

Glucosyltransferase phase variation in *Streptococcus gordonii* modifies adhesion to saliva-coated hydroxyapatite surfaces in a sucrose-independent manner

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Phase variation of *Streptococcus gordonii* between high (Spp⁺) and low (Spp⁻) levels of glucosyltransferase (GTF) activity resulted in the greater adhesion of Spp⁻ strains to saliva-coated hydroxyapatite (S-HA) in a washed-cell adhesion test. Specific GTF mutants did not show this response. Although washed Spp⁺ cells produced 5-fold or more glucan from sucrose than Spp⁻ cells did under the conditions of the adhesion test, sucrose elevated the adhesion of both phenotypes to hydroxyapatite (HA) equally, but had no effect on adhesion to S-HA. This effect was not sucrose-specific, however, because equimolar amounts of other carbohydrates and NaCl elevated adhesion of both Spp types to levels similar to those seen with sucrose. Adhesion did not correlate with relative changes in cell hydrophobicity. These results suggest that, in addition to changes in GTF activity, other changes relevant to adhesion may occur during Spp phase variation.

Key words: *Streptococcus gordonii*; glucosyltransferase; phase variation; saliva; sucrose

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We recently described phase variation in glucosyltransferase (GTF) activity of *Streptococcus gordonii* (15) (previously classified as *Streptococcus sanguis* (9)). Normally *S. gordonii* grows on sucrose-agar as hard, cohesive colonies (Spp⁺). Broth cultures of Spp⁺ cells spontaneously give rise to soft, noncohesive colonies (Spp⁻) at frequencies of 10⁻⁴ to 10⁻³. Spp⁻ cultures have 10–30% of the GTF activity of Spp⁺ cultures and in turn give rise to Spp⁺ revertants at the same 10⁻⁴ to 10⁻³ frequencies (15). *S. gordonii* and *S. sanguis* are initial colonizers of the tooth surface (3) and potentially may have significant influences on colonization of the dental plaque by other bacteria. Additional bacteria can attach directly or indirectly to *S. gordonii* and *S. sanguis* cells via lectin-like interactions (10, 11), or bind to glucans produced by these streptococci (2). Although glucan synthesis has been associated with a spectrum of activities connected with colonization and maintenance of mutans streptococci (7),

the role that GTF and glucans play in the ecology of *S. gordonii* and *S. sanguis* is less well defined. We describe here results of our initial characterization of the adhesive phenotypes of Spp⁺ and Spp⁻ variants by measuring their ability to attach to hydroxyapatite (HA) and saliva-coated hydroxyapatite (S-HA) surfaces under nongrowing conditions. Strain Challis was used because GTF phase variation is described in greatest detail in this strain (15) and strain Challis is more suited to the ongoing genetic studies of this phenomenon. Additionally, strain Challis has little ability to attach to HA or to S-HA surfaces, and therefore, changes in adhesion caused by the level of GTF activity should be more evident.

Washed-cell adhesion assays (1, 4), which measure initial, reversible and subsequent irreversible adhesion (5), were done with cells from late-log broth cultures (A₅₂₀ of 1.9) grown anaerobically in the presence of ³H-thymidine. Cells grown in Todd-Hewitt (Difco Labora-

tories, Detroit, MI) or chemically defined FMC (16) medium gave similar results. To measure adhesion to S-HA, saliva was collected from healthy donors, heat-treated at 60°C and prepared as described (4). No bacteria were recovered from the saliva on Todd-Hewitt agar plates incubated anaerobically, and no salivary GTF activity was detected on activity gels (12, 15). Saliva was adsorbed to HA beads (BDH Biochemicals, Poole, UK) in polystyrene tubes on a rotating drum (10 rpm, New Brunswick Scientific, Edison, NJ) at room temperature for 90 min. Bacteria were washed twice and resuspended in buffered KCl, pH 6.8 (1), to a concentration of 1 × 10⁸ bacteria/ml. One ml of the bacterial suspension was added to 10 mg of washed HA or S-HA beads and tubes were rotated (10 rpm) for 3.5 h at 37°C. This incubation temperature was used to optimize the activity of cell-associated GTF which synthesized maximum amounts of glucan by 3.5 h. Preliminary experiments under these

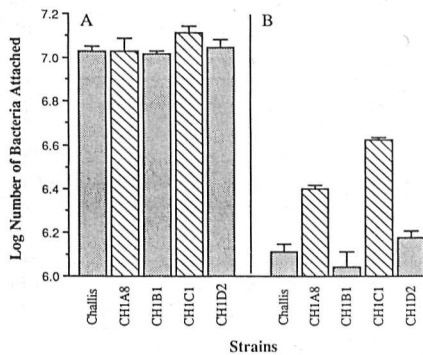


Fig. 1. Adhesion of Spp variants to HA (Panel A) and S-HA (Panel B) beads. Log₁₀ number of bacteria (\pm SD) attached to 10 mg of beads after 3.5 h at 37°C. The strains were derived sequentially as shown (the strain Challis through strain CH1D2). ▨, Spp⁻; ▩, Spp⁺ strains.

conditions showed that maximum adhesion at this subsaturating concentration of bacteria was achieved in 3.5 h. Kinetic studies (1) with cell concentrations of 4×10^6 to 4×10^9 bacteria/ml demonstrated that saturation of the bead surfaces required in excess of 4×10^9 bacteria/ml.

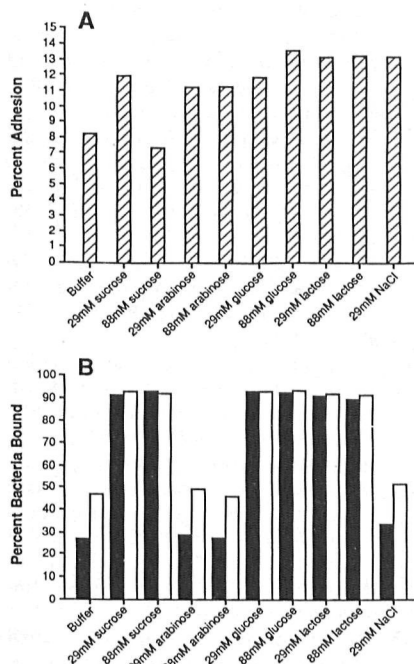


Fig. 2. Changes in adhesion to HA and the relative hydrophobicity of strain Challis caused by various additives. Panel A, percentage of 1×10^8 bacteria attached to HA after 3.5 h at 37°C in the presence of carbohydrates and NaCl; Panel B, percentage of 1×10^8 bacteria adsorbed to octyl-□, and phenyl-■ Sepharose under the same conditions.

Table 1. Elevated adhesion to HA in the presence of sucrose and arabinose

Conditions	Adhesion ^a of strain			
	Challis	G9B	C5	FC1
Buffer	1.02 \pm 0.04	1.81 \pm 0.05	0.91 \pm 0.02	0.43 \pm 0.05
Sucrose (29 mM)	1.40 \pm 0.02	2.61 \pm 0.16	1.16 \pm 0.01	1.01 \pm 0.01
Sucrose (88 mM)	1.23 \pm 0.01	1.92 \pm 0.06	0.97 \pm 0.01	0.83 \pm 0.07
Arabinose (29 mM)	1.40 \pm 0.08	2.30 \pm 0.07	1.28 \pm 0.07	1.05 \pm 0.09

^a Number of bacteria ($\times 10^7 \pm$ SD) attached to 10 mg HA after 3.5 h at 37°C in buffered KCl alone or with added carbohydrate. Strain G9B was obtained from B. Rosan and strains C5 and FC1 were obtained from R. Gibbons. Strains G9B and C5 were classified as *S. gordonii* and strain FC1 as *S. sanguis* based upon their reactions in API-ZYM and LRA osidase tests (Analytab Products, Plainview, NY) according to the criteria of Kilian et al. (9).

After incubation, the beads were rinsed twice to remove unattached bacteria and counted in a scintillation counter to determine the number of bacteria attached to the beads. Unattached bacteria in the supernatant were counted in the same way. Total recovery of the bacteria was $\geq 90\%$. All tests were done in duplicate and experiments repeated at least twice. Results were then analyzed by Student's *t*-test for small samples. No coaggregation of the cells or differences in chain length of the washed cells of either phenotype were seen macroscopically or microscopically in the presence or absence of any compounds added to the assay system.

The Spp status of sequentially derived Spp⁺ and Spp⁻ strains (15) had little influence on the cells' ability to attach to HA (Fig. 1). These results were confirmed with four other independently derived Spp⁻ and three other Spp⁺ isolates (data not shown). Although there was a significant decrease in the adhesion of both phenotypes to S-HA compared to HA ($P < 0.01$, Fig. 1), Spp⁻ isolates were consistently 2–5 times more adhesive to S-HA than were Spp⁺ cells ($P < 0.001$). These results were again confirmed with the other independently derived Spp⁺ and Spp⁻ isolates ($P < 0.003$, data not shown). The increased adhesion of Spp⁻ cells to

S-HA was not related to any changes in the relative cell surface hydrophobicity, as radiolabeled Spp⁻ cells showed the same abilities as Spp⁺ cells to bind to phenyl- and octyl-Sepharose (14) under the incubation conditions of the adhesion test (see below). It is also noteworthy that GTF mutant strains with either a mutation (strain CHA1) or insertion (strain CH82) in the GTF structural gene (Mark Sulavik, personal communication), did not show increased adhesion to S-HA compared to the parental Spp⁺ strain Challis. These results suggest that the increased ability of Spp⁻ cells to attach to S-HA is not caused by reduced GTF activity alone nor by relative changes in hydrophobic properties of the bacterial surfaces.

The influence of GTF activity on the initial adhesion to HA and S-HA was studied by the addition of the substrate sucrose to the buffer in the adhesion test. Sucrose had a concentration-dependent effect on adhesion over the range of 2.9 to 88 mM. Adhesion increased proportionally over the level measured in buffer alone to reach a maximum at 29–36 mM sucrose and then decreased proportionally thereafter. The addition of 29 mM sucrose increased the adhesion of strain Challis to HA ($P < 0.01$) and also elevated the adhesion ($P < 0.01$) of the Spp⁺ *S. gor-*

Table 2. Influence of Spp type and carbohydrates on adhesion to HA

Strain	Spp type	Glucan ^a	Adhesion ^b in the presence of:			
			Buffer alone	Sucrose 29 mM	Sucrose 88 mM	Arabinose 29 mM
Challis	+	5.81 \pm 1.48	0.56 \pm 0.02	0.87 \pm 0.01	0.64 \pm 0.01	0.75 \pm 0.01
CH1C1	-	0.73 \pm 0.27	0.80 \pm 0.01	1.15 \pm 0.05	0.96 \pm 0.06	0.98 \pm 0.02
CH1D2	+	8.21 \pm 0.93	0.61 \pm 0.01	0.98 \pm 0.09	0.70 \pm 0.01	0.91 \pm 0.01

^a ng of total glucan (\pm SD) synthesized from 1.5 mM sucrose by 1×10^8 washed cells under the conditions of the adhesion test as determined by [¹⁴C-glucose]sucrose incorporation (8). Total recovery of unincorporated and incorporated radiolabel was $> 90\%$ indicating that glucans were not lost in the assay. ^b Number of bacteria ($\times 10^7 \pm$ SD) attached to 10 mg HA after 3.5 h at 37°C in buffered KCl alone or with added carbohydrate.

donii strains G9B and C5 and of the Spp⁺ *S. sanguis* strain FC1 (Table 1). This effect was not sucrose-specific, however, because equimolar concentrations of the irrelevant, nonmetabolizable carbohydrate, arabinose, produced a similarly elevated response (Table 1). Higher sucrose concentrations (88 mM) depressed adhesion of all stains to levels below those of 29 mM sucrose ($P < 0.01$). Over this concentration range, the addition of sucrose did not affect the adhesion of any of these Spp⁺ strains to S-HA.

Similarly, the addition of 29 mM glucose or fructose, examined as the constituents of sucrose, or lactose, an inhibitor of some streptococcal cell interactions (10), or NaCl, which was used to measure ionic effects, all increased adhesion of strain Challis to HA to levels indistinguishable from that caused by 29 mM sucrose. The effects of these 3 metabolizable carbohydrates differed from that of sucrose, however, in that 88 mM concentrations did not depress adhesion (Fig. 2A). Bacteriostatic levels of chloramphenicol (100 µg/ml), present throughout the test, did not inhibit carbohydrate-enhanced adhesion, suggesting that de novo protein synthesis was not required for this response. Although the addition of metabolizable carbohydrates to the test system consistently increased the relative cell surface hydrophobicity compared with that of cells in buffer alone, this increase did not occur in the presence of a nonmetabolizable carbohydrate such as arabinose nor in the presence of NaCl (Fig. 2B). pH did not affect adhesion or relative hydrophobicity over the ranges found in these tests (pH 5.5 to 6.8). The relative hydrophobicity of the Spp⁻ cells in the presence of these carbohydrates and NaCl was similar to that of the Spp⁺ strains. Thus, again changes in relative cell surface hydrophobicity did not correlate with changes in adhesion (Fig. 2A).

Cell surface GTF activities of the washed Spp⁺ and Spp⁻ cells were confirmed by activity gels (15) and [¹⁴C-glucose]sucrose incorporation into glucans (8). Under the incubation conditions of the adhesion test, the relative amounts of glucan synthesized from sucrose by the washed Spp⁻ cells were significantly reduced compared with that of Spp⁺ cells (Table 2), and consistent with the GTF activities of cell-free culture supernatants (15). However, de-

spite this difference, sucrose elevated the adhesion of Spp⁻ strains, such as CH1C1, to HA as much as it elevated adhesion of the Spp⁺ parental strain Challis and revertant strains such as CH1D2 (Table 2). Again, this effect was not sucrose-specific, as arabinose also increased adhesion (Table 2). As was found with the parental Spp⁺ strains (see above), neither carbohydrate increased the adhesion of Spp⁻ strains or their Spp⁺ revertants to S-HA.

Thus, in classical, washed-cell adhesion tests, which measure initial binding of non-growing bacteria to substrata (1, 4), adhesion to HA is not influenced by Spp phase variation and the glucans produced from sucrose by cell-bound GTF do not appear to affect initial adhesion to HA or S-HA. However, the increased adhesion of Spp⁻ cells to S-HA may be significant and indicate that concomitant phenotypic changes occur during Spp phase variation in addition to changes in GTF activity. It has been postulated that variations in adhesive properties may facilitate colonization of nonshedding surfaces (6). Spp⁻ phase variants of *S. gordonii* may be associated with such ecologically relevant events, similar to those proposed for phase variation in some other bacteria (13). Studies to further investigate differences between the 2 phenotypes are in progress.

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