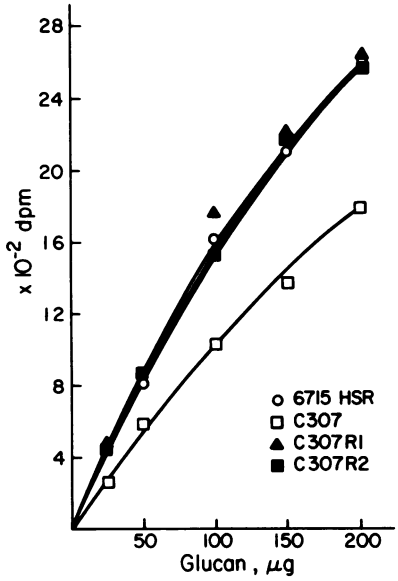

FIG. 3. *Absorption of water-soluble glucan by resting cells of 6715 HSR, C307, C307R1, and C307R2 by using a glucan preparation from strain C4.*

Cell-associated GTF activities. The higher aggregation activity of resting cells of C307 in the presence of either sucrose or dextran T2000 and their increased ability to adsorb to saliva-treated HA beads suggest that these resting cells may differ in their cell-associated GTF content. Accordingly, an experiment was performed to compare their cell-associated GTF content. The

results (Table 6) indicate that the GTF content of C307 cells was lower than that of the parent and the three revertant strains.

Growth and acid production. Although the ability of C307 to adsorb to saliva-treated HA beads and to undergo aggregation was greater than 6715 HSR, the higher incidence of caries produced by C307 could be attributed to its faster rate of growth and acid production from sucrose. The results (Table 7) show that the rates of growth of C307, 6715 HSR, and revertants as measured by acid production during the logarithmic phase of growth were similar. Also, the terminal amount of acid produced as measured by pH determinations and the amount of sodium hydroxide consumed in 18-h sucrose cultures were all similar.

Amount of serotype *g* antigen on cells of revertants of C307. Previous studies have demonstrated that C307 possesses less than 1% of the serotype-specific *g* antigen normally found on *S. mutans* 6715 HSR cells (10). No detectable *g* antigen is present in 0.6 mg of cells of C307 when compared to an equivalent amount of cells of 6715 HSR (Table 8). The dry weight of cells used to absorb the IgG anti-serotype *g* antibody ranged from 0.05 to 200 mg, and even at the highest concentration of cells used, no *g* antigen was detected in C307. The present study however, demonstrated that the revertants of C307 possess serotype *g* antigen. C307R1 exhibits higher levels of the serotype-specific antigen than *S. mutans* 6715 HSR, whereas C307R2 and C307R3 possess levels of serotype *g* antigen which were similar to those normally found on *S. mutans* 6715 HSR.

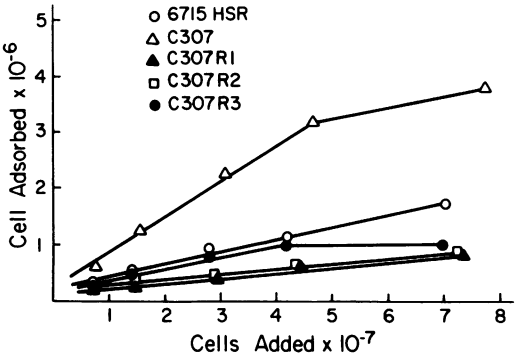

FIG. 4. *Adsorption of 6715 HSR, C307, C307R1, C307R2, and C307R3 cells to saliva-treated HA beads.*

TABLE 6. *Cell-associated GTF activity of 6715 HSR, C307, C307R1, C307R2, and C307R3*

Strain	Cell-associated GTF activity[a]	
	Total units	Sp act (μmol/mg of protein per h)
6715 HSR	10.82 ± 0.96	0.42 ± 0.04
C307	8.26 ± 1.81	0.29 ± 0.07
C307R1	10.29 ± 2.11	0.38 ± 0.09
C307R2	11.10 ± 1.59	0.38 ± 0.05
C307R3	10.31 ± 1.56	0.40 ± 0.06

[a] The values shown are the means of triplicate samples ± standard deviation. The activities presented are based on 400 ml of PD-glucose cultures.

TABLE 7. *Acid production by 6715 HSR, C307, C307R1, C307R2, and C307R3*

Strain	Acid production[a]	
	Milliequivalents per h	Milliequivalents 18 h[b]
C307	0.032	0.58 (4.6)
C307R1	0.030	0.58 (4.5)
C307R2	0.029	0.54 (4.6)
C307R3	0.028	0.56 (4.6)
6715 HSR	0.029	0.54 (4.6)

[a] Milliequivalents of acid produced per 10 ml of PD-sucrose culture per h during logarithmic phase of growth.

[b] Acid production and pH values (within parentheses) of cultures after 18 h of incubation.

TABLE 8. *Quantitative absorption of IgG anti-serotype g antibody with either S. mutans 6715 HSR, C307, C307R1, C307R2, and C307R3*

Dry weight of cells (mg)[a]	% absorption[b] of:				
	6715 HSR	C307	C307R1	C307R2	C307R3
0.05	21.0	0.0	22.5	22.5	25.0
0.10	43.5	0.0	60.0	42.5	43.5
0.20	78.0	0.0	92.5	76.5	80.0
0.40	100.0	0.0	100.0	100.0	100.0
0.60	100.0	0.0	100.0	100.0	100.0
200.00	100.0	0.0	100.0	100.0	100.0

[a] The range of cells used was from 0.05 mg to 200 mg. Because the results obtained with cells between 0.4 to 200 mg were identical, the results obtained with cells between 0.6 to 200 mg have been omitted.

[b] The absorption was determined by the formula [(amount of IgG anti-serotype *g* antibody in supernatant fluids after absorption) ÷ amount of IgG anti-serotype *g* antibody added to reaction mixture)] × 100, as determined by radial immunodiffusion with monospecific goat anti-rabbit gamma globulin.

DISCUSSION

The caries pattern obtained with the parent strain 6715, as with other *S. mutans* serotypes, is normally manifested by lesions on the buccal, sulcal, and proximal surfaces. Mutant C307, which was shown to be defective in the serotype *g* antigen, was found to produce an unusual

pattern of caries in gnotobiotic rats. The caries lesions not only occurred on the buccal, sulcal, and proximal surfaces but also on the lingual surfaces. The presumptive revertants of C307 were of lower virulence, producing fewer caries on the buccal, sulcal, proximal, and lingual surfaces. The biochemical results obtained with parent strain 6715 HSR, mutant C307, and the revertants did not reveal a clear and obvious relationship between their biochemical traits and caries patterns.

As compared with 6715 HSR, mutant C307 showed similar or slightly less extracellular and cell-associated GTF activities, plaque formation, adherence to glass surfaces, and lower rates of growth and acid production. However, notable differences in traits seen were the increased ability of C307 to adsorb to saliva-treated HA and to aggregate in the presence of sucrose or dextran and its low, soluble glucan-binding activity. In the case of the three revertants, these same biochemical features were similar to those of 6715 HSR.

Although there was little difference in the ability of any of these strains to adhere to glass surfaces, mutant C307 showed an enhanced ability to adsorb to saliva-treated HA beads. Several cell surface mediators of adherence have been proposed including teichoic acids (28) and substances which interact with blood group-reactive salivary mucins (9). Recently, Staat et al. (30) showed that adherence to saliva-treated HA was mediated by a protein. The adherence could be inhibited by a protein-binding lectin from *Persea americana* or by proteolytic enzymes. The serotype antigen is known to exist both in the cell wall and as a microcapsular structure (3). It is possible that the increased HA binding seen with C307 could be due to an increase in the availability of such receptors caused by cell surface changes associated with the loss of the serotype antigen and the associated microcapsule. This point regarding the microcapsule content in C307 must be investigated.

Mutant C307 exhibits a marked ability to aggregate in the presence of sucrose and T2000 dextran, yet possesses lower levels of cell-associated GTF and lower glucan-binding activity as compared with the other strains. Models for cellular aggregation which have been proposed (5, 29) involve the participation of cell-bound GTF, dextran, and nonenzyme dextran receptors. According to one of the models (29), the loss of the type-specific polysaccharide could lead C307 cells to bind less GTF. A similar role for the type-specific polysaccharide has not been proposed for the nonenzyme dextran receptor. It has been suggested that the nonenzyme dextran receptors play a minor role in aggregation induced by high-molecular-weight dextran (5). However, the data obtained with C307 suggest that the increased aggregation is not due to higher levels of cell-associated GTF but rather to mediation by nonenzyme dextran receptors which may be more accessible or less sterically hindered on the altered surface of this mutant and thus more effective in mediating direct cell-to-cell dextran-induced aggregation. The importance of direct cell-to-cell dextran-mediated aggregation has been shown in the work of Wu-Yuan et al. (32). It is likely that sucrose-induced aggregation would also be affected by more accessible dextran receptors which could bind newly synthesized glucan and enhance cell-to-cell binding. Moreover, if C307 does not contain a microcapsule, the lack of this structure may also enhance the direct cell-to-cell interaction. That the dextran receptors are more accessible rather than more abundant is supported by the fact that the binding of [^{14}C]glucan is lower in this mutant than in the other strains.

Although Keyes reported (14) lingual caries in rats fed diets containing skim milk, other than this substrate, this unusual caries involvement could not be attributed to any one property of C307. It is possible that the enhanced property of cells of C307 to adsorb to saliva-treated HA beads may be important for these cells to colonize lingual surface with a higher efficiency; however, revertants of C307, particularly R1, which still caused high lingual caries incidence, adsorb to saliva-treated HA beads at lower rates, similar to the parent type 6715 HSR. It is suggested that most of the changes in traits seen in C307, when compared with those of 6715 HSR, can be attributed to an altered cell surface which is brought about by the loss of the *g* antigen. A similar pleiotropic effect (colonial morphology, growth rate, cell size, etc.) due to a defect in a cell surface component, teichoic acid, in a mutant of *Staphylococcus aureus* has been reported (26). In the case of the revertants, the restoration of the *g* antigen was also accompanied by the attendant restoration of the in vitro parent-type virulence factors. However, the restoration of *g* antigen did not eliminate the property of lingual caries activity. It is possible that there are factors associated with caries formation which are still obscure. Perhaps when a method for genetic analysis of *S. mutans* becomes available, additional studies on the revertants of C307 could be performed.

Mukasa and Slade (23) demonstrated that antibody globulin to the *a-d* site of *S. mutans* strain HS-6 effectively inhibited the adherence of these cells to glass surfaces without affecting GTF activity. Subsequently, these authors showed that anti-*a-d* and anti-dextran antibod-

ies inhibited the adsorption of GTF (24). These results suggested to them that the serotype antigen or a site adjacent to the serotype antigen may play a role as the recognition site for adherence; moreover, GTF probably binds this site. The work done with C307 and its revertants suggests that the *g* antigen of 6715 HSR is not involved in adsorption of cells of 6715 to saliva-treated HA, adherence of cells to glass surfaces or cell aggregation.

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