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Differences in the Adsorptive Behavior of Human Strains of *Actinomyces viscosus* and *Actinomyces naeslundii* to Saliva-Treated Hydroxyapatite Surfaces

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Human strains of *Actinomyces viscosus* and *A. naeslundii* differ in the time of their appearance and in their patterns of colonization in the mouth. Strains of these organisms were found to differ in their abilities to adsorb to saliva-treated hydroxyapatite (S-HA) surfaces, thought to mimic the teeth, and these differences parallel their patterns of colonizing the dentition. Thus, strains of *A. viscosus* tended to adsorb in higher numbers to hydroxyapatite (HA) treated with saliva of older children and adults than with saliva of younger children (ages 6 to 11). These salivary changes may account for the increased frequency with which this organism can be isolated from the mouths of children as they grow older. In contrast, strains of *A. naeslundii* and *Streptococcus mutans* did not show a preference for attaching to either type of S-HA. Strains of *A. viscosus* also generally adsorbed in higher numbers than *A. naeslundii* to HA treated with adult saliva; this may explain why higher proportions of *A. viscosus* are usually recoverable from the teeth of adults, even though *A. naeslundii* is generally present in higher proportions in saliva. Significant variation was noted between strains and between saliva samples collected from different donors. The differences in adsorptive behavior of strains of these species suggests that they are binding to different receptors in the salivary glycoprotein coating on HA surfaces. Adsorption of *A. naeslundii* ATCC 12104 was enhanced when S-HA was pretreated with neuraminidase, but this had little effect upon the adsorption of other *Actinomyces* strains tested. Adsorption of strain ATCC 12104 to S-HA was also strongly inhibited by fructose and sucrose and weakly inhibited by glucose, maltose, galactose, and lactose. However, other strains of *A. naeslundii* tested were affected less, or not at all, by these sugars. Adsorption of two strains of *A. viscosus* was not affected by any of the sugars or amines tested.

Actinomyces viscosus and *A. naeslundii* are attracting considerable interest because they are regularly present in dental plaques (5, 9) and are associated with gingivitis (17, 20) and cemental dental caries (19) in humans. Furthermore, they have been shown to induce periodontal pathology and root surface caries in experimental animals (12, 16, 18). Strains of these organisms isolated from humans are closely related (10, 14, 15; A. L. Coykendall and A. J. Munzenmaier, Int. Assoc. Dent. Res., abstr. no. 989, 1979; in fact, they are often distinguished solely on the basis of catalase activity (20). This has prompted several investigators to question whether they should be considered separate species.

Analyses of the guanine/cytosine ratios of their deoxyribonucleic acid and deoxyribonucleic acid-deoxyribonucleic acid hybridization studies have substantiated their close relationship (A. L. Coykendall and A. J. Munzenmaier, Int. Assoc. Dent. Res., abstr. no. 989, 1979). This is further supported by cluster analyses which suggested that the degree of difference between strains designated as *A. viscosus* or *A. naeslundii* is similar to that between the two recognized serotypes of *A. israelii* (10).

However, despite the apparent similarity of human strains of *A. viscosus* and *A. naeslundii*, Ellen (5) noted that they seem to differ in their patterns of oral colonization. Thus, catalase-negative strains considered *A. naeslundii* could often be isolated from the mouths of predentate infants, whereas catalase-positive strains resembling *A. viscosus* could not. Also, strains of *A. naeslundii* were present in saliva or plaque samples from all 3- to 4-year-old children examined, but the isolation frequency of strains of *A. viscosus* increased more slowly with age and the organism was only detected in about half of the 7- to 8-year-old children examined (5). In addition, plaque samples from older children (5) and adults (9) always contained higher proportions of *A. viscosus*, whereas *A. naeslundii* was more prevalent in saliva.

The differing patterns of colonization of the dentition by human strains considered to be *A. viscosus* or *A. naeslundii* could reflect differences in their abilities to attach to salivary pellicles coating the teeth. The present investigation was therefore initiated to compare the adherence of these organisms to hydroxyapatite (HA) surfaces treated with saliva of children and adults.

MATERIALS AND METHODS

Cultures and cultural conditions. *A. viscosus* LY7, CK8, and ATCC M100, *A. naeslundii* ATCC 12104 and 55N, and *A. odontolyticus* ATCC 17982 were obtained from H. V. Jordan, Forsyth Dental Center. Freshly isolated strains were obtained from samples of human dental plaque and whole saliva as described by Ellen and Balcerzak-Raczkowski (6). The organisms were identified on the basis of cellular morphology, catalase activity, nitrate reduction, ability to be agglutinated with a lectin contained in commercial mannose preparations (8), and by their reaction with high dilutions of reference antisera prepared to *A. viscosus* T14 and *A. naeslundii* ATCC 12104 (10). All strains were maintained on Trypticase soy blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) and in Trypticase soy broth (BBL); cultures were incubated in Brewer jars filled with 80% N_2, 10% CO_2, and 10% H_2 at 35°C.

Adsorption of *Actinomyces* strains to saliva-treated HA. The ability of strains of *A. viscosus* and *A. naeslundii* to attach to saliva-treated HA (S-HA) was determined with methods previously described (3). Stationary-phase cells harvested from Trypticase glucose broth cultures containing 10 μC of [^{3}H]thymidine per ml were washed three times and suspended in 0.05 M KCl containing 1 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ at pH 6 (buffered KCl). Samples (10 mg) of buffered KCl-equilibrated spheroidal HA beads were treated with 0.5 ml of clarified whole saliva by continuous inversion for 2 h at room temperature. The beads were then washed three times and incubated for 90 min with 2.5×10^7 washed [^{3}H]thymidine-labeled *Actinomyces* cells suspended in 0.5 ml of buffered KCl. The actinomycete suspensions were dispersed by repeated passage through a 27-gauge syringe needle immediately before use. Microscopic observations indicated that the suspensions existed as single cells or as small aggregates of two to three organisms. After incubation, the beads with attached bacteria were washed three times with buffered KCl, and the number of adherent organisms was determined by scintillation counting as previously described (3). All assays were performed in duplicate, and most experiments were repeated at least twice.

Collection of saliva. Samples of whole unstimulated saliva were collected in containers over ice from children and adults. The saliva was heated at 56°C for 30 min to inactivate degradative enzymes and then clarified by centrifugation at 10,000 × *g* for 10 min (3). The saliva was stored at −70°C until used for treating the HA beads.

Effect of neuraminidase treatment of S-HA and of the presence of various sugars and amines on adsorption of *A. viscosus* and *A. naeslundii*. The effect of neuraminidase treatment of S-HA was studied by using reaction mixtures which contained 30 mg of HA beads treated with 1.5 ml of clarified whole saliva collected from a single adult of blood type AB. The washed S-HA beads were incubated for 1 h with 1.5 ml of enzyme (500 μm/ml, Sigma Chemical Co., St. Louis, Mo.) derived from *Vibrio cholerae* in buffered KCl at pH 5. They were then washed twice with buffered KCl at pH 6.0 and incubated for 1 h with 1.5 ml of a washed suspension of actinomycetes cells adjusted to an optical density at 550 nm of 0.6, which represented ca. 10^8 organisms per ml, as based upon a standard curve relating optical density to actinomycete cell number. After incubation, the number of unadsorbed actinomycetes remaining in the liquor was determined by measuring the turbidity of the supernatant liquor at 550 mμ by using a Gilford spectrophotometer. Under the conditions used, from 40 to 60% of the total actinomyces cells added generally became associated with the S-HA beads. This method is similar to that previously used for studying bacterial adsorption to saliva-treated enamel powder (13); however, the HA beads did not contain "fines" which required dissolving with ethylenediaminetetraacetic acid. Control bacterial suspensions were incubated without S-HA beads, with untreated S-HA beads, and with S-HA beads which had been treated with heat-inactivated neuraminidase. To determine whether the presence of various sugars and amines could affect the attachment of *Actinomyces* strains to S-HA, we incorporated these compounds into similar reaction mixtures at a final concentration of 0.1 M.

RESULTS

Adsorption of *A. viscosus* and *A. naeslundii* strains to HA treated with saliva from children and adults. Initial experiments compared the ability of *A. viscosus* LY7 and *A. naeslundii* ATCC 12104 to attach to HA beads which had been pretreated with samples of saliva obtained from 6- to 11-year-old children, teenagers, and adults (Table 1). For comparative purposes, a recently isolated serotype *c* strain of *Streptococcus mutans* was also studied. Although *A. naeslundii* ATCC 12104 attached in somewhat higher numbers to S-HA than did *S. mutans* H12, neither organism showed a preference for attaching to HA which had been treated with saliva from the children or from the teenagers and adults (Table 1). In contrast, *A. viscosus* LY7 attached in 4- to 5-fold-higher numbers to HA which had been pretreated with saliva from the teenagers and adults than with saliva from the children (Table 1). The adherence of this strain was also greater than that of either *A. naeslundii* ATCC 12104 or *S. mutans* H12 to all of the S-HA surfaces studied.

The adsorption of these and other reference *Actinomyces* strains (*A. viscosus* LY7, CK8, and

TABLE 1. *Bacterial adsorption to HA pretreated with whole saliva from humans of various ages*

Age of saliva donors	No. of samples	No. of cells (×10⁶) adsorbed/10 mg of S-HA		
		A. viscosus LY7	*A. naeslundii* 12104	*S. mutans* H12
6–11	6	4.4 ± 0.6[a, b] (2.2–6.2)[c]	1.0 ± 0.1[b] (0.9–1.3)	0.44 ± 0.1[b] (0.3–0.7)
16–17	4	19.1 ± 5.6 (7.8–24.8)	0.61 ± 0.2 (0.4–1.0)	0.27 ± 0.04 (0.2–0.4)
25–45	6	15.2 ± 1.7 (11.2–18.4)	1.4 ± 0.2 (1.1–2.1)	0.61 ± 0.2 (0.2–1.1)

[a] $P < 0.01$ (t test).
[b] Mean ± standard error of the mean.
[c] Numbers in parentheses are the range.

ATCC M100; *A. naeslundii* ATCC 12104 and 55N; and *A. odontolyticus* ATCC 17982) to HA pretreated with saliva from an 11-year-old child or with saliva from a 28-year-old adult was also compared (Table 2). Each of the three *A. viscosus* strains and the strain of *A. odontolyticus* attached in 2- to 10-fold-higher numbers to HA pretreated with saliva from the adult than with saliva from the child. However, the attachment of *A. naeslundii* ATCC 12104 and 55N did not increase to a similar extent to HA pretreated with saliva from the adults (Table 2).

As a group, the strains of *A. viscosus* tended to adsorb in higher numbers than the other *Actinomyces* strains tested to HA treated with saliva from the adult (Table 2), though some strain variation was noted. For example, *A. viscosus* ATCC M100 attached in lower numbers to both types of S-HA than did strains LY7 and CK8. Similarly, *A. naeslundii* 55N showed a higher avidity for the S-HA surfaces than did *A. naeslundii* ATCC 12104.

Adsorption of freshly isolated strains of *A. viscosus* and *A. naeslundii* to HA treated with adult saliva. The adsorption of three freshly isolated strains of *A. viscosus* and *A. naeslundii* to HA pretreated with saliva from four adults was compared with reference strains LY7 and ATCC 12104 (Table 3). As a group, the *A. viscosus* strains again tended to adsorb in higher numbers to the S-HA surfaces than did the strains of *A. naeslundii*, although variations among strains and exceptions were noted. For example, the adherence of freshly isolated *A. naeslundii* strain P3 was greater than that of other *A. naeslundii* strains to HA treated with three of the four saliva samples tested. In addition, all of the *A. naeslundii* strains attached comparably to the *A. viscosus* strains to HA treated with saliva from donor 3, but not to HA treated with saliva of the other individuals tested (Table 3). It was also found that HA

TABLE 2. *Adsorption of Actinomyces strains to HA treated with saliva from a child and an adult*

Organism	No. of cells (×10⁶) adsorbed/10 mg of HA treated with:		Relative adsorption (adult saliva/child saliva)
	Saliva of an 11-year-old child	Saliva of a 28-year-old adult	
A. viscosus			
LY7	0.9 ± 0.12[a]	9.2 ± 0.03[a]	10.2
CK8	4.2 ± 0.49	10.5 ± 0.56	2.5
ATCC M100	0.4 ± 0.02	2.0 ± 0.03	5.0
A. naeslundii			
ATCC 12104	0.3 ± 0.03	0.5 ± 0.08	1.7
55N	1.4 ± 0.23	2.0 ± 0.06	1.4
A. odontolyticus			
ATCC 17982	0.2 ± 0.02	2.0 ± 0.45	10.0

[a] Mean ± standard error of the mean.

treated with saliva from two individuals (donors 2 and 4) adsorbed higher numbers of *A. viscosus* cells than did HA treated with saliva from other donors. Since these assays were run simultaneously using the same bacterial cell suspensions, and they were duplicated, the differences observed do not appear to be attributable to experimental variation; rather, they appear to reflect differences in the composition of the saliva samples.

Effect of various sugars and amines on adsorption of strains of *A. naeslundii* and *A. viscosus* to S-HA. The ability of various sugars and amines to inhibit the adsorption of strains of *A. viscosus* and *A. naeslundii* to S-HA was studied in an effort to elucidate the nature of the receptors involved. Adsorption of *A. naeslundii* ATCC 12104 was moderately inhibited by fructose and sucrose, and weakly inhibited by glucose, maltose, galactose, and lactose (Table 4). Adsorption of freshly isolated *A. naeslundii* L13 was weakly inhibited by glucose, galactose, sucrose, and, possibly, lactose, but *A. naeslundii* L7 and *A. viscosus* LY7 and V9 were not significantly affected by any of the sugars tested (Table 4). Similarly, the adsorption of none of the strains tested was affected by 10 mg/ml of dextran (Pharmacia, Uppsala, Sweden) of molecular weights 10,000 and 20,000, or by 0.1 M spermine, putrescine, lysine, ammonium chloride, or iodoacetate (Table 4).

Effect of neuraminidase pretreatment of S-HA on adsorption of *A. viscosus* or *A. naeslundii*. The ability of strains of *A. viscosus* and *A. naeslundii* to attach to erythrocytes has been shown to be dependent upon or enhanced by neuraminidase which may be either synthe-

TABLE 3. *Adsorption of A. viscosus and A. naeslundii strains to HA treated with saliva of adults*

Organism	Source	No. of cells ($\times 10^6$) adsorbed/10 mg of HA treated with saliva from:			
		Donor 1	Donor 2	Donor 3	Donor 4
A. viscosus					
LY7	Plaque	9.0 ± 1.28[a]	24.3 ± 0.04[a]	7.0 ± 0.21[a]	21.1 ± 0[a]
V10	Plaque	12.6 ± 0.03	19.6 ± 0.09	10.5 ± 0.08	23.3 ± 0.12
V9	Plaque	12.9 ± 0.13	22.3 ± 0.15	10.9 ± 0.04	22.7 ± 0.14
V15	Plaque	10.5 ± 0.05	22.9 ± 0.14	8.9 ± 0.75	21.4 ± 0
	Mean	11.3 ± 0.92	22.3 ± 0.98	9.3 ± 0.89	22.5 ± 0.52
A. naeslundi					
ATCC 12104	Sinus	2.6 ± 1.7	5.5 ± 2.85	1.5 ± 0.06	1.3 ± 0.21
L13	Saliva	5.2 ± 0.67	8.7 ± 0.75	16.1 ± 0.01	16.5 ± 0.17
L7	Saliva	4.7 ± 0.82	11.5 ± 2.36	12.4 ± 0.03	12.9 ± 0.10
P3	Plaque	10.0 ± 0.02	19.8 ± 0.22	10.9 ± 0.08	21.9 ± 0.15
	Mean	5.6 ± 1.56[b]	11.4 ± 3.06[b]	10.2 ± 3.11	13.2 ± 4.36[c]

[a] Mean ± standard error of the mean.
[b] $P < 0.05$.
[c] $P < 0.10$.

TABLE 4. *Effect of 0.1 M sugars and amines on adsorption of strains of A. naeslundii and A. viscosus to S-HA*

Substance[a]	% Adsorption relative to buffer				
	A. naeslundii strain			*A. viscosus* strain	
	12104	L13	L7	LY7	V9
Buffer	100	100	100	100	100
Glucose	68	64	91	110	92
Galactose	73	70	101	109	101
Fructose	20	87	116	103	100
Sucrose	37	74	109	118	99
Lactose	75	79	115	105	105
Maltose	60	90	125	107	107

[a] 0.1 M fucose, mannose, xylose, rhamnose, glucosamine, galactosamine, mannosamine, *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylmannosamine, spermine, putrescein, L-lysine, NH_4Cl, iodoacetate, and 10 mg of dextran of molecular weight 20,000 and 10,000 per ml gave values similar to buffer controls for all strains.

sized by the organisms or provided exogenously (4, 7). Consequently, the effect of neuraminidase pretreatment of the S-HA beads on the subsequent adsorption of *Actinomyces* strains was determined. Neuraminidase pretreatment exerted a moderate to slight enhancing effect upon adsorption of the three *A. naeslundii* strains tested, but *A. viscosus* LY7 and V9 were unaffected (Table 5). It is not known whether the effect of neuraminidase pretreatment was the result of enzymatic action in removing sialic acid residues, or whether the enzyme became bound to the S-HA and promoted actinomycete adsorption.

TABLE 5. *Effect of pretreatment of S-HA with neuraminidase on adsorption of strains of A. viscosus and A. naeslundii*

S-HA pretreatment	% Adherence relative to buffer controls				
	A. viscosus strain		*A. naeslundii* strain		
	LY7	V9	ATCC 12104	L13	L7
Buffer control	100	100	100	100	100
500 μm of neuraminidase per ml	97	111	143	122	121
Heat-inactived neuraminidase	112	105	105	101	107

DISCUSSION

The present study has shown that strains of *A. viscosus* and *A. naeslundii* often adhere differently to S-HA surfaces which mimic the teeth, and their adherent properties appear to parallel their differing patterns of oral colonization. Thus, strains of *A. viscosus* generally adsorbed in higher numbers than strains of *A. naeslundii* to pellicles formed from samples of adult saliva; this may explain why higher proportions of *A. viscosus* than *A. naeslundii* are generally recoverable from the teeth of older children and adults, even though *A. naeslundii* is usually present in higher proportions in saliva to which the teeth are exposed (5, 9). In addition, strains of *A. viscosus* tended to adsorb more avidly to HA treated with saliva from teenaged children and adults than with saliva from younger children. In contrast, strains of *A. naeslundii* or *S. mutans* did not exhibit a preference for either

type of S-HA surface. *A. naeslundii* is able to effectively colonize oral mucosal surfaces since it can be isolated from the mouths of some predentate infants (5). Its ability to colonize the mucosa in addition to the teeth is probably responsible for its higher concentrations found in saliva of humans of all ages. These attributes, i.e., high salivary concentrations and ability to colonize mucosal surfaces, help to explain why infants and young children who are exposed to salivary contamination from parents and siblings generally become colonized by this organism before colonization by *A. viscosus*. However, the composition of saliva appears to change with age, and the increased avidity of *A. viscosus* strains to pellicles formed from the saliva of older children and adults would be expected to increase the likelihood of such individuals becoming infected by this organism. This may account for the increasing frequency with which this organism can be isolated from the mouths of children as they grow older.

It is of interest to note that rodent strains of *A. viscosus*, which are recognized to be genetically and serologically distinct from the human strains studied in the present investigation (10; A. L. Coykendall and A. J. Munzenmaier, Int. Assoc. Dent. Res., abstr. no. 989, 1979), display differences in their ability to colonize old versus young rats (1). However, the increased susceptibility of rats older than 30 days of age to infection by these organisms appears to be attributable to a reduction of enamel epithelium in the fissures of their recently erupted molar teeth, rather than to an alteration in the composition of their saliva (1).

The different adsorptive behaviors of human strains of *A. viscosus* and *A. naeslundii* to S-HA surfaces noted in the present study suggest that these organisms are binding to different salivary receptors in the pellicle on HA surfaces. This was also suggested by earlier studies of adsorption isotherms which indicated that there were higher numbers of binding sites on HA treated with adult saliva for *A. viscosus* T14 than for *A. naeslundii* ATCC 12104 (3). The observation that certain sugars inhibit the adsorption of some strains of *A. naeslundii* but not those of *A. viscosus* also supports this. The adsorption of both *Actinomyces* species to S-HA surfaces has features which are quite different than those for *S. mutans*, and thus still different receptors appear to be involved in attachment of this organism. For example, the adsorption of *S. mutans* strains of several serotypes to S-HA is inhibited by various amines and iodoacetate (11), whereas these compounds did not affect adsorption of any of the *Actinomyces* strains studied. In addition, lactose, a β-galactoside, weakly inhibited adsorption of *A. naeslundii* ATCC 12104, but this sugar had no effect on the adsorption of the eight *S. mutans* strains tested; in contrast, adsorption of all *S. mutans* was affected by α-galactosides.

Strains considered to be *A. viscosus* or *A. naeslundii* proved to be heterogeneous in a number of characteristics. They differed among and between species in their adherence to HA surfaces pretreated with saliva of different individuals. Differences were also noted in the patterns of inhibition caused by various sugars among the three strains of *A. naeslundii* studied (Table 4). Other investigators have also noted heterogeneity among strains. For example, strains differ in their abilities to hemagglutinate horse, sheep, or human erythrocytes (7), and they also segregate into several clusters by numerical taxonomy (10).

It has recently been demonstrated that the hemagglutinating activity of strains of *A. viscosus* or *A. naeslundii* is either dependent upon or enhanced by neuraminidase, and it is uniformly inhibited by β-galactosides. Neuraminidase is thought to remove terminal sialic acid residues from cell surface glycoproteins which thereby exposes β-galactoside moieties to which the organisms attach via surface fimbriae. The coaggregation of *Actinomyces* strains with strains of *S. sanguis* is also inhibited by β-galactosides (2). However, the attachment of these organisms to S-HA surfaces appears to involve more complex interactions. This is indicated by the observation that β-galactosides only weakly affected the adsorption of two of three strains of *A. naeslundii* to S-HA, and it had no effect upon the two strains of *A. viscosus* (Table 4). These results are consistent with the report of Wheeler et al. (21), who also noted that adsorption of *A. viscosus* T14V to S-HA was not affected by lactose. In addition, pretreatment of the S-HA with neuraminidase did not strongly enhance adsorption of four of the five strains tested. Collectively, these observations suggest that strains of *A. viscosus* and *A. naeslundii* possess other surface ligands in addition to those which bind to β-galactosides which are involved in their attachment to S-HA surfaces.

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