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Influence of Growth Medium on Adsorption of *Streptococcus mutans*, *Actinomyces viscosus*, and *Actinomyces naeslundii* to Saliva-Treated Hydroxyapatite Surfaces

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The influence of the growth medium on the ability of strains of *Streptococcus mutans*, *Actinomyces viscosus* and *A. naeslundii* to attach to saliva-treated hydroxyapatite (S-HA) surfaces was studied. Preliminary experiments indicated that cells of each species harvested in lag, log, and early stationary phases of growth adsorbed comparably to S-HA; thus, early stationary phase cells were used in all subsequent assays. Strains were grown in chemically defined medium (CDM), in CDM supplemented with gastric mucin or with filter-sterilized or ^{60}Co-irradiated saliva from human donors of blood types A, B, or O, and in Trypticase soy broth (BBL Microbiology Systems) and Todd-Hewitt broth. Adherence of *S. mutans* H12 to S-HA tended to vary when the streptococci were grown in saliva-supplemented CDM, but the number of cells which attached was generally within twofold of that of CDM-grown cells. Attachment of *A. viscosus* S2 and LY7 and of *A. naeslundii* S4 and L13 was generally similar when grown in CDM or in CDM supplemented with saliva, but it tended to increase for organisms grown in CDM supplemented with gastric mucin. None of the strains studied appeared to destroy the blood group reactivity of the added salivary components, and they attached equally well to HA treated with homologous or heterogous saliva from that present in the medium in which they were grown. The *A. viscosus* strains adsorbed in 25 to 40% higher numbers to HA treated with blood type B saliva than with type A saliva, irrespective of the medium used for growth. *S. mutans* H12 cells displayed α- and β-glucosidase and α-galactosidase activity; the *Actinomyces* strains exhibited these activities plus β-galactosidase when grown in all media. However, the levels of these glycoside hydrolases did not correlate with cell adsorption to S-HA. The apparent weak influence of the growth medium on attachment of *S. mutans* was studied further. Strains of *S. mutans* isolated from the saliva of five human donors were made resistant to streptomycin, grown in CDM, and then added to new saliva samples from the respective donors from which they were obtained. The in vitro-grown cells were found to attach to S-HA comparably to *S. mutans* cells present naturally in the saliva.

Strains of indigenous *Streptococcus*, *Lactobacillus*, *Neisseria*, *Veillonella*, and *Bacteroides* species have been shown to attach to surfaces of the mouth in a highly specific manner, and the experimentally determined affinity of an organism for given oral surface parallels its natural colonization (6, 7, 9, 10, 18). Bacterial adherence is thought to be necessary for colonization of the mouth because it prevents organisms from being washed away by oral secretions (9, 10). The specificity of attachment suggests that bacteria possess a recognition system which is capable of interacting with specific surface components of host tissues. This system is thought to involve lectin-like bacterial ligands which bind to saccharide receptors of glycoproteins on epithelial cell surfaces or in the enamel pellicle on the teeth (12, 12a).

Different environmental conditions often produce changes in the surface components of bacteria, and therefore the bacterial ligands involved in attachment might be inducible. Such a possibility could account for the observation that laboratory-cultured oral bacteria usually only persist for short periods of time when introduced into the mouths of volunteers (10, 20). Therefore, the influence of the growth environment on the adherence of *Streptococcus mutans* and *Actinomyces* species to saliva-treated spheroidal hydroxyapatite (S-HA) surfaces which mimic the teeth was studied. In addition, the possible association of certain glycoside hydro-

lases, including those which degrade blood group substances (15, 16), with adherence was investigated.

MATERIALS AND METHODS

Cultures and cultural conditions. *S. mutans*, H12, *Actinomyces viscosus* LY7 and S2, and *Actinomyces naeslundii* L13 and S4 were obtained from the culture collection of the Forsyth Dental Center. All cultures were maintained by weekly transfer in Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) and on tryptic soy blood agar plates (Scott Laboratories, Inc., Fiskesville, R.I.). Cultures were incubated at 35°C in Brewer jars filled with 80% N_2, 10% H_2, and 10% CO_2.

For experimental purposes, the organisms were grown in a chemically defined medium (CDM) described by Socransky et al. (J. Dent. Res. **52**(special issue A):88) with limiting glucose (0.1%) or in CDM supplemented with either 0.2% gastric mucin (Wilson and Co., Chicago, Ill.) or 10% whole clarified saliva from donors of blood types A, B, or O. All organisms were transferred at least three times in the respective medium before assay. For comparison, the adherence of organisms grown in Todd-Hewitt broth (Gibco Diagnostics, Madison, Wis.) and TSB was also studied.

Saliva collection and sterilization. Samples of whole, unstimulated saliva were collected in ice-chilled containers from individuals of blood types A, B, and O who were secretors. The saliva was heated at 60°C for 30 min to inactive degradative enzymes, clarified by centrifugation (10,000 × *g* for 10 min), and frozen (−20°C) before use (2, 3). Saliva samples added to CDM were either filter sterilized (0.22 μm, Millipore Corp., Bedford, Mass.) or lyophilized and irradiated with 4 to 6 Mrad of ^{60}Co (courtesy of U.S. Army Laboratory, Natick, Mass.) before addition to the filter-sterilized CDM. Gastric mucin solutions were filter sterilized.

Bacterial adherence to S-HA. Bacterial attachment to S-HA beads was studied essentially as described by Clark et al. (2). Reaction mixtures (0.5 ml) contained 20 mg of S-HA and 2.5×10^7 [^{3}H]thymidine-labeled cells of *S. mutans* or 5×10^7 cells of the *Actinomyces* strains in 0.05 M KCl containing 1 mM phosphate (pH 6.0), 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ (buffered KCl.). The higher concentration of *Actinomyces* cells was used because it was just below that required to saturate the S-HA, and ca. 40% of the available organisms became adsorbed. At lower concentrations, most (i.e., ca. 80%) of the actinomycetes became bound.

Tubes without beads were also treated with saliva and then incubated with bacterial suspensions; these no-bead controls were used to determine bacterial adsorption to the walls of the tubes. All assays were done in duplicate or triplicate unless noted otherwise.

Glycoside hydrolase assays. Washed cells (1.5 mg [dry wt]/ml) prepared from cultures grown in the respective media were assayed for glycosidase activities using *p*-nitrophenyl sugar derivatives (Calbiochem, San Diego, Calif.) as substrates as described by Kilian (17). The following enzyme activities were assayed in the corresponding buffer systems: α- and β-glucosidase–0.067 M phosphate buffer (pH 6.2); α- and β-galactosidase–0.067 M phosphate buffer (pH 7.0); α-mannosidase–0.1 M acetate buffer (pH 4.0); β-glucuronidase–0.067 M phosphate buffer (pH 7.0); α-fucosidase–0.067 M phosphate buffer (pH 7.0); β-*N*-acetyl glucosaminidase–0.1 M acetate buffer (pH 5.3).

Equal volumes of bacterial cell suspensions and 20 mM substrate solutions were mixed and allowed to react at 37°C. Samples (0.5 ml) were removed at 0-, 15-, 30-, and 60-min intervals and added to 0.5 ml of 1 M K_2CO_3 to stop the reaction. Boiled bacterial suspensions were added to substrate solutions and sampled in a similar manner to determine the level of nonenzymatic release of *p*-nitrophenol. After centrifugation (12,000 × *g* for 2 min), the optical densities of the supernatants were measured at 420 nm in a Gilford 300-N spectrophotometer to determine the amount of *p*-nitrophenol released.

Blood group substance determinations. Organisms were tested for their ability to destroy blood group-reactive mucins present in saliva added to CDM. Cultures grown in CDM alone and in saliva-supplemented CDM were centrifuged, and 25-μl samples of the resulting supernatant liquors were serially diluted in 0.01 M phosphate-buffered saline, pH 7.2, in micro-titration plates. Samples of uninoculated media were used as controls. Each dilution was then incubated with 25-μl samples containing 2 hemagglutinating units of anti-A or anti-B blood typing serum (Hyland Laboratories, Inc., Costa Mesa, Calif.) for 1 h with continuous vibration at room temperature (15). A 2% human erythrocyte suspension (25 μl) (Hyland) of appropriate blood type was then added, and after 1 h of incubation at room temperature, the mixtures were examined for agglutination by determining their settling patterns by using a dissecting microscope. Agglutination was confirmed by microscopic examination of wet-mount preparations at ×400 magnification. Between 24 and 48 ng of typical salivary mucin preparations was required to inhibit hemagglutination in the assay as used (8).

Comparison of the adherence of in vitro and in vivo-grown *S. mutans* to S-HA. The level of *S. mutans* present in the saliva of five humans was determined by using mitis salivarius bacitracin (MS-B) agar (14), and isolates were obtained from each individual. Streptomycin-resistant (Sm^r) mutants were obtained from each strain by plating young TSB cultures on Trypticase soy agar plates containing 5% sheep blood and 500 μg of streptomycin-sulfate (Sigma Chemical Co., St. Louis, Mo.) per ml. Resulting colonies were transferred on blood agar plates containing 1,000 μg of antibiotic per ml to obtain mutants resistant to this level.

For study, the Sm^r *S. mutans* strains were transferred three times in CDM. Streptococci harvested from stationary-phase cultures were washed once and suspended in buffered KCl before use. Hydroxyapatite beads (20 mg) were treated with previously collected samples of clarified whole saliva from the respective donors as described previously (2) and then washed twice with buffered KCl to remove excess saliva. Immediately before each attachment assay a fresh saliva sample which contained naturally occurring *S. mutans* was collected from each respective donor. The donors

were instructed to brush their teeth without a dentifrice before expectorating ca. 5 ml of saliva; this was required to increase the number of naturally present *S. mutans* cells. The saliva mixtures were then incubated with the appropriate S-HA beads for 90 min at room temperature with continuous rotation. The beads were washed five times with 1.5-ml samples of 2% Trypticase in 0.85% NaCl (TNS) and ground in sterile glass tissue grinders with TNS. Samples were diluted with TNS and plated in duplicate on MS-B agar for enumeration of total *S. mutans*, on MS-B agar supplemented with 200 μg of streptomycin sulfate per ml for enumeration of Sm^r *S. mutans*, and on mitis salivarius agar (Difco) for enumeration of total streptococci and *S. salivarius*. Samples of each saliva which had not been incubated with S-HA beads were also cultured in a similar manner for comparison.

RESULTS

Effect of growth phase and storage on bacterial adherence to S-HA. In initial experiments, the adherence of CDM-grown cells of *S. mutans* H12, *A. viscosus* S2, and *A. naeslundii* S4 cells harvested in different growth phases to S-HA was determined. *S. mutans* had a much shorter generation time (0.5 h) and adsorbed in lower numbers to S-HA than did the *Actinomyces* strains (generation time, 4 h). However, no marked differences were noted in the adherence of cells harvested from lag through early stationary phases of growth (Table 1).

Prefrozen cells of all strains attached as well as or better than freshly harvested bacteria (Table 2). Consequently, prefrozen early stationary phase cells were used for all subsequent adherence assays; such preparations were also less variable and more convenient.

Effect of the growth medium on attachment of *S. mutans*, *A. viscosus*, and *A. naeslundii* to S-HA. The *Actinomyces* strains grown in all media studied attached in several-fold-higher numbers to S-HA than did *S. mutans* H12 (Table 3). This reflects their greater innate capacity for adsorbing to S-HA (2) in addition to their twofold-higher cell concentrations used in the adherence assays. Some differences were noted in the adherence of organisms harvested from the different media studied. For example, *S. mutans* H12 cells adsorbed in somewhat higher numbers to S-HA when grown in CDM supplemented with filter-sterilized saliva from donors 1 and 2 (blood types A and B, respectively) than when grown in CDM alone (Table 3). In contrast, the adherence of H12 cells grown in CDM supplemented with ^{60}Co-sterilized saliva was decreased. Filter sterilization of saliva was found to remove ca. 75% of the blood group reactive mucins, as judged by a hemagglutination inhibition assay. Since these components are known to bind to bacterial cells and impair attachment to S-HA (11), the decreased adherence of H12 cells grown in the presence of ^{60}Co-sterilized saliva may reflect increased levels of bound mucin on their surfaces. A similar situation may exist with TSB- and Todd-Hewitt broth-grown *S. mutans* cells which also adsorbed less well than CDM-grown streptococci to S-HA. These natural media contain blood group-reactive components which also become bound to *S. mutans* cells during growth (13).

TABLE 1. *Effect of growth phase on adherence of S. mutans and Actinomyces species to S-HA*

Organism	No. of cells adsorbed ($\times 10^6$) ± SE[a]/20 mg of S-HA when harvested from:			
	Lag phase	Early log phase	Late-log phase	Early stationary phase
S. mutans H-12	8.4 ± 0.1	7.9 ± 0.7	8.5 ± 0.8	8.0 ± 0.1
A. viscosus S2	24.8 ± 1.4	28.3 ± 0.8	25.1 ± 0.2	24.8 ± 0.1
A. naeslundii S4	19.6 ± 1.5	23.2 ± 3.7	18.4 ± 0.6	24.0 ± 0.8

[a] SE, Standard error of the mean.

TABLE 2. *Adherence of freshly harvested and prefrozen bacteria to S-HA*

Organism	No. of cells adsorbed ($\times 10^6$) ± SE[a]/20 mg of S-HA	
	Freshly harvested	Prefrozen
S. mutans H12	2.3 ± 0.1	4.5 ± 0.1
A. viscosus S2	40.0 ± 1.8	35.6 ± 0.1
A. viscosus LY7	30.5 ± 1.5	32.6 ± 0.1
A. naeslundii S4	21.5 ± 0.5	24.7 ± 0.3
A. naeslundii L13	27.5 ± 0.5	29.5 ± 0.5

[a] SE, Standard error of the mean.

Differences were also noted in the adherence of *A. viscosus* and *A. naeslundii* cells grown in the media studied to S-HA, but they tended to be relatively small (Table 3). In general, the differences between cells grown in CDM and those grown in other media were usually less than twofold for all strains studied.

Glycoside hydrolase levels of bacteria grown in different media. Washed *S. mutans* H12 cells harvested from all media studied exhibited α- and β-glucosidase and α-galactosidase activities, whereas the *Actinomyces* strains showed these activities plus β-galactosidase (Table 4). However, no correlations were evident between the levels of these enzyme activities and cell adherence (Table 4). These enzymes were elaborated by organisms grown in CDM alone, and, therefore, are apparently formed constitutively. This is also suggested by the observation that cells of the *Actinomyces* strains grown in the media studied exhibited β-galac-

TABLE 3. *Attachment of bacteria grown in different media to S-HA*

Growth medium	No. of cells adsorbed ($\times 10^6$) ± SE[a]/20 mg of S-HA				
	S. mutans	*A. viscosus*		*A. naeslundii*	
	H12	S2	LY7	S4	L13
CDM alone	4.6 ± 0.4	27.7 ± 2.5	25.7 ± 1.9	24.5 ± 5.4	27.7 ± 2.7
CDM + filter-sterilized gastric mucin	2.3 ± 0.3	35.5 ± 1.5	34.5 ± 0.5	37.1 ± 0.4	27.5 ± 0.5
CDM + filter-sterilized saliva 1[b]	8.3 ± 0.9	26.0 ± 0.5	28.9 ± 0.4	34.6 ± 2.4	11.3 ± 1.1
CDM + filter-sterilized saliva 2	6.5 ± 0.2	33.0 ± 2.9	31.5 ± 0.5	12.1 ± 1.8	20.2 ± 0.8
CDM + filter-sterilized saliva 3	3.3 ± 0.2	33.0 ± 2.0	29.5 ± 0.5	17.0 ± 1.0	23.7 ± 0.6
CDM + ^{60}Co-sterilized saliva 1[b]	2.4 ± 0.1	18.0 ± 0.5	23.5 ± 4.4	20.5 ± 2.5	30.8 ± 0.2
CDM + ^{60}Co-sterilized saliva 2	3.9 ± 0.1	16.5 ± 0.5	20.5 ± 1.5	20.0 ± 2.0	26.5 ± 0.5
CDM + ^{60}Co-sterilized saliva 3	2.5 ± 0.1	16.7 ± 0.1	18.0 ± 2.0	17.5 ± 2.5	40.1 ± 0.8
TSB	1.9 ± 0.3	22.0 ± 1.0	26.0 ± 1.0	21.4 ± 0.3	22.5 ± 0.5
Todd-Hewitt Broth	1.9 ± 0.1	21.5 ± 0.5	27.0 ± 2.0	16.0 ± 1.0	22.5 ± 3.5

[a] SE, Standard error of the mean.
[b] Saliva samples 1, 2, and 3 were from donors of blood types A, B, and O, respectively.

TABLE 4. *Cell-associated glycosidase activities of bacteria grown in different media*

Organism	Growth medium	Relative adherence to S-HA[a]	Enzyme activity (μM *p*-nitrophenol released/μg [dry wt]/h)			
			α-Glucosidase	β-Glucosidase	α-Galactosidase	β-Galactosidase
S. mutans H12	CDM	100	193	234	190	0
	CDM + saliva 1[b]	181	157	208	143	0
	CDM + saliva 2	141	293	322	139	0
	CDM + saliva 3	72	190	234	143	0
	CDM + G mucin	49	139	270	132	0
A. viscosus S2	CDM	100	157	49	171	104
	CDM + saliva 1	86	153	29	41	73
	CDM + saliva 2	110	245	43	110	269
	CDM + saliva 3	110	147	49	150	150
	CDM + G mucin	120	211	53	110	220
A. viscosus LY7	CDM	100	214	172	110	24
	CDM + saliva 1	104	153	174	98	12
	CDM + saliva 2	112	208	233	159	17
	CDM + saliva 3	105	196	285	211	12
	CDM + G mucin	123	294	209	110	49
A. naeslundii S4	CDM	100	76	61	110	98
	CDM + saliva 1	115	147	43	85	86
	CDM + saliva 2	40	122	73	134	172
	CDM + saliva 3	56	208	104	74	129
	CDM + G mucin	123	159	98	58	159
A. naeslundii L13	CDM	100	226	184	153	100
	CDM + saliva 1	35	122	196	92	+[c]
	CDM + saliva 2	64	190	245	159	+
	CDM + saliva 3	74	147	159	110	+
	CDM + G mucin	89	134	110	76	+

[a] Hydroxyapatite pretreated with saliva from donor of blood type A.
[b] Saliva and gastric mucin supplements were sterilized by filtration; saliva samples 1, 2, and 3 were obtained from donors of blood types A, B, and O, respectively.
[c] Positive for β-galactosidase activities; values were not determined.

tosidase, but *S. mutans* H12 did not, even though it will form this enzyme when grown in the presence of an appropriate inducer such as lactose. Cells of none of the strains tested had detectable levels of α-mannosidase, β-glucuronidase, α-fucosidase, or β-*N*-acetyl glucosaminidase activities. Also, the hemagglutination inhibition titers of supernatant liquors derived from cultures grown in CDM supplemented with ^{60}Co-sterilized saliva were the same as those for uninoculated broth (titer = $\log_2$ of 6). This suggests that the organisms studied did not destroy blood group A- or B-reactive groups of the salivary mucins. However, 50% or more of the added

mucin would have to be degraded to be detected with the assay used.

Bacterial adsorption to hydroxyapatite treated with homologous and heterologous saliva. Bacteria grown in CDM supplemented with saliva from individuals of blood type A or B did not appear to exhibit increased adherence to hydroxyapatite treated with samples of homologous saliva (Table 5). However, there was a tendency for the *Actinomyces* strains to adsorb in higher numbers to salivary pellicles formed of saliva from the donor of blood type B, irrespective of the medium used for growth (Table 5). Consequently, the adherence of the organisms to hydroxyapatite coated with saliva from three different donors of blood types A and B, respectively, was studied. Consistently higher numbers of TSB-grown *A. viscosus* strain S2 and LY7 cells adsorbed to hydroxyapatite treated with saliva from donors of blood type B than with that from individuals of blood type A (Table 6). This difference was statistically significant ($P < 0.02$). However, the adherence of *A. naeslundii* S4 and L13, and of *S. mutans* H12, was similar to both types of S-HA. The greater affinity of the *A. viscosus* strains for blood type B salivary pellicles does not appear to be related to the growth medium because cells harvested from all media studied exhibited this effect (data not shown).

Adherence of in vitro and naturally grown *S. mutans* to S-HA. Freshly isolated Sm^r strains of *S. mutans* cultured in CDM were added to saliva samples containing naturally present *S. mutans* cells, and their ability to attach to S-HA beads was compared. The pro-

TABLE 5. *Bacterial adsorption to hydroxyapatite treated with homologous and heterologous saliva*

Organism	Growth medium	No. of cells adsorbed ($\times 10^6$) ± SE[a]/20 mg of S-HA	
		Type A S-HA	Type B S-HA
S. mutans H12	CDM	4.5 ± 0.4	4.4 ± 1.1
	CDM + type A saliva	2.4 ± 0.1	2.3 ± 0.1
	CDM + type B saliva	3.9 ± 0.1	4.6 ± 0.4
A. viscosus S2	CDM	25.0 ± 1.0	31.0 ± 1.0
	CDM + type A saliva	18.0 ± 0.5	23.5 ± 1.5
	CDM + type B saliva	16.5 ± 0.5	21.5 ± 1.5
A. viscosus LY7	CDM	23.5 ± 0.5	30.0 ± 4.0
	CDM + type A saliva	23.5 ± 4.5	29.5 ± 3.5
	CDM + type B saliva	20.5 ± 1.5	27.0 ± 2.0
A. naeslundii S4	CDM	19.0 ± 0.5	22.0 ± 1.0
	CDM + type A saliva	20.5 ± 2.5	35.5 ± 3.5
	CDM + type B saliva	20.5 ± 2.0	36.5 ± 0.5
A. naeslundii L13	CDM	24.5 ± 0.5	31.0 ± 1.0
	CDM + type A saliva	30.8 ± 0.1	37.0 ± 1.0
	CDM + type B saliva	26.5 ± 0.5	25.5 ± 1.5

[a] SE, Standard error of the mean.

TABLE 6. *Bacterial attachment to hydroxyapatite treated with saliva of individuals of blood types A and B*

Saliva sample	No. of cells[a] adsorbed ($\times 10^6$) ± SE[b]/20 mg of S-HA				
	A. viscosus		*S. mutans*	*A. naeslundii*	
	S2	LY7	H12	S4	L13
1 Type A	17.2 ± 0.2	20.1 ± 1.5	4.3 ± 0.4	8.5 ± 0.5	17.7 ± 2.7
2 Type A	21.9 ± 1.3	29.5 ± 0.2	4.1 ± 0.1	17.5 ± 0.5	24.1 ± 0.8
3 Type A	21.3 ± 0.5	28.0 ± 2.0	6.4 ± 0.2	10.5 ± 0.01	20.1 ± 1.8
Mean	20.1 ± 1.0[c]	25.9 ± 2.0[c]	5.0 ± 0.5	12.1 ± 1.8	20.6 ± 1.5
1 Type B	25.2 ± 0.4	32.2 ± 2.3	4.3 ± 0.1	10.4 ± 0.01	19.6 ± 1.0
2 Type B	27.8 ± 1.7	33.3 ± 3.7	6.4 ± 0.6	15.8 ± 0.9	24.7 ± 0.2
3 Type B	25.1 ± 0.4	37.4 ± 1.0	7.6 ± 0.4	13.5 ± 0.3	24.7 ± 0.1
Mean	26.0 ± 0.5[c]	34.3 ± 1.5[c]	6.1 ± 0.6	13.2 ± 1.0	22.9 ± 1.1

[a] Cells grown in TSB.

[b] SE, Standard error of the mean.

[c] Adherence differences between blood type A and B saliva statistically significant at $P < 0.02$ level (Student's *t* test).

portions of in vitro-grown Sm^r *S. mutans* of the total *S. mutans* recovered from the S-HA were found to be similar to the proportions of these organisms in the saliva to which the S-HA beads were exposed (Table 7). The organisms recovered from the beads appear to represent adsorbed cells rather than contamination with free salivary organisms because the proportions of *S. salivarius* of the total streptococci recovered from the beads were much lower than in the saliva to which the beads were exposed (Table 7).

DISCUSSION

A variety of bacteria are able to become associated with oral surfaces by ionic and other physical forces of relatively low specificity (12a, 20), but these are usually insufficient to result in colonization. More firm attachment is thought to entail the formation of polymeric bridges, possibly involving lectin-like ligands of the bacterium which bind to specific saccharides of glycoproteins or glycolipid receptors on epithelial cells or teeth (12, 12a). The bacterial ligands could be components such as pili, sugar-binding proteins, or surface-bound enzymes involved in glycoprotein degradation. The latter two types of components are often formed inducibly, which raises the possibility that the ligands involved in bacterial attachment to teeth might also be inducible. If this were the case, studies of the adherent properties of bacteria grown in vitro in the absence of appropriate glycoprotein inducers might be of little significance since such cells would not reflect their in vivo capabilities.

The present study demonstrated that cells of *S. mutans*, *A. viscosus*, and *A. naeslundii* grown in different media do exhibit differences in their ability to attach to S-HA surfaces which mimic the teeth. However, the effects observed were smaller than those usually attributed to inducible systems. For example, organisms grown in complex natural media such as TSB or Todd-Hewitt broth or in defined media supplemented with human saliva generally exhibited no more than twofold differences in their numbers which attached to S-HA, compared to cells grown in chemically defined medium alone. In addition, bacteria harvested from different phases of growth appeared to possess similar adhesive properties. These findings are consistent with those of Cisar et al. (1), who noted that the coaggregation properties of a variety of actinomycetes and streptococci were not markedly affected by the medium in which the organisms were cultivated, or by the physiological age of the bacterial cells. These observations suggest that the ligands involved in the attachment of the organisms studied to S-HA are formed constitutively. This was also suggested by the marked capacity of the *Actinomyces* strains to attach to S-HA when grown in unsupplemented CDM. Thus, ca. 40% of the available cells of the *A. viscosus* and *A. naeslundii* strains studied adsorbed to S-HA at the bacterial concentrations employed, and at lower concentrations, over 80% of the available organisms attached. In the case of *S. mutans* H12, ca. 10% of the available CDM-grown streptococci became associated with the S-HA. This appears to be due to the organism's innate lower binding capacity, rather than to the lack of induction of surface ligands because *S. mutans* cells grown in the presence of salivary components or in complex media tended to adsorb less well. This is probably because their surface ligands become partially saturated by medium glycoproteins (8).

That the ligands involved in attachment to S-HA are formed constitutively by *S. mutans* was also supported by studies which compared the adherence of Sm^r mutants of *S. mutans* grown in CDM with *S. mutans* cells which were present naturally in saliva. The laboratory-cultivated strains attached to S-HA comparably to the naturally grown organisms, indicating that their adherent properties are similar.

The *S. mutans*, *A. viscosus*, and *A. naeslundii* strains studied also appeared to form certain cell-bound glycoside hydrolases constitutively. However, the levels of the enzyme activities measured did not seem to correlate with the adherent properties of the organisms. Nevertheless, it is of interest that the attachment of *S. mutans* cells to S-HA is inhibited by melibiose, an α-galactoside (13), and the organism synthesizes α-galactosidase constitutively. Similarly, the attachment of *A. viscosus* and *A. naeslundii* cells to erythrocytes is inhibited by β-galactosides (4, 5), and all strains studied formed β-galactosidase constitutively.

No differences were detected in the capacity of the bacteria studied to attach to hydroxyapatite which was coated with the same or with a heterologous sample of saliva from that present

TABLE 7. *Comparative attachment of in vitro and naturally grown S. mutans cells to S-HA*

Saliva donor	% *S. salivarius* of total streptococci:		% In vitro-grown Sm^r *S. mutans* of total *S. mutans*:	
	In saliva	Attached to S-HA	In Saliva	Attached to S-HA
1	19	3	43	51
2	10	0.3	44	46
3	16	0.3	47	52
4	15	1	89	87
5	12	0	31	34

in the medium in which they were grown. This further suggests that there is not a special relationship between the synthesis of ligands involved in adherence to S-HA and components of the growth environment. However, it was noted that *A. viscosus* cells tended to adsorb in higher numbers to hydroxyapatite treated with saliva from donors of blood type B than from those of blood type A. This effect occurred irrespective of the medium in which the cells were grown. This suggests the possibility that individuals of blood type B may become infected with *A. viscosus* strains more readily than individuals of blood type A; further studies are needed to test this possibility.

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LITERATURE CITED

1. **Cisar, J., P. Kolenbrander, and F. McIntire.** 1979. Specificity of coaggregation reactions between human oral streptococci and strains of *A. viscosus* or *A. naeslundii*. Infect. Immun. **24**:742–752.
2. **Clark, W. B., L. L. Bammann, and R. J. Gibbons.** 1978. Comparative estimates of bacterial affinities and adsorption sites on hydroxyapatite surfaces. Infect. Immun. **19**:846–853.
3. **Clark, W. B., and R. J. Gibbons.** 1977. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. Infect. Immun. **18**:514–523.
4. **Costello, A. H., J. O. Cisar, P. E. Kolenbrander, and O. Gabriel.** 1979. Neuraminidase-dependent hemagglutination of human erythrocytes by human strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. Infect. Immun. **26**:563–572.
5. **Ellen, R. P., E. D. Fillery, K. H. Chan, and D. A. Grove.** 1980. Sialidase-enhanced lectin-like mechanism for *Actinomyces viscosus* and *Actinomyces naeslundii* hemagglutination. Infect. Immun. **27**:335–343.
6. **Ellen, R. P., and R. J. Gibbons.** 1979. Parameters affecting the adherence and tissue tropisms of *Streptococcus pyogenes*. Infect. Immun. **9**:85–91.
7. **Fuller, T.** 1973. Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. J. Appl. Bacteriol. **36**:131–139.
8. **Gibbons, R. J., and J. V. Qureshi.** 1978. Interaction of *Streptococcus mutans* and other bacteria with blood group-reactive substances, p. 163–181. *In* M. Stiles, W. Loesche, and T. O'Brien, (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. 1. Information Retrieval, Inc., Washington, D.C.
9. **Gibbons, R. J., and J. van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
10. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
11. **Gibbons, R. J., and J. V. Qureshi.** 1978. Selective binding of blood group-reactive salivary mucins by *Streptococcus mutans* and other oral organisms. Infect. Immun. **22**:665–671.
12. **Gibbons, R. J.** 1979. On the mechanisms of bacterial attachment to teeth, p. 267–274. *In* I. Kleinberg, S. A. Ellison, and I. D. Mandel, (ed.), Saliva and Dental Caries (a special supplement to Microbiology Abstracts). Information Retrieval Inc., Washington, D.C.

12a. **Gibbons, R. J.** 1980. Adhesion of bacteria to surfaces of the mouth, p. 351–388. *In* R. Berkeley, J. Lynch, J. Melling, P. Rutter, and B. Vincent (ed.), Microbial adhesion to surfaces. Ellis Horwood Ltd., London.

13. **Gibbons, R. J., and J. V. Qureshi.** 1979. Inhibition of adsorption of *Streptococcus mutans* strains to saliva-treated hydroxyapatite by galactose and certain amines. Infect. Immun. **26**:1214–1217.
14. **Gold, O., H. V. Jordan and J. van Houte.** 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. **18**:1357–1364.
15. **Hoskins, L. C., and E. T. Boudling.** 1976. Degradation of blood group antigens in human colon ecosystems. I. *In vitro* production of ABH blood group-degrading enzymes by enteric bacteria. J. Clin. Invest. **57**:63–73.
16. **Hoskins, L. C., and E. T. Boulding.** 1976. Degradation of blood group antigens in human colon ecosystems. II. A gene interaction in man that affects the fecal population density of certain enteric bacteria. J. Clin. Invest. **57**:74–82.
17. **Kilian, M.** 1978. Rapid identification of *Actinomycetaceae* and related bacteria. J. Clin. Microbiol. **8**:127–133.
18. **Liljemark, W. F., and R. J. Gibbons.** 1971. Ability of *Veillonella* and *Neisseria* species to attach to oral surfaces and their proportions present indigenously. Infect. Immun. **4**:264–268.
19. **Rolla, S., S. A. Robrish, and W. H. Bowen.** 1977. Interaction of hydroxyapatite with *Streptococcus mutans* and *Streptococcus sanguis*. Acta Pathol. Microbiol. Scand. Sect. B. **85**:341–346.
20. **Ruangsri, P., and D. Orstavik.** 1977. Effect of the acquired pellicle and of dental plaque on the implantation of *Streptococcus mutans* on tooth surfaces in man. Caries Res. **11**:204–210.