Amylase-binding proteins A (AbpA) and B (AbpB) differentially affect colonization of rats' teeth by *Streptococcus gordonii*

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*S. gordonii* produces two α-amylase-binding proteins, AbpA and AbpB, that have been extensively studied *in vitro*. Little is known, however, about their significance in oral colonization and cariogenicity (virulence). To clarify these issues, weanling specific pathogen-free Osborne-Mendel rats, TAN : SPFOM(OM)BR, were inoculated either with wild-type strains FAS4-S or Challis-S or with strains having isogenic mutations of *abpA*, *abpB*, or both, to compare their colonization abilities and persistence on the teeth. Experiments were done with rats fed a sucrose-rich diet containing low amounts of starch or containing only starch. The mutants and wild-types were quantified *in vivo* and carious lesions were scored. In 11 experiments, *S. gordonii* was a prolific colonizer of the teeth when rats were fed the sucrose (with low starch)-supplemented diet, often dominating the flora. Sucrose-fed rats had several-fold higher recoveries of inoculants than those eating the sucrose-free, starch-supplemented diet, regardless of inoculant type. The strain defective in AbpB could not colonize teeth of starch-only-eating rats, but could colonize rats if sucrose was added to the diet. Strains defective in AbpA surprisingly colonized better than their wild-types. A double mutant deficient in both AbpA and AbpB (*abpA*/*abpB*) colonized like its wild-type. Wild-types FAS4-S and Challis-S had no more than marginal cariogenicity. Notably, in the absence of AbpA, cariogenicity was slightly augmented. Both the rescue of colonization by the AbpB− mutant and the augmentation of colonization by AbpA− mutant in the presence of dietary sucrose suggested additional amylase-binding protein interactions relevant to colonization. Glucosyltransferase activity was greater in mutants defective in *abpA* and modestly increased in the *abpB* mutant. It was concluded that AbpB is required for colonization of teeth of starch-eating rats and its deletion is partially masked if rats eat a sucrose-starch diet. AbpA appears to inhibit colonization of the plaque biofilm *in vivo*. This unexpected effect *in vivo* may be associated with interaction of AbpA with glucosyltransferase or with other colonization factors of these cells. These data illustrate that the complex nature of the oral environment may not be adequately modelled by *in vitro* systems.

**INTRODUCTION**

Amylase, the most abundant enzyme in saliva, binds specifically and with high affinity to amylase-binding streptococci (ABS). They are early colonizers of the tooth and numerous in human supragingival dental plaque biofilm (Scannapieco *et al.*, 1994; Tseng *et al.*, 1992; Tanzer *et al.*, 2001b). It has been speculated that ABS such as *Streptococcus gordonii*, abundant on the teeth of humans, make a significant contribution to the process of dental caries (Scannapieco *et al.*, 1993). However, a recent comprehensive review of the literature on human dental caries suggests, if anything, an inverse relationship of *S. gordonii* colonization with caries prevalence and incidence (Tanzer *et al.*, 2001b). Studies in rats initially free of ABS fed a high sucrose diet suggest that some *S. gordonii* may be modestly cariogenic (Tanzer *et al.*, 2001a). ABS, especially *S. gordonii*, are being considered as delivery vehicles for mucosal vaccines (Bolken *et al.*, 2002; Oggioni *et al.*, 1999; Pozzi *et al.*, 1992, 1994; Sharma *et al.*, 1999). It is thus of

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Abbreviation: ABS, amylase-binding streptococci.
value to understand the conditions for their colonization of the mouth and their cariological significance.

Only animals that secrete significant $\alpha$-amylase in their saliva are colonized by ABS, thus suggesting the importance of amylase-binding in oral colonization by these bacteria (Scannapieco et al., 1994). Salivary amylase retains its enzymic activity while bound to ABS and may facilitate dietary bacterial starch utilization (Douglas, 1990; Rogers et al., 2001; Scannapieco et al., 1990). Amylase binds to high affinity receptors on S. gordonii that cluster around surface cell division sites (Scannapieco et al., 1992). Bacterial cells in exponential growth bind more amylase than stationary-phase ones (Rogers et al., 2001; Scannapieco et al., 1992). Amylase-binding components of 20 and 82 kDa are released into culture liquors of S. gordonii strains (Douglas, 1990; Gwynn & Douglas, 1994). These findings suggest a multi-component, cell-surface-associated amylase receptor that is released into the in vitro milieu as a culture matures or, it may be speculated, into the fluids of the oral cavity as growth progresses in situ in the dental plaque biofilm.

Recently, the genes (abpA and abpB) encoding these two amylase-binding proteins have been identified, cloned and sequenced (Rogers et al., 1998, 2001; Brown et al., 1999; Li et al., 2002). They are recognized in the recent draft publication of the genome of S. gordonii (http://www.tigr.org). Except for preliminary reports (Tzaner et al., 2002a, b), no data have been published, however, on the role of the amylase-binding proteins in colonization in vivo or on caries. This paper clarifies the impact of single and double mutations of abpA and abpB on the colonization of rats’ teeth by S. gordonii, on competition for the tooth surface and on dental caries.

**METHODS**

**Micro-organisms.** The strains used in the present study and their relevant phenotypes are listed in Table 1. The constructions of the mutants have been detailed previously (Rogers et al., 1998, 2001; Brown et al., 1999; Li et al., 2002). All strains were maintained at $-70$°C in 15% glycerol-supplemented fluid thiglycollate medium (Difco) supplemented with meat extract and excess CaCO$_3$ (TMM). After subculture, purity and phenotype were confirmed. As an additional screen for undetected phenotypic changes other than those desired, comparisons of growth rates in chemically defined medium [FMC (Terlecki et al., 1975) supplemented with 2% glucose] were made for strains Challis-S, Challis-ST, Challis-SE and Challis-STE at 37°C using a Bausch and Lomb Spectronic 21 spectrophotometer with 1.5 cm light path. Additionally, bacterial proteins were extracted from the cell surface with SDS and culture supernatants were evaluated by SDS-PAGE stained with Coomassie brilliant blue, and by Western blots probed with antiserum to S. gordonii to seek any changes of protein profiles other than expected ones (Rogers et al., 1998; Li et al., 2002).

**Experimental animals, diets, inocula and study designs.** The experimental animal procedures used in this study are slight modifications of those detailed previously (Tanzner, 1979; Tanzner et al., 1985a, b, 2001a). Importantly, the colony of TAN: SPFOM(OM) BR rats has an indigenous flora free of $\alpha$-amylase-binding bacteria, as tested by an $^{125}$I-amylase binding assay and radioautography (Tseng et al., 1992; Tanzer et al., 2001a). These rats, like others (Chauncey et al., 1963), secrete abundant salivary $\alpha$-amylase and they are readily colonized upon inoculation by wild-type S. gordonii when the rats eat either a sucrose-rich/starch-containing diet (diet 2000) or one that is free of sucrose but starch-rich (diet 2000CS). The rat colony is also free of the potentially cariogenic mutants streptococci, as demonstrated during 32 years of experiments by repeated culturing of the dentition of progeny after eating a sucrose-rich diet. Experiments detailed herein were approved by the Institutional Animal Care and Use Committee of the University of Connecticut Health Center.

Inocula were prepared by growth to exponential phase in TMM and adjusted to an OD$_{600}$ (1 cm light path) of 2.0 in that medium. Each rat was orally inoculated with 200 μl culture (approximately 10$^3$ cells) at 22 days old, 1 day after weaning and provision of either of two test diets. One group on each test diet remained uninoculated. Diet 2000 contains 56% confectioners’ sugar (97% powdered sucrose/3% powdered cornstarch); diet 2000CS contains 56% cornstarch in lieu of confectioners’ sugar. These diets were consumed, as was sterile demineralized water, ad libitum, for the duration of the experiments.

A total of 11 experiments were performed. The experiments compared colonization and persistence of wild-type S. gordonii (FAS4-S or Challis-S) with their respective abpA mutants, termed FAS4-ST and Challis-ST (Rogers et al., 2001; Li et al., 2002). An abpB mutant (Challis-SE) was constructed from the more transformable strain, Challis-S, as was a double mutant (Challis-STE) defective in both abpA and abpB (Li et al., 2002). We shall detail those two experiments that are the most informative and complete with respect to the Challis-S mutants.

In one experiment (Fig. 1) 10 groups were studied simultaneously. Five groups of rats were fed diet 2000 while another five groups were fed diet 2000CS. All animals originated from several litters born on the same day. There were 10 randomly assigned rats per group. One group consuming each of the diets remained uninoculated. After 1 day of consumption of test diets and demineralized water, the other groups were inoculated with either Challis-S or one of the following of its mutants: abpA mutant Challis-ST, abpB mutant Challis-SE or abpA/abpB double mutant Challis-STE.

In another experiment (Fig. 2) 8 groups of rats were similarly studied while eating either the sucrose-rich or starch-rich diets. The weanling rats were either uninoculated or inoculated with either the wild-type Challis-S, its abpA mutant Challis-ST or equally and simultaneously inoculated with both the wild-type and its abpA mutant, thus enabling the study of competition between this pair.

During these experiments, on either day 22 or 21 after inoculation, rats’ teeth were swabbed for recovery of the inoculants and total flora. On

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*Em, erythromycin; St, streptomycin; Tc, tetracycline. Strains were constructed as described in Rogers et al. (2001) and Li et al. (2002).
either day 42 or 41 after inoculation, respectively, animals were euthanized and the molar teeth of one hemi-mandible were excised en bloc, sonified to dislodge and disperse their floras and cultured as detailed below. The methods used prior to plating were described previously (Tanzer et al., 1985a, b, 2001a).

Recovery of inoculants. In all experiments samples were spiral plated onto the following non-selective and selective agars: Trypticase soy sheep blood agar (BA, for total recoverable flora); *mitis salivarius* agar (MS, for total streptococci, including possible cross/extraneous contaminants and revertants); MS supplemented with 200 μg streptomycin sulfate ml⁻¹ (MSS, for FAS4-ST and Challis-S, as well as FAS4-ST and Challis-ST); MS supplemented with both 200 μg streptomycin sulfate ml⁻¹ and 10 μg tetracycline hydrochloride ml⁻¹ (MSST, for FAS4-ST and Challis-ST); MS with 10 μg erythromycin ml⁻¹ (MSE, for Challis-SE); and MS supplemented with all three of the antibiotics (MSSTE, for Challis-STE). Recoveries were expressed as both absolute c.f.u. values and as relative values, the percentage of total recoverable c.f.u. on BA that was the inoculant, on extracted teeth. Data could be expressed only in relative terms from the swab samples of the teeth, due to the variable size of such samples. In doubly inoculated animals, Challis-S c.f.u. recoveries were computed as equal to (CFU_MSS–CFU_MSSST).

Carious lesion scoring. Forty-two or 41 days after inoculation, in the two experiments, animals were euthanized by anaesthetic overdose. Specimens were prepared, randomly coded and blindly scored (Tanzer, 1979; Tanzer et al., 2001a). Only after scoring was completed and raw data entered into the laboratory computer were the random number codes broken.

Statistical analyses. Bacteriological as well as caries score data were statistically analysed as described previously (Tanzer, 1979; Tanzer et al., 2001a) by ANOVA. Statistically significant differences among the group means were isolated using the Fisher LSD procedure (Snedecor & Cochran, 1967; SPSS version 10.1, Chicago, IL, USA). Bacterial recoveries, expressed as percentages, were arcsine-transformed to improve normalcy of distribution before parametric analysis. c.f.u. numbers and caries scores were analysed without transformation. To compute growth rates of cultures and possible differences among them, equations were fitted, slopes computed and differences among them, equations were fitted, slopes computed and transformed to improve normalcy of distribution before parametric analysis. c.f.u. numbers and caries scores were analysed without transformation. To compute growth rates of cultures and possible differences among them, equations were fitted, slopes computed and transformed to improve normalcy of distribution before parametric analysis.

Glucosyltransferase activity of wild-type and mutants. GluG activity of wild-type and *abpA, abpB* and *abpA*/*abpB* mutants was initially explored. Relative amounts of Glu enzyme activity were determined by measuring enzyme activity in polyacrylamide gels as
described by Vickerman & Minick (2002). Briefly, the wild-type and mutant strains were grown in broth to late exponential phase, as determined by optical density. Equal amounts of cell-free supernatants were run on SDS-PAGE gels. After electrophoresis, gels were incubated overnight in 3% sucrose and the resulting glucan bands were visualized by staining with periodic acid and pararosaniline. The intensity of the stained bands was densitometrically assessed and reflected the relative activities of the Gtf protein.

RESULTS

Growth characteristics of strains and phenotype
Strains Challis-S, Challis-ST, Challis-SE and Challis-STE had generation times that were nearly the same \([\text{OD} = 64 \pm 2 \text{ SEM}(\text{min})]\) and had instantaneous growth rates during exponential phase falling within the 95% confidence intervals of one another. The cultures grew to virtually the same stationary-phase optical density \((1.58 \pm 1.69)\). This suggested that no detectable energy-yielding, anabolic or catabolic changes had occurred among the mutants. No qualitative phenotypic changes were detected, except for the sought-after amylase-binding defects and their associated antibiotic resistance markers, other than increased Gtf activity of Challis-ST, Challis-SE and Challis-STE, as described below. No changes in surface or supernatant protein profiles were detected, other than of the expected AbpA and AbpB.

Colonization and competition studies
A total of 11 in vivo experiments were done. In no case was an uninoculated animal observed to harbour one of the inoculants and in no case was evidence of reversion of mutant to wild-type phenotype noted. In no experiment were the weight gains of the diet 2000-fed rats different from those of rats eating diet 2000CS, within that experiment. This was as expected, because the diets are isocaloric.

In all experiments \(S. gordonii\) strains colonized rats fed a high sucrose/low cornstarch diet better than rats fed the diet containing only cornstarch. At the termination of the experiments, in sonicates of the molar teeth of one hemimandible, \(abpA\) mutants, \(abpA/abpB\) mutants and their respective wild-types colonized the rats’ teeth and were generally recovered in 5–6.5-fold higher numbers when the animals ate the sucrose/starch diet than when they ate the starch-only diet (Fig. 3 and Fig. 4). This directional difference was also observed intra-experimentally in swab samples of the dentition (data not shown).

The \(abpB\) mutant (Challis-SE) failed to colonize the teeth if the animals ate the starch-supplemented diet (Fig. 3). It was recovered intra-experimentally in extremely low percentages from a few rats, but was not detectable in swabs of most animals inoculated by it. However, if the rats ate the sucrose/starch diet, the \(abpB\) mutant did colonize the teeth, albeit at significantly lower levels \((P<0.001)\) than its wild-type on a c.f.u. basis and equal levels \((P=0.866)\) on a relative basis. Clearly, colonization by the \(abpB\) mutant was ‘rescued’ by sucrose supplementation of the diet.

It was unexpected that while the wild-type strains colonized the teeth very well, ranging from 20 to 70% of the total recoverable flora in the series of experiments, when comparisons were made with their \(abpA\) mutants, the latter colonized even better, if the animals ate the sucrose-rich diet (Fig. 3). This difference was statistically significant in 3 of 11 experiments, despite the ability of the wild-type cells to colonize the teeth very well, ranging from 20 to 70% of the total recoverable flora (Fig. 3). It was unexpected that while the wild-type strains colonized the teeth very well, ranging from 20 to 70% of the total recoverable flora in the series of experiments, when comparisons were made with their \(abpA\) mutants, the latter colonized even better, if the animals ate the sucrose-rich diet (Fig. 3). This difference was statistically significant in 3 of 11 experiments, despite the ability of the wild-type cells to colonize the teeth very well, both on a c.f.u. basis and equal levels \((P=0.866)\) on a relative basis. Clearly, colonization by the \(abpB\) mutant was ‘rescued’ by sucrose supplementation of the diet.

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![Fig. 3.]( downloading from www.microbiologyresearch.org by IP: 54.70.40.11 On: Tue, 11 Dec 2018 04:56:56)
In the two experiments in which it was tested, double mutant abpA/abpB (Challis-STE) colonized as well or better than its wild-type progenitor (Challis-S) and better than the abpB mutant (Challis-SE), independent of dietary carbohydrate (Fig. 3).

We sought further evidence of the advantage of abpA mutant Challis-ST versus its wild-type Challis-S using a competition paradigm. We felt that the abpB mutant would not be useful for such experiments, because it could not even compete well with the indigenous flora of the rats. For this study, with rats eating either the sucrose-starch-supplemented diet or the starch-only supplemented diet, weanlings were inoculated with either the wild-type, abpA or, simultaneously and equally, with both strains (Fig. 2). In the cornstarch-only group, the absolute count recoveries of the wild-type and the abpA mutant cells were not different, but the abpA mutant constituted a higher proportion of the total recoverable flora than its wild-type \( (P=0.028) \) (Fig. 4). For the sucrose-fed rats, the absolute recoveries of the wild-type and abpA mutant were not different, but approached significant difference as a percentage of the total recoverable flora \( (P=0.122) \). For the doubly infected rats, there was no difference in the colonization abilities of these two strains, if the rats ate the starch-only diet. By contrast, if animals ate the high sucrose diet, the abpA mutant and wild-type strains not only colonized at much higher levels than in the starch-only group \( (P<0.001) \), but the abpA mutant also competed better for colonization of the teeth than its wild-type on an absolute count basis \( (P<0.001) \) and constituted 85% of all bacteria, compared to 8% for the wild-type \( (P<0.001) \). In aggregate, the wild-type and the AbpA mutant accounted for ~94% of all bacteria on the teeth.

Within any experiment there were either no differences of caries scores between uninoculated rat groups and those inoculated by S. gordonii wild-types FAS4-S or Challis-S or slight ones. If there were statistically significant increases in caries scores, they were biologically unimpressive. However, caries scores were sometimes higher (Fig. 5) in

Fig. 4. Colonization and competition for colonization of weanling rats’ teeth by the designated S. gordonii strain. Diets are shown on the x axis. Absolute counts are presented on the left and percentages of total recoverable flora are on the right. Uninoculated animals were free of Challis or other S. gordonii strains. All animals were free of mutans streptococci. The experimental design and caries scores are depicted in Figs 2 and 6, respectively.

Fig. 5. Bar diagram of hemi-mandibular enamel caries scores as a function of tooth surface category, plotted according to the recommendations of Rinehimer & Tanzer (1980). To the left of the midline, fissure (sulcal, light grey) lesion scores are plotted; to the right of the midline, smooth surface lesion scores are plotted according to the designated morphological categories (buccolingual, dark grey; molar, white; approximal, black). Data represent mean values ± SEM for each experimental group \( (n=10) \). Inoculants are identified on the left. The experimental design and bacterial recoveries are depicted in Figs 1 and 3, respectively.
Because the in vivo S. gordonii deficient evidence of enhanced GtfG activity in AbpA-again, no contrasts among the low scores of the starch-fed colonization by the deficient strain. Evidence of enhanced GtfG activity in AbpA-AbpB-deficient strain (Challis-SE). There was also a suggestion of a modest increase of GtfG activity in the AbpA expression in some way modulates GtfG activity. Preliminary results (Fig. 7) revealed that the relative Gtf activity of AbpA- and AbpA/AbpB-deficient mutants was greater than either that of wild-type (Challis-S) or its AbpB-deficient strain (Challis-SE). There was also a suggestion of a modest increase of GtfG activity in the AbpB-deficient strain.

**DISCUSSION**

We sought to (a) determine the relative contributions made by S. gordonii-specific salivary amylase-binding surface proteins to colonization of the in vivo plaque biofilm on teeth of an animal model that produces abundant salivary amylase and (b) characterize the cariogenicity of S. gordonii strains under conditions maximizing their colonization of the teeth. Our previous work identified two streptococcal proteins that bind salivary amylase. The 20 kDa AbpA appears to be the major protein responsible for amylase binding to the cell surface (Rogers et al., 1998). The 82 kDa AbpB interacts specifically with amylase, but does not appear essential for amylase binding to the cell surface of S. gordonii in vitro (Li et al., 2002). In the present work we tested strains of S. gordonii made deficient in one or both of these proteins in a rat model of human oral colonization.

The ecological consequences of amylase binding to S. gordonii are clearly more complex than initially envisioned. For example, while AbpA-deficient mutants adhere less well to amylase-coated hydroxyapatite and are defective in biofilm formation in vitro (Rogers et al., 2001), they...
colonize the teeth of rats to a greater extent than their parental strains, especially when the animals are fed a sucrose-rich diet. Although this suggests that AbpA fosters net clearance of \textit{S. gordonii} \textit{in vivo}, previous \textit{in vitro} data did not reveal amylase agglutination of any ABS (Scannapieco \textit{et al}., 1995). It is possible that other important colonization factors are unmasked or redistributed on the cell surface in AbpA-deficient strains \textit{in vivo} and may serve to augment bacterial adhesion to the tooth. Other surface components that might be redistributed or up-regulated in the \textit{abpA} mutant to enhance oral colonization include SspA and SspB (Demuth \textit{et al}., 1996), the sialic-acid-binding protein Hsa (Takahashi \textit{et al}., 2002) and CshA (McNab \textit{et al}., 1996).

The present seemingly disparate observations could be explained if one or both of the Abps of \textit{S. gordonii} not only interact with amylase but with other, as yet undefined, salivary or streptococcal surface proteins to either enhance or inhibit oral colonization. Because \textit{S. gordonii} releases both AbpA and AbpB into its \textit{in vitro} milieu, AbpA may serve to block adhesive interactions in the \textit{in vivo} plaque biofilm and promote net clearance of these bacteria from the oral cavity. Because AbpB is required for \textit{S. gordonii} colonization of teeth in rats fed a starch–only diet, it may augment adhesive interactions and retention in the \textit{in vivo} plaque biofilm. In the presence of sucrose, however, the effect of absence of AbpB is masked, perhaps, by the glucosyltransferase activity of these cells that is implicated as a positive colonization factor (Sulavik \textit{et al}., 1992; Vickerman & Jones, 1995; Vickerman & Minick 2002).

BLAST searching of the AbpB protein sequence suggests its relationship to bacterial di-peptidases (Li \textit{et al}., 2002). The absence of this enzyme activity in the \textit{abpB} mutant strain may block an important nutritional pathway \textit{in vivo} that severely compromises the bacterium’s ability to compete with other oral flora in a salivary environment.

The observation that GtfG activity may be greater in AbpA- and AbpA/AbpB-defective cells than in their wild-type or in the corresponding AbpA-defective strain may help to explain the behaviour \textit{in vivo} of AbpA-defective mutants, but this explanation requires more detailed study and must be regarded as speculative at present.

In view of the two seemingly counter-effects of AbpA and AbpB \textit{vis-a-vis} tooth surface colonization, and the additional putative pro-colonization effect of GtfG, it is not surprising that a double mutant, \textit{abpA/abpB}, colonizes at least as well as its wild-type \textit{in vivo}. Perhaps the anti-colonization effect of AbpA and the pro-colonization effect of Gtf in sucrose-fed rats, effectively mitigate the positive colonization effect of AbpB. While this double mutant binds no amylase \textit{in vitro}, and there is no evidence of another, previously cryptic amylase-binding cell-surface determinant, one cannot presently exclude the possible unmasking or up-regulating of other colonization factors in \textit{S. gordonii} by the double deletion of \textit{abpA} and \textit{abpB}.

Consistent with previous data (Tanzer \textit{et al}., 2001a), \textit{S. gordonii} appears to be either a non-cariogen or, at most, a feeble cariogen, despite its striking ability to prominently colonize the surface of teeth of our rats or humans (Scannapieco \textit{et al}., 1993; Tanzer \textit{et al}., 2001b). The present data suggest that deletion of its \textit{abpA} slightly increases its virulence, and that this cariogenicity increase is associated with its augmented ability to colonize the surface of teeth in the \textit{in vivo} plaque biofilm. The increased cariogenicity observed for the AbpA-deficient mutant may be related to dis-inhibition of GtfG activity; increased GtfG activity may contribute to the observed subtly augmented cariogenicity by this strain as compared to its wild-type. Of course, it is only from sucrose that Gtf of oral streptococci synthesize extracellular or cell-surface glucan homopolymers, and abundant evidence associates this sucrose-dependent phenomenon with cariogenicity in experimental animals and humans (Tanzer \textit{et al}., 1974, 2001b; Tanzer 1979). The association of higher, albeit relatively weak by comparison with the \textit{mutans} streptococci, cariogenicity of \textit{S. gordonii} in rats has been reported recently (Tanzer \textit{et al}., 2001a).

In summary, this study demonstrated that strains of \textit{S. gordonii} defective in AbpB could not colonize starch-only eating rats, but could colonize rats if sucrose was added to the diet. By contrast, strains of \textit{S. gordonii} defective in AbpA colonized better than their wild-type, already good colonizers. A double mutant lacking both AbpA and AbpB colonized like its wild-type. Studies are now in progress to determine the molecular bases for these findings. The strains of \textit{S. gordonii} studied are, at most, feeble cariogen in the presence of sucrose. The results illustrate that the complex nature of the \textit{in vivo} plaque biofilm may not be adequately characterized from \textit{in vitro} model data.

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