RESEARCH REPORTS

Biological

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ABSTRACT

Streptococcus gordonii and *Streptococcus mutans* avidly colonize teeth. *S. gordonii* glucosyltransferase (GtfG) and amylase-binding proteins (AbpA/AbpB), and *S. mutans* glucosyltransferase (GtfB), affect their respective oral colonization abilities. We investigated their interrelationships and caries association in a rat model of human caries, examining the sequence of colonization and non- vs. high-sucrose diets, the latter being associated with aggressive decay in humans and rats. Virulence-characterized wild-types of both species and well-defined mutants of *S. gordonii* with interrupted *abpA* and *gtfG* genes were studied. While both *S. gordonii* and *S. mutans* were abundant colonizers of rat's teeth in the presence of either diet, if inoculated singly, *S. mutans* always out-competed *S. gordonii* on the teeth, independent of diet, strain of *S. mutans*, simultaneous or sequential inoculation, or presence/absence of mutations of *S. gordonii*'s *abpA* and *gtfG* genes known to negatively or positively affect its colonization and to interact *in vitro* with *S. mutans* GtfB. *S. mutans* out-competed *S. gordonii* in *in vivo* plaque biofilm. Caries induction reflected *S. mutans* or *S. gordonii* colonization abundance: the former highly cariogenic, the latter not. *S. gordonii* does not appear to be a good candidate for replacement therapy. These results are consistent with human data.

KEY WORDS: dental plaque biofilm, bacterial interference, glucosyltransferase, amylase binding protein, sucrose, starch.

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Streptococcus mutans Out-competes *Streptococcus gordonii* *in vivo*

INTRODUCTION

The mutans streptococci are strongly cariogenic, while other tooth-colonizing oral streptococci are thought to be either non-cariogenic or minimally so (Hamada and Slade, 1980; Loesche, 1986; Tanzer *et al.*, 2001a). Thus, there is interest in “bacterial interference therapy” to pre-empt establishment or replace mutans streptococci from tooth surfaces by non-cariogenic tooth-colonizing bacteria. This concept is not new (Aly *et al.*, 1982; Sprunt and Leidy, 1982; Tanzer *et al.*, 1985a,b; Hillman *et al.*, 2000). The non-mutans species—formerly classified as *Streptococcus sanguis*, which now includes the species *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus gordonii*—are found in high numbers on supragingival tooth surfaces. *S. sanguis* had been suggested as a replacement organism, since it has been negatively associated with caries (Krasse *et al.*, 1968; de Stoppelaar *et al.*, 1969; Loesche and Syed, 1973; Loesche *et al.*, 1975; Swenson *et al.*, 1976). While recent *in vitro* studies suggest that *S. gordonii* should be capable of such replacement (Kreth *et al.*, 2008; Liu *et al.*, 2011), other *in vivo* microbiological assessments of carious human teeth continue to suggest otherwise (Gross *et al.*, 2010). We know of no human populations free of *S. gordonii* and mutans streptococci that could serve as “clean” study subjects. We therefore tested, in rats initially free of these bacteria, the hypotheses that *S. gordonii* can colonize and persist on the dentition despite simultaneous, previous, or subsequent inoculation by *S. mutans* strains, and *vice versa*. We also tested the dependency of these relationships upon dietary sucrose. We also tested abilities of mutants of *S. gordonii* deficient in genes that encode colonization determinants to affect this relationship.

MATERIALS & METHODS

Bacterial Strains, Plasmids, and Primers

Bacterial strains, plasmids, and primers used in this study are presented in Appendix 2.

Construction of Mutant Strains

Details of the rationales and construction of *S. gordonii* mutant strains of Challis CH1 are presented in Appendix 2. Briefly, a mutant of the wild-type *S. gordonii* Challis strain was constructed having a kanamycin-resistant phenotype to facilitate recovery and quantification during animal experiments. A chromosomal integration site was selected empirically, based upon the gene order of convergent open reading frames at loci SGO_2076 encoding a putative phage integrase and SGO_2077 encoding a hypothetical protein with no identified conserved domains and no known function. Thus, *S. gordonii* strain

Figure 1. General protocols and time-course of events of experiments in rats that compared the abilities of wt *S. mutans* strains with wt and mutant *S. gordonii* strains to colonize teeth and induce caries. There were 10 rats *per* group in all experiments. Inoculum doses within any experiment were the same and ranged among experiments from 10^9 to 10^{10} CFU. We tested bacterial recoveries intra-experimentally by swabbing the teeth on days indicated by arrows, and immediately after euthanasia, at 42 days post-initial inoculation. Colonization status was determined by extraction of mandibular molars *en bloc* from one hemi-mandible and sonication of its teeth. Sonic suspensions of tooth-associated bacteria were differentially quantified by methods detailed in Appendix 2. Animal groups are designated by their inoculation/infection status within the horizontal bars in the Fig. Panel (A) presents the general design of experiments using the “simultaneous inoculation protocol” for either single strains or 2 presumptively competitive strains inoculated simultaneously in equal doses. Panel (B) presents the general design of experiments using the “sequential inoculation protocol” in which single strains were inoculated into the mouths of previously un-inoculated animal groups, and confirmed to have colonized by swab culture on day 8. Animal groups that had been either previously un-inoculated or previously inoculated and established to be heavily colonized were subsequently inoculated with a second strain on day 10.

KS1 was generated by replacement of SGO_2076 and a portion of the downstream intergenic region (bp 2140291-2141556; GenBank CP000725) with an *aphIII* determinant encoding kanamycin resistance.

Strain KS1*gtfG* was constructed with the 2.5-kb dsDNA *XhoI/SstI* fragment of pKS1 carrying the *aphIII* determinant between SGO2076 flanking regions. In this case, the linear fragment was used to transform the CH1 derivative strain CH1Δ*gtfG* in which *gtfG* encoding the single *S. gordonii* glucosyltransferase enzyme had been deleted (Banas *et al.*, 2007).

abpA-deficient mutants in KS1 or KS1*gtfG* were constructed by disruption of *abpA* by an integrated *tetM* gene that encodes tetracycline resistance (EMBL accession no. X56353). After sequence verification, selected transformants were designated strain KS1*abpA*, in which *abpA* was disrupted, and strain KS1*gtfG/abpA*, a double-mutant with both a *gtfG* deletion and *abpA* disruption, respectively.

All mutant strains were compared with *S. gordonii* parental strain Challis for similar colony morphology on blood agar (BA) and Mitis-Salivarius Agar (MSA), Gram stain, and biochemical/metabolic profiles. No differences were noted in growth rates between the parental and mutant strains. To compare amylase-binding activity between wild-type and mutant strains, we used a Western blot amylase ligand-binding assay as previously described (Chaudhuri *et al.*, 2007, 2008). Gtf activity was qualitatively determined in 12% polyacrylamide gels as previously described (Vickerman and Minick, 2002).

In vivo Studies

Experiments with rats were approved by the IACUC, University of Connecticut Health Center. Eight *in vivo* experiments were performed. For details, see Appendix 2. Their designs are depicted in Figs. 1A and 1B. For each experiment, 21-day-old weanlings were randomly assigned from multiple litters born on the same day. In the simultaneous inoculation protocol (A) (Tanzer *et al.*, 1985a), one day after provision of test diet, rats were either inoculated (Experimental Day 0) simultaneously with equal numbers of stipulated *S. gordonii* strain, its isogenic derivative, and an *S. mutans* strain; inoculated with one or the other of them; or remained un-inoculated. In the sequential inoculation protocol (B), by contrast, one day after provision of test diet, weanlings were either inoculated (Experimental Day 0) with one of the test strains or remained un-inoculated. Ten days later, inoculation of the potentially competing species was given both to a previously inoculated group and to a group previously un-inoculated. Some groups remained un-inoculated. In this way, sequentially doubly- and singly-inoculated animals, as well as un-inoculated ones, could be studied.

RESULTS

Cross-contamination among rat groups, reversion of mutants to parental phenotypes, indigenous or unintended extraneous infection, or adverse health effects did not occur. Single pure-culture inoculants were always recovered in high numbers from

Figure 2. Recoveries of inoculants from rats' teeth and associated caries lesion scores 42 days after either simultaneous (panels A, B, C) or sequential (panels D, E, F) inoculations, respectively. Inocula were *S. mutans* and/or *S. gordonii* wild-types and mutants, as stipulated below. In all inoculant groups, there were 10 rats, and all ate a high-sucrose diet. The Y axes of panels A and D depict absolute count recoveries of inoculants from 3 molar teeth of a hemi-mandible as mean $\pm$ SEM $\times 10^7$ CFUs. The Y axes of panels B and E depict those recoveries as mean $\pm$ SEM percent of total recoverable flora. For these bacteriological data (panels A, B, D, and E), variously shaded bars of the histograms and corresponding keys at the tops of these panels identify the recoverants. The Y axes of panels C and F depict mean $\pm$ SEM caries lesion scores for coronal tooth surface categories that are identified by the keys with bar shades above those panels. Caries lesions were assessed on the 3 molar teeth of the contralateral hemi-mandible for each rat. Panels A, B, and C present the data for an experiment using the simultaneous inoculation protocol. All inoculations occurred at experimental day 0. Inoculant groups are designated numerically on the X axis, as:

- 1 – uninoculated
- 2 – *S. mutans* 10449S
- 3 – *S. gordonii* Challis-S
- 4 – *S. gordonii* Challis-ST
- 5 – *S. mutans* 10449S plus *S. gordonii* Challis-S
- 6 – *S. mutans* 10449S plus *S. gordonii* Challis-ST.

Panels D, E, and F present the data for an experiment using the sequential inoculation protocol. Initial inoculations occurred at experimental day 0, and if a sequential inoculation was to occur, it was given at experimental day 10. Thus, inoculant groups are designated numerically on the X axis, as:

- 1 – uninoculated
- 2 – *S. mutans* 10449S
- 3 – *S. gordonii* Challis-S
- 4 – *S. gordonii* Challis-ST
- 5 – uninoculated, then later *S. mutans* 10449S
- 6 – uninoculated, then later *S. gordonii* Challis-S
- 7 – uninoculated, then later *S. gordonii* Challis-ST
- 8 – *S. mutans* 10449S, then later *S. gordonii* Challis-S
- 9 – *S. gordonii* Challis-S, then later *S. mutans* 10449S
- 10 – *S. mutans* 10449S, then later *S. gordonii* Challis-ST
- 11 – *S. gordonii* Challis-ST, then later *S. mutans* 10449S.

swabbed and sonicated teeth. However, for doubly inoculated animals, whether simultaneously or sequentially inoculated by *S. gordonii* and *S. mutans* strains, recoveries were always dominated by *S. mutans* on sonicated molars, independent of sequence of inoculation and sucrose- or sucrose-free diet. Caries scores reflected *S. mutans* predominance.

We previously studied mutants of *S. gordonii* that did not express *abpA* or *gtfG* derived from a spontaneous streptomycin-resistant mutant of *S. gordonii* Challis, strain Challis-S; these strains were used in colonization and caries studies in the rat (Tanzer *et al.*, 2001b, 2003, 2008). Now we examined their abilities to compete with *S. mutans* strains for colonization of rats' teeth. When the genome sequence of Challis CH1 was determined, we constructed strain KS1 with a chromosomally integrated kanamycin resistance gene for selection in animal model studies to verify and extend our initial experiments. Use of strains derived from strain KS1 minimized the possibility that previous observations were influenced by pleiotropic effects of the undefined mutation(s) that conferred streptomycin resistance in strain Challis-S (Appendix 2).

Figs. 2A-2C show colonization by *S. gordonii* strains Challis-S, its *abpA*-defective mutant Challis-ST, and *S. mutans* 10449S, inoculated in equal doses into sucrose-eating rats, either singly or simultaneously (Fig. 1A), and respective caries scores. Molars became heavily colonized (Figs. 2A, 2B) by *S. mutans* or *S. gordonii* strains if inoculated singly, and the Challis-ST mutant tended to establish in slightly higher numbers ($p = 0.116$) than its parent, as previously reported (Tanzer *et al.*, 2003). But when *S. mutans* 10449S was simultaneously inoculated with either *S. gordonii* strain, it greatly outnumbered them in absolute (Fig. 2A) counts recovered ($p < 0.001$). As a percentage of recoverable flora (Fig. 2B), both of these species dominated the flora, but when inoculated simultaneously, *S. mutans* dominated, while *S. gordonii* virtually disappeared ($p < 0.001$). Caries scores (Fig. 2C) reflected colonization status: high *S. mutans*, with high scores (all comparisons, $p < 0.001$), while high *S. gordonii* only slightly augmented scores of un-inoculated rats attributable to their indigenous oral flora ($p < 0.02$). There was no evidence that *S. gordonii* co-colonization inhibited *S. mutans* 10449S cariogenicity.

We then hypothesized that prior colonization of teeth by *S. gordonii* would prevent their displacement by subsequent *S. mutans* inoculation, and *vice versa*. Use of the sequential inoculation protocol (Fig. 1B) and the same inoculants (Figs. 2D-2F) showed that both *S. mutans* and *S. gordonii* were very numerous on molars if they were inoculated singly. They were less numerous if either was inoculated at day 10, but at this time the *abpA* mutant was established in higher numbers than its progenitor (all, $p < 0.001$). By contrast, when initial *S. gordonii* inoculation was followed at day 10 by 10449S inoculation, the 10449S displaced it, on absolute (Fig. 2D) and percentage (Fig. 2E) bases ($p < 0.001$). If rats had been first inoculated by *S. mutans* 10449S, and 10 days later by either *S. gordonii* strain, *S. mutans* was not displaced ($p < 0.001$), and *S. gordonii* was established poorly. The percentage of total recoverable flora represented by the sum of *S. mutans* and *S. gordonii* was very high and virtually the same for each inoculated group. *S. gordonii* strains caused minor augmentation of caries scores (Fig. 2F) ($p < 0.05$), while *S. mutans* caused great augmentation ($p < 0.001$). Inoculation by *S. mutans* at day

10 resulted in slightly lower caries scores than inoculation at day 0 ($p < 0.001$), probably reflecting reduced *S. mutans* challenge-time. There was neither inhibition nor augmentation of cariogenicity of *S. mutans* by prior colonization by *S. gordonii*.

The cariogenic and colonization superiority of *S. mutans* was hypothesized to depend on its well-known production of a variety of extracellular glucans uniquely from sucrose, its potent acidogenesis from sucrose, and other less-defined sucrose-supported functions. To test this, we repeated the simultaneous inoculation protocol using a sucrose-free diet, substituting powdered cornstarch (Appendix 2) for powdered sucrose. For this experiment, we used parental strain *S. gordonii* KS1 and its isogenic *abpA*-deficient mutant (Appendix 2). While KS1 colonized better ($p < 0.001$) than KS1*abpA* and *S. mutans* 10449S on both absolute and relative count bases (Figs. 3A, 3B), all inoculants were recovered in high numbers and percentages from teeth. However, neither *S. gordonii* strain displaced 10449S or became dominant in its presence ($p < 0.001$), although KS1*abpA* colonized better than its KS1 parent ($p < 0.001$). *S. mutans* 10449S was recovered in higher numbers and percentages from rats also inoculated by KS1*abpA* rather than KS1 ($p < 0.001$), suggesting an interactive effect associated with loss of *S. gordonii* AbpA expression. Importantly, the competitive advantage of *S. mutans* appeared independent of sucrose.

The caries scores (Fig. 3C) were very low, as expected in rats eating a high-cornstarch/sucrose-free diet (Tanzer *et al.*, 2003). While total lesion scores were statistically higher for the *S. mutans*-inoculated groups than for either un-inoculated or *S. gordonii*-inoculated groups ($p < 0.05$), they were low by comparison with those observed in experiments with a high-sucrose diet.

While results indicating superiority of *S. mutans* in competition with *S. gordonii* seemed convincing, they could have been influenced by complex interactive effects of AbpA with the Gtfs of both/either *S. gordonii* and/or *S. mutans*, viz. with GtfG and GtfB, or by pleiotropic effects resulting from undefined antibiotic resistance mutations. It was also possible that *S. mutans* strain 10449S might have unusual potency in competition with *S. gordonii*. Therefore, the simultaneous inoculation protocol using *gtfG*- and *abpA*-mutants of *S. gordonii* strain KS1 (Appendix 2), their progenitors, and a different *S. mutans* strain (LT11) was performed in rats fed the high-sucrose diet. Again, single inoculants became very numerous on teeth, but if any *S. gordonii* strain had been simultaneously inoculated with *S. mutans* LT11, only the latter was found in very high numbers and percentages on molars (Figs. 3D, 3E) (all, $p < 0.001$). Also, KS1*gtfG* colonized less well than its progenitor (absolute, $p = 0.027$; percent, $p < 0.001$). The percentage of total recoverable flora constituted by LT11 plus KS1*gtfG**abpA* double-mutant was lower than for all other doubly inoculated rat groups ($p < 0.001$), suggesting complex *in vivo* interactive effects between AbpA and GtfG of *S. gordonii* and/or GtfB of *S. mutans*, as previously reported *in vitro* (Chaudhuri *et al.*, 2007). Nonetheless, in doubly inoculated rats, *S. mutans* LT11 vastly predominated on the surfaces of the teeth. The previously observed superior competitiveness of *S. mutans* strain 10449S, thus, did not appear either unique or attributable to pleiotropic effects.

The caries scores (Fig. 3F) reflected the *S. mutans* LT11 colonization of the rats ($p < 0.001$); *S. gordonii* strains, again,

Figure 3. Recoveries of inoculants from rats' teeth and associated caries lesion scores 42 days after simultaneous inoculation of rats eating sucrose-free diet (panels A, B, C) or eating a high-sucrose diet (panels D, E, F), respectively. Inocula were *S. mutans* and/or *S. gordonii* wild-types and mutants, as stipulated below. There were 10 rats in each group. The Y axes of panels A and D depict absolute count recoveries of inoculants from 3 molar teeth of a hemi-mandible as mean $\pm$ SEM $\times 10^7$ CFUs. The Y axes of panels B and E depict those recoveries as mean $\pm$ SEM percent of total recoverable flora. For these bacteriological data (panels A, B, D, and E), various shaded bars of the histograms and the corresponding keys at the tops of these panels identify the recoverants. The Y axes of panels C and F depict mean $\pm$ SEM caries lesion scores for coronal tooth surface categories that are identified by bar shades above those panels. Caries lesions were assessed on the 3 molar teeth of the contralateral hemi-mandible for each rat. Panels A, B, and C describe the results of a simultaneous inoculation protocol experiment in rats eating a sucrose-free, high-cornstarch diet. All inoculations occurred at experimental day 0. To simplify the strain designations, KS1abpA was substituted for KS1 Ω abpA properly found in the Table of Bacterial Strains used (see Appendix 2). Inoculant groups are designated numerically on the X axis, as:

- 1 – uninoculated
 - 2 – *S. mutans* 10449S
 - 3 – *S. gordonii* KS1
 - 4 – *S. mutans* 10449S plus *S. gordonii* KS1
 - 5 – *S. gordonii* KS1abpA
 - 6 – *S. mutans* 10449S plus *S. gordonii* KS1abpA
- Panels D, E, and F present the data for an experiment using the simultaneous inoculation protocol in rats eating the high-sucrose diet. To simplify strain designations, KS1gtgG was substituted for KS1 Δ gtgG, KS1abpA was substituted for KS1 Ω abpA, and KS1gtgG/abpA was substituted for KS1 Δ gtgG/ Ω abpA, as shown in the Table of Bacterial Strains used (see Appendix 2). Thus, inoculant groups are designated numerically on the X axis, as:
- 1 – Uninoculated
 - 2 – *S. mutans* LT11
 - 3 – *S. gordonii* KS1
 - 4 – *S. gordonii* KS1gtgG
 - 5 – *S. gordonii* KS1abpA
 - 6 – *S. gordonii* KS1abpAgtgG
 - 7 – *S. mutans* LT11 plus *S. gordonii* KS1
 - 8 – *S. mutans* LT11 plus *S. gordonii* KS1gtgG
 - 9 – *S. mutans* LT11 plus *S. gordonii* KS1abpA
 - 10 – *S. mutans* LT11 plus *S. gordonii* KS1abpAgtgG

Figure 4. Recoveries of inoculants from rats' teeth 42 days after simultaneous (panels A and B) inoculations. Inocula were *S. mutans* and/or *S. gordonii* wild-types and mutants, as stipulated below. In all inoculant groups, there were 10 rats. Rats ate the sucrose-free diet. The Y axis of panel A depicts absolute count recoveries of inoculants from 3 molar teeth of a hemi-mandible as mean $\pm$ SEM $\times 10^7$ CFUs. The Y axis of panels B depicts those recoveries as mean $\pm$ SEM percent of total recoverable flora. The variously shaded bars of the histograms and the corresponding keys at the tops of the panels identify the recoverants. To simplify the X axis labels, we substituted the designation KS1gtfG for KS1 Δ gtfG, KS1abpA was substituted for KS1 Δ abpA, and KS1gtfG/abpA was substituted for KS1 Δ gtfG/ Δ abpA, as shown in the Table of Bacterial Strains used (see Appendix 2). Inoculant groups are designated numerically on the X axis, as:

- 1 – uninoculated
- 2 – *S. mutans* 10449S
- 3 – *S. gordonii* KS1
- 4 – *S. gordonii* KS1gtfG
- 5 – *S. gordonii* KS1abpA
- 6 – *S. gordonii* KS1gtfGabpA
- 7 – *S. mutans* 10449S plus *S. gordonii* KS1
- 8 – *S. mutans* 10449S plus *S. gordonii* KS1gtfG
- 9 – *S. mutans* 10449S plus *S. gordonii* KS1abpA
- 10 – *S. mutans* 10449S plus *S. gordonii* KS1abpAgtfG

Caries lesion scores were very low and are not presented here.

caused little or no augmentation of caries scores ($p > 0.05$). Double inoculation by *S. mutans* LT11 plus KS1 or LT11 plus KS1abpA resulted in increased total caries score (both, $p < 0.005$), but double inoculation by LT11 plus either KS1gtfG or KS1gtfGabpA did not.

Finally, to confirm that the advantage of *S. mutans* over *S. gordonii* was not sucrose-dependent, using KS1 and its site-specific constructs, KS1gtfG and KS1gtfGabpA, we carried out the simultaneous inoculation protocol using the cornstarch, sugar-free diet in which extracellular glucan synthesis was impossible. All singly inoculated strains heavily colonized the molar teeth. When any of the KS1 strains were simultaneously inoculated with *S. mutans* 10449S, the latter dominated the numbers and percentages on teeth (all comparisons, $p < 0.002$) (Figs. 4A, 4B). KS1 and its gtfG and abpA deletion mutants colonized comparably ($p > 0.05$). However, the double-mutant KS1gtfGabpA colonized less well than KS1 ($p = 0.027$) on an absolute count basis (Fig. 4A). The percentage of total recoverable flora represented by the sum of *S. mutans* and *S. gordonii* ranged from 70 to 100%. Only the combined inoculum of

S. mutans 10449S + *S. gordonii* KS1gtfG evidenced any decreased ability to colonize (Fig. 4B) ($p < 0.001$). Remarkably, the *S. mutans* strain 10449S out-competed all *S. gordonii* site-specific constructs in rats fed the sugar-free, high-cornstarch diet. The caries scores were minimal, as expected for this sucrose-free diet (data not shown).

DISCUSSION

We addressed the relationships *in vivo* of 2 plaque biofilm streptococci, *S. mutans* and *S. gordonii*, as a function of the presence/absence of dietary sucrose, and explored possible pre-emption or displacement of *S. mutans* on teeth through bacterial interference. A well-defined rat dental caries model was used, because much literature shows its relevance to predicting behavior of oral flora on human teeth and predicting results of caries-preventive intercessions (Tanzer, 1986), and because healthy mutans-free and *S. gordonii*-free rats maintained in our barrier-breeding facility provided “clean” control un-colonized rats, harboring normal rodent gut flora. We know of no human populations free of *S. gordonii* or other amylase-binding oral streptococci and mutans streptococci that could serve as study subjects, were one to contemplate such studies.

This report describes studies that revealed consistent patterns: (A) No strain of *S. gordonii* (either wild-type or mutants deficient in gtfG, abpA, or gtfGabpA) could compete with *S. mutans* for colonization in the plaque biofilm; (B) failure to compete is evident when these bacteria are inoculated in very large, equal numbers, either simultaneously or sequentially, after a 10-day period allowed for their establishment; (C) superiority of *S. mutans*' colonization ability is not unique to strain 10449S; it is also apparent for *S. mutans* strain LT11; and (D) the superiority of *S. mutans* colonization is independent of whether rats are fed a high-sucrose diet or an otherwise identical sucrose-free, high-starch diet. The effect, therefore, cannot be attributed to the production of extracellular glucans (Tanzer et al., 1974; Kuramitsu, 1993; LeBlanc, 1994) by *S. mutans* or to other sucrose-dependent phenomena. It must have another, as-yet-undefined, basis(es).

In the presence of a sucrose diet, *S. mutans* colonization induces high caries lesion scores for all categories of surfaces of the crowns of teeth. Induction of lesions by *S. gordonii* wt and various mutants, when inoculated singly, is modest, and sometimes lesion scores are not significantly greater than those for un-inoculated control rats. In the presence of a starch diet, caries lesion induction associated with inoculation by either *S. mutans* or *S. gordonii* strains is low: for *S. mutans*, statistically significant, but biologically unimpressive; for *S. gordonii*, virtually none, consistent with previous observations (Tanzer, 1979; Tanzer et al., 2001a, 2003).

A recent study using an *in vitro* biofilm model indicated that hydrogen peroxide produced by *S. gordonii* inhibited *S. mutans* growth, suggesting that it should inhibit colonization of *in vivo* dental plaque biofilm by *S. mutans* (Kreth et al., 2008). However, the present *in vivo* plaque biofilm studies in rats with normal salivary secretion showed no inhibition of *S. mutans* by *S. gordonii*, consistent with previous literature on human dental plaque (Krasse et al., 1968; de Stoppelaar et al., 1969; Folke et al., 1972; Loesche and Syed, 1973; Loesche et al., 1975; Staat et al., 1975; van Houte and Duchin, 1975; Swenson et al., 1976; Emilson and

Westergren, 1979; Tanzer *et al.*, 2001a). Furthermore, previous reports of high peroxidase levels in saliva of rats and humans (Nickerson *et al.*, 1957; Morioka *et al.*, 1969; Ihalin *et al.*, 2006) also suggest the impotence of *S. gordonii*-produced peroxide to inhibit *S. mutans*. Recent observations on advanced caries lesions in young human teeth, using bacterial 16S sequence analysis methods, are consistent with the present data, and indicate that *S. gordonii* diminishes greatly in caries-associated plaque biofilm, while *S. mutans* persists (Gross *et al.*, 2010).

In summary, *S. mutans* out-competes *S. gordonii* on teeth, independent of host sucrose diet, sequence of inoculation, strain of *S. mutans*, and the presence or absence of mutations of *abpA* and *gtfG* genes of *S. gordonii* implicated in its colonization and interactions with *S. mutans* glucans. The mechanisms that explain these observations are yet unknown. *S. gordonii* does not appear to be a good candidate for replacement therapy against caries, even though, by itself, it is an excellent colonizer of the teeth.

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