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Salivary-Agglutinin-Mediated Adherence of *Streptococcus mutans* to Early Plaque Bacteria

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Interspecies binding is important in the colonization of the oral cavity by bacteria. *Streptococcus mutans* can adhere to other plaque bacteria, such as *Streptococcus sanguis* and *Actinomyces viscosus*, and this adherence is enhanced by saliva. The salivary and bacterial molecules that mediate this interaction were investigated. Salivary agglutinin, a mucinlike glycoprotein known to mediate the aggregation of many oral streptococci in vitro, was found to mediate the adherence of *S. mutans* to *S. sanguis* or *A. viscosus*. Adherence of *S. mutans* to saliva- or agglutinin-coated *S. sanguis* and *A. viscosus* was inhibited by antibodies to the bacterial agglutinin receptor. Expression of the *S. sanguis* receptor (SSP-5) gene in *Enterococcus faecalis* increased adhesion of this organism to saliva- or agglutinin-coated *S. sanguis* and *A. viscosus*. This interaction could be inhibited by antibodies to the agglutinin receptor. The results suggest that salivary agglutinin can promote adherence of *S. mutans* to *S. sanguis* and *A. viscosus* through interactions with the agglutinin receptor on *S. mutans*.

The mutans group of streptococci, in particular *Streptococcus mutans* and *Streptococcus sobrinus*, are known etiological agents of dental caries. The mechanisms by which these organisms initially colonize tooth surfaces are not fully understood; however, adherence to the salivary pellicle or to existing plaque is considered important (9, 13, 25). *S. mutans*, but not *S. sobrinus*, can adhere to high-molecular-weight salivary mucins and acidic proline-rich proteins that constitute part of the enamel salivary pellicle (10, 11). In addition, both *S. mutans* and *S. sobrinus* can adhere to strains of *Streptococcus sanguis*, *Streptococcus mitis*, and *Actinomyces viscosus*, the predominant organisms during early plaque formation (13). The adherence of *S. mutans* serotype c, the most common oral isolate, to other plaque bacteria is enhanced by saliva, whereas the adherence of *S. sobrinus* is unaffected by saliva (13). The nature of the salivary molecules and their bacterial receptors involved in the interbacterial binding of *S. mutans* has not been determined.

A number of salivary components, such as mucins, proline-rich proteins, histatins, lysozyme, fibronectin, and secretory immunoglobulin A (sIgA), have been found to interact with *S. mutans* (1, 7, 10, 11, 17). Included among these is salivary agglutinin, a high-molecular-weight, mucin-like glycoprotein that causes aggregation of cells in suspension (18, 23). Salivary agglutinin can also bind to hydroxyapatite and mediate adherence of *S. mutans* to this solid support (15). Agglutinin interacts with specific streptococcal surface proteins that possess carbohydrate-binding activity (4, 6). The genes encoding the *S. mutans* and *S. sanguis* agglutinin receptors have been cloned and sequenced (5, 14, 20). These studies have revealed that the *S. mutans* receptor, variously known as P1, B, I/II, PAc, and IF, is genetically and antigenically related to the *S. sanguis* receptor protein SSP-5 (6) and also to SpaA from *S. sobrinus* (26). Introduction of the SSP-5 gene into a nonaggregating strain of *Enterococcus faecalis* transforms the organism to an aggregation-positive phenotype (3). The possibility of a role for salivary agglutinin and its bacterial receptor in saliva-mediated interspecies binding of *S. mutans* has not been addressed.

In the present study, the effect of salivary agglutinin on the adherence of *S. mutans* and *S. sobrinus* to *S. sanguis* and *A. viscosus* was investigated. The adherence characteristics of SSP-5-positive transformed *E. faecalis* were also examined.

MATERIALS AND METHODS

Bacteria and culture conditions. *A. viscosus* NC3 is an oral isolate provided by B. J. Moncla, University of Washington. *S. sanguis* (*gordonii*) G9B, *S. mutans* KPSK2 (serotype c), *S. sobrinus* 6715 (serotype g), and *E. faecalis* S161 were from the culture collection maintained at the University of Pennsylvania, School of Dental Medicine. *E. faecalis* S161EB-5 was generated by transformation of *E. faecalis* S161 with shuttle vector pAM401 (27) containing a 5.3-kbp insert encoding the SSP-5 antigen (3). *E. faecalis* S161-401 was generated by transformation of S161 with pAM401 that did not contain a streptococcal insert. Bacteria were grown overnight under anaerobic conditions (85% N_2, 10% H_2, 5% CO_2) at 37°C in Trypticase peptone broth supplemented with 5 g of yeast extract per liter and 0.5% glucose. Numbers of bacteria were determined in a Klett-Summerson photometer. To metabolically label bacteria, 10 μCi of [^{3}H]thymidine (Amersham Corp., Arlington Heights, Ill.) per ml was added to the medium. Resulting specific activities varied between 7×10^{-5} and 1×10^{-4} cpm per cell.

Collection of saliva and purification of salivary agglutinin. Whole paraffin-stimulated saliva was collected from several healthy volunteers, pooled, clarified by centrifugation (10,000 × *g*, 10 min), and stored at −70°C. Salivary agglutinin was purified from parotid saliva by ion-exchange and gel permeation chromatography as described previously (4). Samples of purified agglutinin were tested for aggregating activity by the method of Ericson et al. (8).

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TABLE 1. Effect of saliva and agglutinin on adherence of *S. mutans* KPSK2 and *S. sobrinus* 6715 to *S. sanguis* G9B and *A. viscosus* NC3[a]

Test organism	Mean no. (10^7) of test organisms bound ± SD					
	S. sanguis G9B			*A. viscosus* NC3		
	Control	Saliva	Agglutinin	Control	Saliva	Agglutinin
S. mutans KPSK2	1.0 ± 0.2	4.7 ± 0.6	9.1 ± 1.2	1.8 ± 0.2	5.8 ± 0.4	9.8 ± 0.7
S. sobrinus 6715	1.7 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	4.6 ± 0.5	4.5 ± 0.3	2.1 ± 0.2

[a] Input cell number for test organisms, 2×10^8. Values are means ± standard deviation ($n = 6$). Control, binding in the absence of saliva or agglutinin. Otherwise, *S. sanguis* and *A. viscosus* were incubated with whole saliva or salivary agglutinin before assay as described in Materials and Methods.

Production of antibodies. Antibodies to SSP-5 were produced as described by Demuth et al. (3). Female BALB/c mice were immunized intraperitoneally with 0.2 mg of purified SSP-5 in Freund's complete adjuvant. The mice were boosted twice at 14-day intervals and bled 14 days later. The IgG fraction from preimmune and immune sera was obtained by affinity chromatography over protein A-Sepharose. Monospecific anti-P1 IgG was provided by K. W. Knox. Anti-lipoteichoic acid (LTA) IgG was prepared as reported previously (21).

Interbacterial binding assay. Adherence of *S. mutans*, *S. sobrinus*, and *E. faecalis* strains (test organisms) to *S. sanguis* and *A. viscosus* (base organisms) was determined by a modification of the nitrocellulose blot assay developed by Lamont and Rosan (13). In brief, cells of *S. sanguis* and *A. viscosus* were suspended in buffered KCl (5 mM KCl, 2 mM K_2PO_4, 1 mM $CaCl_2$ [pH 6.0]), and 10^8 cells were deposited on nitrocellulose paper in a dot-blot apparatus. The blot was washed in KCl containing 0.1% Tween 20 (KCl-Tween) and incubated for 2 h at room temperature with radiolabeled test organisms suspended in KCl-Tween. After unbound organisms were removed by washing, the experimental areas of the nitrocellulose were excised, and the amount of interbacterial binding was quantified by scintillation spectroscopy. To control for nonspecific background binding, test organisms were added to wells containing nitrocellulose without base organisms.

To examine the influence of saliva and agglutinin on binding, nitrocellulose containing base organisms was incubated with 50 μl of saliva diluted 1:5 in KCl or with 50 μl of agglutinin suspended in KCl at twice its original concentration in parotid saliva. After 1 h at room temperature, the base organism blots were washed three times in KCl-Tween. Adherence of test organisms to saliva- or agglutinin-treated base organisms was determined as described above.

Antibody inhibition of binding was investigated by incubating the test organisms with antibody prior to assay. Test organisms suspended in KCl were incubated with 20 μg of the relevant antibody per ml for 1 h at 37°C. Bacteria were collected by centrifugation (10,000 × *g*, 10 min) and resuspended in KCl-Tween, and adherence to untreated, saliva-treated, or agglutinin-treated base organisms was measured as described above.

RESULTS

Effect of saliva and agglutinin on the adherence of *S. mutans* and *S. sobrinus* to *S. sanguis* and *A. viscosus*. In order to investigate their influence on binding, whole saliva or salivary agglutinin was incubated with immobilized *S. sanguis* and *A. viscosus* prior to addition of the mutans streptococci. As shown in Table 1, *S. mutans* adhered to the base organisms in the absence of saliva at a relatively low level, with approximately 5% of input cells adhering to *S. sanguis* and 9% adhering to *A. viscosus*. Whole saliva produced a fivefold enhancement of *S. mutans* binding to *S. sanguis* and a threefold enhancement of binding to *A. viscosus*. Purified salivary agglutinin increased binding to *S. sanguis* ninefold and binding to *A. viscosus* fivefold. In contrast, binding of *S. sobrinus* to the base organisms was not promoted by either whole saliva or salivary agglutinin.

Effect of P1 antibodies on the adherence of *S. mutans* and *S. sobrinus*. To address the involvement of the agglutinin receptor in *S. mutans* adherence, inhibition of binding by antibodies to P1 was assessed. P1 IgG inhibited adherence of *S. mutans* to saliva-coated *S. sanguis* and *A. viscosus* by 95 and 70%, respectively (Table 2). P1 IgG also reduced adherence of *S. mutans* to *S. sanguis* in the absence of saliva by 80%. Control nonimmune and anti-LTA antibody had no effect on *S. mutans* binding (not shown). Adherence of *S. sobrinus* to both untreated and saliva-treated *S. sanguis* and *A. viscosus* was unaffected by P1 antibodies.

Adherence of *E. faecalis* S161EB-5. The adherence characteristics of *E. faecalis* S161EB-5, which expresses functional cell surface SSP-5, were investigated. As shown in Table 3, adherence of S161EB-5 to both *S. sanguis* and *A. viscosus* was enhanced by whole saliva and salivary agglutinin in a manner similar to that seen with *S. mutans* (Table 1). Saliva

TABLE 2. Effect of P1 IgG on the adherence of *S. mutans* KPSK2 and *S. sobrinus* 6715 to *S. sanguis* G9B and *A. viscosus* NC3[a]

Test organism	Incubation with anti-P1 before assay[b]	Mean no. (10^6) of test organisms bound ± SD			
		G9B	G9B + saliva	NC3	NC3 + saliva
S. mutans KPSK2	−	12.3 ± 2.4	54.6 ± 7.1	18.1 ± 3.7	60.4 ± 7.6
	+	2.4 ± 0.3	2.7 ± 0.4	16.8 ± 2.9	17.9 ± 2.5
S. sobrinus 6715	−	18.4 ± 2.7	23.2 ± 3.1	48.2 ± 5.9	51.5 ± 5.8
	+	14.3 ± 3.2	21.9 ± 4.0	47.5 ± 3.6	59.9 ± 7.7

[a] Input cell number for test organisms, 2×10^8. Values are means ± standard deviation ($n = 6$).
[b] +, test organisms incubated with anti-P1 IgG before assay as described in Materials and Methods; −, control with normal rabbit IgG.

TABLE 3. Adherence of *E. faecalis* S161EB-5 and S161-401 to *S. sanguis* G9B and *A. viscosus* NC3[a]

Test organism	Mean no. (10^6) of test organisms bound ± SD					
	S. sanguis G9B			*A. viscosus* NC3		
	Control	Saliva	Agglutinin	Control	Saliva	Agglutinin
E. faecalis S161EB-5	7.4 ± 0.7	18.1 ± 2.3	57.6 ± 4.7	9.9 ± 0.8	17.4 ± 1.6	39.1 ± 4.1
E. faecalis S161-401	3.0 ± 0.2	5.4 ± 0.5	9.5 ± 0.9	7.6 ± 0.9	7.3 ± 0.8	8.1 ± 0.7

[a] See Table 1, footnote *a*.

and agglutinin had comparatively little effect on the adherence of *E. faecalis* S161-401, the control strain that contains pAM401 without the streptococcal insert (Table 3).

Effect of SSP-5 antibodies on the adherence of *E. faecalis* S161EB-5. The ability of antibodies to SSP-5 to inhibit binding of *E. faecalis* S161EB-5 was examined. Adherence of S161EB-5 to saliva-treated *S. sanguis* and *A. viscosus* was inhibited 83 and 89%, respectively, by SSP-5 IgG (Table 4). SSP-5 IgG also inhibited S161EB-5 adherence to agglutinin-treated *S. sanguis* and *A. viscosus* by 87 and 86%, respectively. S161EB-5 binding to *S. sanguis* in the absence of saliva was inhibited 65% by SSP-5 IgG. Adherence of *S. mutans* to untreated and saliva- and agglutinin-treated *S. sanguis* and *A. viscosus* was similarly affected by SSP-5 IgG. SSP-5 IgG had no influence on the adherence of *E. faecalis* S161-401 and *S. sobrinus* to untreated and saliva- and agglutinin-treated *S. sanguis* and *A. viscosus* (not shown).

DISCUSSION

Bacterial colonization of tooth surfaces depends on a complex series of interactions with host and other bacterial factors. The ability to adhere to the tooth is one important attribute for potential colonizers. *S. mutans* and *S. sobrinus* can adhere to the enamel salivary pellicle (9) and to organisms that constitute early bacterial plaque, such as *S. sanguis* and *A. viscosus* (13). Adherence of *S. mutans* serotype c strains, the most common isolates from the human oral cavity (16), to *S. sanguis* and *A. viscosus* is enhanced by whole saliva (13). The nature of the salivary and bacterial molecules that participate in this interaction was investigated in the present study.

Adherence of *S. mutans* KPSK2, a serotype c strain, to *S. sanguis* and *A. viscosus* was enhanced by purified salivary agglutinin. Thus, it would appear that agglutinin can promote the interbacterial binding of these organisms. The involvement of other salivary molecules cannot be ruled out by these observations, and an assessment of the role of agglutinin relative to that of other molecules is complicated by the difficulty of measuring the amounts of agglutinin contributed to whole saliva by the major glands. This problem could be addressed by using glandular saliva in the binding assay, and such studies are under way.

Salivary agglutinin is a large (~400-kDa subunit molecular weight), acidic, mucinlike glycoprotein found in parotid and submandibular saliva (5). Agglutinin interacts with *S. mutans* and other oral streptococci such as *S. sanguis* in suspension to produce bacterial aggregates. Nevertheless, the precise function(s) of agglutinin in the oral cavity remains undetermined. Agglutinin-mediated bacterial aggregation may represent a nonimmunologic mechanism that promotes clearance of organisms from the oral cavity (19). Agglutinin may also be involved in the initial adherence of *S. mutans* to salivary pellicle. Purified agglutinin can bind to hydroxyapatite and mediate the attachment of *S. mutans* (15). In addition, agglutinin receptor-deficient mutants have a reduced capacity to adhere to saliva- or agglutinin-coated hydroxyapatite (12, 15). However, other studies have suggested that saliva-mediated adherence to hydroxyapatite and saliva-mediated aggregation are distinct processes that involve different mechanisms (22). Furthermore, in addition to salivary agglutinin, a variety of other salivary molecules, for example, a high-molecular-weight submandibular mucin and proline-rich proteins, appear to promote *S. mutans* adhesion to hydroxyapatite (10, 11). Thus, the involvement of agglutinin in the in vivo adherence of *S. mutans* to the salivary pellicle remains speculative. The current data do suggest, however, that agglutinin can bind to bacteria that are already present on the tooth surface and subsequently mediate *S. mutans* adherence. *S. sobrinus*, on the other hand, does not appear to interact with agglutinin in this manner. Neither whole saliva nor purified agglutinin enhanced the binding of this organism to *S. sanguis* or *A. viscosus*. Indeed, agglutinin showed some inhibition of *S. sobrinus* adherence to *A. viscosus*, indicating that the presence of high levels of agglutinin may interfere with binding between these organisms. These results are consistent with previous studies

TABLE 4. Effect of SSP-5 IgG on the adherence of *E. faecalis* S161EB-5 and *S. mutans* KPSK2 to *S. sanguis* G9B and *A. viscosus* NC3[a]

Test organism	Incubation with anti-SSP-5 before assay[b]	Mean no. (10^6) of test organisms bound ± SD					
		S. sanguis G9B			*A. viscosus* NC3		
		Control	Saliva	Agglutinin	Control	Saliva	Agglutinin
E. faecalis S161EB-5	−	8.2 ± 1.0	20.3 ± 1.9	52.6 ± 5.3	10.4 ± 0.8	18.9 ± 1.8	42.0 ± 5.7
	+	2.9 ± 0.3	3.5 ± 0.3	6.9 ± 0.8	9.5 ± 0.7	2.1 ± 0.2	5.9 ± 0.6
S. mutans KPSK2	−	9.8 ± 0.8	50.5 ± 6.7	85.2 ± 9.9	20.8 ± 1.6	63.5 ± 7.7	91.3 ± 8.4
	+	3.3 ± 0.5	6.1 ± 0.9	9.4 ± 0.8	21.3 ± 2.0	15.5 ± 2.9	24.8 ± 3.6

[a] See Table 1, footnote *a*.
[b] +, test organism incubated with anti-SSP-5 IgG before assay as described in Materials and Methods; −, control with normal mouse IgG.

showing that *S. sobrinus* 6715 is insensitive to agglutinin-mediated aggregation (18).

The streptococcal receptor for agglutinin is a high-molecular-weight surface protein that occurs, with slight variation in composition and binding specificity, in all of the mutans streptococci (except *S. rattus*) and *S. sanguis* (4, 6, 24). The nomenclature of this molecule is not uniform; it is known as MSL-1, P1, B, I/II, PAc, and IF (in *S. mutans*), SpaA (in *S. sobrinus*), and SSP-5 (in *S. sanguis*) (2, 6, 12, 14, 24, 26). Antibodies to P1 or to the recombinant SSP-5 protein inhibited *S. mutans* binding to saliva- or agglutinin-coated *S. sanguis* and *A. viscosus*. Thus, the agglutinin receptor appears to mediate the saliva-dependent binding of *S. mutans* to the organisms present in early plaque. The results, however, do not exclude the possibility that other interactive molecules on *S. mutans* may also be involved in interspecies attachment.

Adherence of *S. sobrinus* to saliva- or agglutinin-coated *S. sanguis* and *A. viscosus* did not appear to be mediated by the agglutinin receptor. Both P1 and SSP-5 antibodies failed to inhibit binding of *S. sobrinus*, and neither saliva nor agglutinin enhanced adherence. This result may be explained by quantitative differences in P1-SpaA expression between *S. mutans* and *S. sobrinus* or functional differences between the SpaA molecule from *S. sobrinus* and the agglutinin receptor proteins from *S. mutans* and *S. sanguis*. In support of the latter concept, no specific salivary molecules that interact with SpaA have been identified, and the function of SpaA may be more related to sucrose-dependent aggregation (2).

Corroboration of the role of the agglutinin receptor in *S. mutans* adherence was provided by engineered strains of *E. faecalis*. Expression of the SSP-5 gene in *E. faecalis* produced a phenotype capable of elevated binding to saliva- or agglutinin-coated *S. sanguis* and *A. viscosus*. Furthermore, this interaction was inhibited by SSP-5 antibodies. In the oral cavity, therefore, interactions between the agglutinin and its bacterial receptor may serve to promote colonization of *S. mutans* by mediating adherence to existing bacterial plaque. Thus, the functions of agglutinin may include clearance through aggregation, interbacterial adherence, and possibly adherence to tooth surfaces. Which of these diverse functions is operative at a particular time may depend on the species, serotype, or strain of bacteria; host factors, such as the amount of agglutinin secreted or heterogeneity in the carbohydrate moieties of agglutinin; or the prevailing environmental conditions. Another implication of these results is that since the SSP-5 gene was isolated from a strain of *S. sanguis*, the SSP-5 molecule of *S. sanguis* may be involved in saliva-mediated adherence to other *S. sanguis* strains or to *A. viscosus*. The existence and significance of these potential interactions require further investigation.

An additional role for the agglutinin receptor may be in the saliva-independent attachment of *S. mutans* to *S. sanguis*. Both P1 and SSP-5 antibodies reduced *S. mutans* adherence to *S. sanguis*. Furthermore, transformation of *E. faecalis* with the SSP-5 gene enhanced binding of the transformed organism to *S. sanguis*, a reaction that could be inhibited with SSP-5 antibodies. Although these phenomena may be the result of steric or nonspecific effects, it is possible that *S. sanguis* expresses surface carbohydrates that are recognized by the agglutinin receptor. Further studies are required to clarify this point.

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REFERENCES

1. **Babu, J. P., and M. K. Dabbous.** 1986. Interaction of salivary fibronectin with oral streptococci. J. Dent. Res. **65**:1094–1100.
2. **Curtiss, R.** 1986. Genetic analysis of *Streptococcus mutans* virulence and prospects for an anticaries vaccine. J. Dent. Res. **65**:1034–1045.
3. **Demuth, D. R., P. Berthold, P. S. Leboy, E. E. Golub, C. A. Davis, and D. Malamud.** 1989. Saliva-mediated aggregation of *Enterococcus faecalis* transformed with a *Streptococcus sanguis* gene encoding the SSP-5 surface antigen. Infect. Immun. **57**:1470–1475.
4. **Demuth, D. R., C. A. Davis, A. M. Corner, R. J. Lamont, P. S. Leboy, and D. Malamud.** 1988. Cloning and expression of a *Streptococcus sanguis* surface antigen that interacts with a human salivary agglutinin. Infect. Immun. **56**:2484–2490.
5. **Demuth, D. R., E. E. Golub, and D. Malamud.** 1990. Streptococcal-host interactions. Structural and functional analysis of a *Streptococcus sanguis* receptor for a human salivary glycoprotein. J. Biol. Chem. **265**:7120–7126.
6. **Demuth, D. R., M. S. Lammey, M. Huck, E. T. Lalley, and D. Malamud.** 1990. Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. Microb. Pathogen. **9**:199–211.
7. **Douglas, C. W. I., and R. R. B. Russell.** 1984. The adsorption of human salivary components to strains of the bacterium *Streptococcus mutans*. Arch. Oral Biol. **29**:751–757.
8. **Ericson, T., K. Pruitt, and H. Wedel.** 1975. The reaction of salivary substances with bacteria. J. Oral Pathol. **4**:307–323.
9. **Gibbons, R. J., L. Cohen, and D. I. Hay.** 1986. Strains of *Streptococcus mutans* and *Streptococcus sobrinus* attach to different pellicle receptors. Infect. Immun. **52**:555–561.
10. **Gibbons, R. J., and D. I. Hay.** 1989. Adsorbed salivary acidic proline-rich proteins contribute to the adhesion of *Streptococcus mutans* JBP to apatitic surfaces. J. Dent. Res. **68**:1303–1307.
11. **Kishimoto, E., D. I. Hay, and R. J. Gibbons.** 1989. A human salivary protein which promotes adhesion of *Streptococcus mutans* serotype c strains to hydroxyapatite. Infect. Immun. **57**:3702–3707.
12. **Koga, T., N. Okahashi, I. Takahashi, T. Kanamoto, H. Asakawa, and M. Iwaki.** 1990. Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutants of *Streptococcus mutans* serotype c. Infect. Immun. **58**:289–296.
13. **Lamont, R. J., and B. Rosan.** 1990. The adherence of mutans streptococci to other oral bacteria. Infect. Immun. **58**:1738–1743.
14. **Lee, S. F., A. Progulske-Fox, and A. S. Bleiweis.** 1988. Molecular cloning and expression of a *Streptococcus mutans* major surface protein antigen, P1 (I/II), in *Escherichia coli*. Infect. Immun. **56**:2114–2119.
15. **Lee, S. F., A. Progulske-Fox, G. W. Erdos, D. A. Piacentini, G. Y. Ayakawa, P. J. Crowley, and A. S. Bleiweis.** 1989. Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1 (I/II). Infect. Immun. **57**:3306–3313.
16. **Loesche, W. J.** 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. **50**:353–380.
17. **MacKay, B. J., L. Denepitiya, V. J. Iacono, S. B. Krost, and J. J. Pollock.** 1984. Growth-inhibitory and bactericidal effects of human parotid salivary histidine-rich polypeptides on *Streptococcus mutans*. Infect. Immun. **44**:695–701.
18. **Malamud, D., B. Appelbaum, R. Kline, and E. E. Golub.** 1981. Bacterial aggregating activity in saliva: comparisons of bacterial species and strains. Infect. Immun. **31**:1003–1006.
19. **Mandel, I. D.** 1987. Nonimmunologic aspects of caries resistance. J. Dent. Res. **55**:C22–C31.

20. **Okahashi, N., C. Sasakawa, M. Yoshikawa, S. Hamada, and T. Koga.** 1989. Molecular characterization of a surface protein antigen gene from serotype c *Streptococcus mutans*, implicated in dental caries. Mol. Microbiol. **3**:673–678.
21. **Rosan, B.** 1973. Antigens of *Streptococcus sanguis*. Infect. Immun. **7**:205–211.
22. **Rosan, B., D. Malamud, B. Appelbaum, and E. E. Golub.** 1982. Characteristic differences between saliva-dependent aggregation and adhesion of streptococci. Infect. Immun. **35**:86–90.
23. **Rundegren, J.** 1986. Calcium-dependent salivary agglutinin with reactivity to various oral bacterial species. Infect. Immun. **53**:173–178.
24. **Russell, R. R. B.** 1980. Distribution of cross-reactive antigens A and B in *Streptococcus mutans* and other oral streptococci. J. Gen. Microbiol. **118**:383–388.
25. **Staat, R. H., S. D. Langley, and R. J. Doyle.** 1980. *Streptococcus mutans* adherence: presumptive evidence for protein-mediated attachment followed by glucan-dependent cellular accumulation. Infect. Immun. **27**:675–681.
26. **Takahashi, I., N. Okahashi, C. Sasakawa, M. Yoshikawa, S. Hamada, and T. Koga.** 1989. Homology between surface protein antigen genes of *Streptococcus sobrinus* and *Streptococcus mutans*. FEBS Lett. **249**:383–388.
27. **Wirth, R., F. Y. An, and D. B. Clewell.** 1986. Highly efficient protoplast transformation system for *Streptococcus faecalis* and a new *Escherichia coli-S. faecalis* shuttle vector. J. Bacteriol. **165**:831–836.