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Streptococcus gordonii's sequenced strain CH1 glucosyltransferase determines persistent but not initial colonization of teeth of rats

J.M. Tanzer[a,*], A.M. Thompson[a,1], L.P. Grant[a,1], M.M. Vickerman[b], F.A. Scannapieco[b]

[a] University of Connecticut Health Center, Farmington, CT, USA
[b] Department of Oral Biology, School of Dentistry, University of Buffalo, Buffalo, NY 04214, USA

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ABSTRACT

Objective: Extracellular glucan synthesis from sucrose by *Streptococcus gordonii*, a major dental plaque biofilm bacterium, is assumed important for colonization of teeth; but this hypothesis is un-tested *in vivo*.

Methods: To do so, we studied an isogenic glucosyltransferase (Gtf)-negative mutant (strain AMS12, *gtf*G^{-}) of *S. gordonii* sequenced wild type (WT, strain Challis CH1, *gtf*G^{+}), comparing their *in vitro* abilities to grow in the presence of glucose and sucrose and, *in vivo*, to colonize and persist on teeth and induce caries in rats. Weanling rats of two breeding colonies, TAN:SPFOM(OM)BR and TAN:SPFOM(OMASF)BR, eating high sucrose diet, were inoculated with either the WT (*gtf*G^{+}), its isogenic *gtf*G^{-} mutant, or reference strains of *Streptococcus mutans*. Control animals were not inoculated.

Results: *In vitro*, the *gtf*G^{-} strain grew at least as rapidly in the presence of sucrose as its WT *gtf*G^{+} progenitor, but formed soft colonies on sucrose agar, consistent with its lack of insoluble glucan synthesis. It also had a higher growth yield due apparently to its inability to channel carbon flow into extracellular glucan. *In vivo*, the *gtf*G^{-} mutant initially colonized as did the WT but, unlike the WT, failed to persist on the teeth as shown over time. By comparison to three *S. mutans* strains, *S. gordonii* WT, despite its comparable ecological success on the teeth, was associated with only modest caries induction. Failure of the *gtf*G^{-} mutant to persistently colonize was associated with slight diminution of caries scores by comparison with its *gtf*G^{+} WT.

Conclusions: Initial *S. gordonii* colonization does not depend on Gtf-G synthesis; rather, Gtf-G production determines *S. gordonii*'s ability to persist on the teeth of sucrose-fed rats. *S. gordonii* appears weakly cariogenic by comparison with *S. mutans* reference strains.

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* *Corresponding author at*: Division of Oral Medicine, Department of Oral Health and Diagnostic Science, University of Connecticut School of Dental Medicine, University Connecticut Health Center, Farmington, CT 06030-1605, USA. Tel.: +1 860 679 2855; fax: +1 860 679 4760.
E-mail addresses: tanzer@nso.uchc.edu (J.M. Tanzer), AnThompson@nso2.uchc.edu (A.M. Thompson), sybilcreek@yahoo.com (L.P. Grant), mmv4@buffalo.edu (M.M. Vickerman), fas1@acsu.buffalo.edu (F.A. Scannapieco).
[1] Present address: Department of Oral Health and Diagnostic Science, University of Connecticut School of Dental Medicine, University Connecticut Health Center, Farmington, CT 06030-1605, USA.
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1. Introduction

Streptococcus gordonii, a species now officially distinguished taxonomically from *Streptococcus sanguis*,[1,2] binds α-amylase and is one of the "pioneer" species numerically abundant on teeth of salivary α-amylase-secreting hosts only, including humans and rats.[3–5] Extracellular glucan synthesis from sucrose by glucosyltransferase (Gtf) has long been associated with the ability of the formerly termed sanguis streptococci to colonize the teeth.[6–8] This belief probably grew from findings that had previously established an analogous relationship with regard to the extracellular glucans synthesized by mutans streptococci.[9–11]

The single *S. gordonii* Gtf enzyme, whose gene is designated *gtf*G,[12] makes both α-1,6- and α-1,3-linked extracellular glucans from sucrose,[13–16] analogous to the glucans produced by the mutans streptococci. Also, the mutans streptococci and *S. gordonii* Gtf enzymes share chemical structure characteristics of sucrose hydrolysis and glucan binding domains. The glucans produced by the *S. gordonii* Gtf and the glucan binding capabilities of the enzyme itself have been postulated to play roles in oral biofilm formation.[17] Although a role of extracellular glucans in surface colonization has been demonstrated *in vitro* based on the ability of glucan-synthesizing *S. gordonii* to accumulate on saliva-coated hydroxyapatite beads in the presence of sucrose,[18–20] this has never been directly assessed *in vivo*.

Other factors have been demonstrated to influence tooth surface colonization by *S. gordonii*: *viz.* its colonization of rats is complexly influenced by two cell-surface α-amylase binding proteins in a sucrose-independent but sucrose-augmented fashion.[21] Still other cell-surface proteins have been implicated in sucrose-independent colonization, based mostly on data from binding to saliva-coated hydroxyapatite,[22–24] and a very short-term *in vivo* study that could not have discriminated between oral mucosal and tooth surface colonization in mice eating a sucrose-free as-well-as a possibly sucrose-contaminated (molasses) diet, using mutants with defects of cell-surface-associated polypeptides CshA and CshB.[25]

The present study addresses the role of Gtf-G in colonization of the teeth of rats by use of a well-characterized, sequenced *S. gordonii* strain[26] and its isogenic mutant that is deficient in the enzyme Gtf-G that, thus, cannot synthesize extracellular glucan.[27] It therefore tests the hypothesis that Gtf-G is seminal to sucrose-associated colonization and persistence in the biofilm on the teeth. It simultaneously seeks to assess whether the production of glucans by *S. gordonii* influences their cariogenicity.

2. Materials and methods

2.1. Bacterial strains and growth studies

S. gordonii strains Challis CH1 and its isogenic mutant AMS12[27] were studied. Strain AMS12 was constructed by allelic exchange in which an internal 1.7-kb HindIII fragment of the structural gene, *gtf*G, was replaced with a lacZ/erythromycin resistance determinant. The resulting gene encodes a truncated ∼560-amino acid protein with no Gtf activity.[12]

Strain phenotypes were confirmed qualitatively [API 20 Strep, bioMérieux, Marcy l'Etoile, France] and growth rates were measured in chemically defined (protein-free) FMC[21,28] medium to seek any undetected phenotypic changes as well as expected behaviours. Because of the tendency of extracellular glucan-synthesizing bacteria to clump in the presence of sucrose, thus making growth analysis by turbidimetric methods problematic, growth rates were assessed by analysis of bacterial protein.[29,30] For some *in vivo* studies, comparisons with *Streptococcus mutans* strains BM71,[31] NCTC-10449S[32] and LT11[33] were also made. All cultures were maintained at −70 °C before study. After recovery from rats, colonies resembling inoculants by their phenotypes on appropriate agar media were re-identified by API 20 strips and, furthermore, chromosomal DNA isolated from the recovered strains using standard molecular biology techniques gave similar restriction fragment length profiles; DNA probes designed to anneal to the chromosomal regions flanking *gtf*G hybridized to the expected sized fragments in Southern blot analyses.[34]

2.2. Experimental design, animals, diet, inoculation, recovery of bacteria

Three *in vivo* experiments were done. Their designs are depicted in Fig. 1A–C. They were approved by the Institutional Animal Care and Use Committee of the fully accredited Center for Laboratory Animal Care, University of Connecticut Health Center. Procedures were slight modifications of those previously detailed.[32,35] Specifically, weanling rats of two different specific pathogen free Osborne-Mendel (SPFOM) rat colonies, designated TAN:SPFOM(OM)BR and TAN:SPFOM(OMASF)BR, were studied. Importantly, both colonies have an indigenous flora free of α-amylase-binding bacteria, as tested by ^{125}I-amylase binding and fluorescent antibody to amylase-binding protein assays, and also free of mutans streptococci, as demonstrated by repeated culturing of the dentition of progeny after provision of a sucrose-rich diet.[21,36] The TAN:SPFOM(OMASF)BR colony was derived by Caesarean delivery of pups of TAN:SPFOM(OM)BR females; these pups were foster-fed by germ-free rats and subsequently inoculated by the so-called altered Schaedler flora (ASF)[37] typical of rodent gut.[38] The resultant gnotobiotic breeding colony was expanded in the gnotobiotic facility, then housed in our strict barrier facility and further expanded to enable experiments.[37]

For those experiments, weanling 21-day-old rats of the TAN:SPFOM(OM)BR colony were randomly distributed to groups of equal size, as stipulated for each experiment, and fed diet 2000, containing 56% sucrose[21,36] and, 1 day later, inoculated with either CH1 or AMS12. As a positive control *S. mutans* BM71 was used to inoculate an additional group and as a negative control one group was not inoculated (Fig. 1A). Inocula were of equal doses (∼10^9 CFU), as confirmed by counting of CFU after spiral plating. To be assured that the more complex oral flora of rat colony TAN:SPFOM(OM)BR, by comparison with the TAN:SPFOM(OMASF)BR colony[52] was not interfering with the colonization of the inoculants or the development of carious lesions, two experiments using the latter colony of rats were also done. Again, one group remained un-inoculated. The design of one of them also included simultaneous study of three strains of known virulent *S. mutans*, BM71, 10449S and LT11, as

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