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Lactose-Reversible Coaggregation Between Oral Actinomycetes and *Streptococcus sanguis*

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Freshly isolated strains of oral actinomycetes were obtained from human dental plaque and were tested for the ability to coaggregate with common laboratory stock strains of *Streptococcus sanguis*. Strains belonging to the genera *Actinomyces*, *Arachnia*, *Bifidobacterium*, and *Bacterionema* were isolated. Only members of the genus *Actinomyces* coaggregated with the streptococci, and only *Actinomyces viscosus* and *Actinomyces naeslundii* exhibited lactose-reversible interactions. A total of 61 strains, consisting of all of the *A. viscosus* isolates and 86% of the *A. naeslundii* isolates, coaggregated; 87% exhibited lactose-reversible coaggregation. On the basis of this property and the altered ability of strains to coaggregate after heat treatment of the cells, we delineated four coaggregation groups. The other 13% of the strains constituted a fifth group, which was characterized by a pattern of closely related interactions that were not reversed by lactose. Compared with previously characterized coaggregation properties determined with stock culture strains of actinomycetes, more than 80% of these fresh isolates exhibited identical coaggregation properties. Thus, most of the coaggregation between freshly isolated oral actinomycetes and streptococci involves lactose-reversible cell-cell interactions, which suggests that such coaggregation is mediated by a network of lectin-carbohydrate interactions between complementary cell surface structures on the two cell types.

Interactions between different bacterial species that inhabit the same ecological niche may be very important in the establishment of certain bacterial microcommunities. Dental plaque represents one such microcommunity, which is characterized by a complex and highly organized set of interactions among oral bacteria as well as between bacteria and solid surfaces (11). Of the several examples of interactions among oral bacteria (1, 7, 8, 13), the coaggregation of *Actinomyces* species and *Streptococcus* species has received the greatest attention (2, 3, 5, 10, 12). Gibbons and Nygaard observed coaggregation of several bacterial pairs, including *Actinomyces viscosus* and *Actinomyces naeslundii* with *Streptococcus sanguis* strains (7). By testing the coaggregation of human *A. naeslundii* isolates with *S. sanguis* and *Streptococcus mitis* strains, Ellen and Balcerzak-Raczkowski (5) found that exposure to heat or to proteolytic enzymes reduced the ability of the actinomycetes, but not the streptococci, to react with untreated cells of other types. These results suggested a role for cell surface proteins on the actinomycetes in mediating coaggregation. McIntire and colleagues examined the mechanism of coaggregation between *A. viscosus* T14V and *S. sanguis* 34 and discovered that this interaction was reversed by lactose and was dependent on calcium ions (12). Thus, coaggregation appeared to result from a lectin-carbohydrate interaction between surface components.

In a recent survey, the properties of coaggregation reactions between a variety of oral *Streptococcus* and *Actinomyces* species were used to define four groups of streptococci and two groups of actinomycetes (3). The parameters examined were (i) detectable coaggregation when the two cell types were mixed, (ii) the effect of heating cells on the ability of the heated cell type to coaggregate, and (iii) the effect of adding lactose to reverse the coaggregation. Group A actinomycetes had lactose-nonreversible interactions with both group 1 and group 2 streptococci and lactose-reversible coaggregations with group 3 and group 4 streptococci, but only if the group 4 streptococcal cells were heated before they were mixed with the actinomycetes. Group B actinomycetes were not able to coaggregate with group 1 streptococci, showed lactose-nonreversible coaggregation with group 2 streptococci, and had lactose-reversible inter-

actions with both group 3 and group 4 streptococci. All coaggregations which were lactose reversible were abolished by heating the actinomycete cells but not by heating the streptococci, whereas coaggregations which were not lactose reversible were prevented by heating the streptococci but not by heating the actinomycetes. Of all the strains tested, only human strains of *A. viscosus* and *A. naeslundii* coaggregated with *S. sanguis* and *S. mitis* strains.

All of the *Actinomyces* species and most of the *Streptococcus* species used in the previous study were stock culture strains that had been transferred an unknown number of times in various laboratories. To further our study of the interactions among different oral bacteria, we examined fresh human isolates of oral actinomycetes for the ability to coaggregate with oral streptococci and compared their coaggregation properties with those of the previously characterized stock culture strains.

MATERIALS AND METHODS

Bacterial strains. Previously characterized actinomycetes and streptococci (3) served as reagent strains and were used to define the coaggregation properties of the new oral actinomycetes used in this study. All of the following reagent strains used were of human origin: *A. viscosus* strains MG1, T14V, and T14AV; *A. naeslundii* strains WVU45 (ATCC 12104), I, and W1544; and *S. sanguis* strains DL1 (Challis), H1, 34, and J22.

Procedure for isolating fresh strains of oral actinomycetes. Each human plaque sample was taken with a sterile curette from the posterior lingual surface of either the first or the second molar at the gingival margin. The samples were transferred immediately to freshly steamed fluid thioglycolate broth (Difco Laboratories, Detroit, Mich.) containing 20% beef infusion. Dilutions were made in the same medium, and 0.1-ml portions were spread onto blood agar plates prepared with Columbia agar base (BBL Microbiology Systems, Cockeysville, Md.). These plates were incubated anaerobically at 37°C under an atmosphere containing H_2, CO_2, and N_2 (10:10:80) by using Gas-Paks (BBL Microbiology Systems). After 5 days, isolated colonies were transferred to fresh Columbia blood agar plates and streaked twice for purity. Isolated colonies were transferred to fluid thioglycolate broth containing 20% beef infusion and 5% calcium carbonate. When a moderate amount of growth was visible (1 to 5 days), 1 ml was transferred into 10 ml of complex broth medium containing 0.2% glucose as the carbon source (3). These cultures were used to inoculate 100 ml of the same medium to obtain cells for coaggregation experiments.

Identification of isolates. The new isolates of *Actinomyces* and related organisms were subjected to a battery of 51 taxonomic tests. The following tests were used, as described previously (17): Gram stain reaction; branching morphology, as judged by gram-stained smears from colonies; dark-field observation of broth cultures; hydrogen peroxide decomposition; and relative surface growth in an aerobic environment containing 10% carbon dioxide and 80% nitrogen. Gas chromatographic analyses of metabolic end products were performed by the methods described by Holdeman et al. (9) except that we used a stainless steel column (6 feet [1.83 m] by 0.25 in. [0.64 cm]) packed with 15% S-1220 containing 1% phosphoric acid on Chromosorb WAW (100/120 mesh; Supelco, Inc., Bellefonte, Pa.).

The inocula for the biochemical tests were from 2-day cultures in prereduced, anaerobically sterilized peptone yeast extract medium containing Tween 80 (9). Inocula (1.5 to 2.0 ml) were suspended in 100 ml of the same basal medium immediately after the medium had been autoclaved and cooled to room temperature; 2 ml of the suspended inoculum was then dispensed into each culture tube containing 0.2 ml of a 10× concentrate of substrate in distilled water. A 0.5-ml amount of inoculum was added to 5 ml of milk and 5 ml of gelatin medium (9). The culture tubes, which had loose-fitting caps, were incubated in an anaerobic atmosphere containing 10% H_2, 10% CO_2, and 80% N_2, which was achieved by evacuation and replacement. Acid production was measured by using a pH meter (Beckman Instruments, Inc., Irvine, Calif.) after incubation at 35°C for 6 days. Assays for indole production, reduction of nitrate and nitrite, and deamination of arginine were performed as described by Cowan (4). Hydrolysis of esculin, gelatin, and starch, reactions in milk, and the deamination of urea were assayed by the methods of Holdeman et al. (9).

On the basis of the above-described tests, 51 characters were coded 1 for positive or 0 for negative. Numerical analyses were performed by using the Jaccard similarity coefficient, which excludes negative matches (14), and programs UMDTAXON 6 and IGPS 2, which were generously provided by R. R. Colwell, University of Maryland. Microbial clusters were formed by an unweighted average linkage method (16). Computations were performed at the Locke Computer Center, University of Washington. Included in the numerical analysis were the following reference strains: *A. viscosus* ATCC 19246, *A. naeslundii* ATCC 12104 and ATCC 19039, *Actinomyces israelii* ATCC 12103 and ATCC 23860, *Actinomyces meyeri* VPI 12031, *Actinomyces odontolyticus* CDC W1514, and *Bifidobacterium adolescentis* CDC X573 (*Actinomyces eriksonii* ATCC 15423).

Coaggregation assay. The conditions of the bacterial growth and coaggregation assay have been described previously (3). The bacterial cells were grown in a complex medium containing glucose as the carbon source, and then they were harvested in the mid to late exponential phase of growth, washed, and suspended in coaggregation buffer, which consisted of the following [dissolved in 0.001 M tris(hydroxymethyl)aminomethane, pH 8.0]: $CaCl_2$, 10^{-4} M; $MgCl_2$, 10^{-4} M; NaN_3, 0.02%; and NaCl, 0.15 M. Coaggregation was determined by the visual assay method (3) by mixing equal volumes of cell suspensions (about 10^{10} cells per ml) of a fresh oral isolate and an appropriate reagent streptococcal strain. After overnight incubation of this mixture at room temperature, the degree of coaggregation was assigned a score from 0 to 4. The

absence of coaggregation (scored as 0) appeared as a homogeneous cell suspension. A score of 4 was given to a mixture with a clear supernatant fluid and large coaggregates which settled immediately to the bottom of the tube. Each freshly isolated oral actinomycete strain was tested against each of four reagent stock strains of *S. sanguis*, and the coaggregation pattern was compared with the patterns obtained by mixing pairs of reagent stock actinomycetes with these streptococci.

Heat treatment of cells. Actinomycetes and streptococci at cell densities of about 10^{10} cells per ml in coaggregation buffer were heated separately at 85°C for 30 min and then cooled in a water bath at room temperature. Coaggregation assays were performed with heated and unheated cells of each type.

Effect of lactose and ethylenediaminetetraacetic acid on coaggregation. Reversal of coaggregation was determined by adding to a suspension of coaggregated bacteria either 1 M lactose or 0.01 M ethylenediaminetetraacetic acid to give final concentrations of 0.06 M and 0.6 mM, respectively. Reversal of coaggregation was defined as the complete disappearance of bacterial coaggregates.

RESULTS

Plaque samples were obtained from 16 individuals (age range, 13 to 48 years). In a preliminary test, 363 fresh oral isolates were examined for the ability to coaggregate with reagent actinomycetes and streptococci. Of these isolates, 157 (43%) exhibited some pattern of coaggregation with the reagent stock strains. Thus, it appears that coaggregation is a common property of many oral bacteria.

Diagnostic identity and numerical taxonomy of fresh isolates. There were 112 isolates which were identified as gram-positive rods. Each of these was tested for 51 characteristics, including the following: evidence of branching when examined by dark-field illumination; catalase reaction; relative growth under aerobic (10% CO_2 plus air) and anaerobic (10% H_2–10% CO_2–80% N_2) atmospheres; 41 fermentation and biochemical reactions; and end product analysis of glucose fermentation. Presumptive identification of each isolate was accomplished by (i) including a reference culture within the cluster obtained by numerical analysis or (ii) consulting the keys provided in the *Anaerobe Laboratory Manual* (9). Numerical analyses with the Jaccard similarity coefficient resulted in seven clusters or phena containing three or more isolates with ≥60% similarity. The rather low similarity value of 60% was used to define clusters because of the metabolic variability observed with most strains of *A. viscosus* and *A. naeslundii* (Williams, unpublished data). A simplified dendrogram is shown in Fig. 1.

Because strains of *A. viscosus* and *A. naeslundii* share a large number of characteristics, the presence of catalase activity in *A. viscosus* remains the primary distinguishing feature between these two species. On the other hand, isolates within each of these species exhibit widely varying characteristics, and for this reason it has been proposed that the isolates be split into typical and atypical categories (6). In this study, we observed a similar intraspecies heterogeneity, as well as interspecies relatedness among isolates (Table 1). Intraspecies heterogeneity was manifested in the distribution of isolates of these two species into five of the seven clusters and into the miscellaneous group of unrelated bacteria. Interspecies relatedness was evident in that two clusters, clusters 1 and 3, contained members of both *A. viscosus* and *A. naeslundii* and no cluster was composed solely of *A. viscosus* isolates. In addition, interspecies relatedness was observed in the American Type Culture Collection reference strains *A. viscosus* ATCC 19246 and *A. naeslundii* ATCC 12104, which were placed in cluster 1. Cluster 1 consisted solely of *A. viscosus* and *A. naeslundii* isolates, and these isolates were obtained from 12 of the 16 plaque samples. Cluster 2 contained seven *A. naeslundii* isolates from a single sample. The numbers of plaque samples providing isolates that belonged to the remaining clusters were as follows: cluster 3, 7 samples; cluster 4, 1 sample; cluster 5, 2 samples; cluster 6, 7 samples; cluster 7, 7 samples; and unclustered group X, 6 samples. Cluster 3 was composed of *Bifidobacterium* species and *A. naeslundii*, as well as three strains of *A. viscosus*. Although the *Bifidobacterium* species exhibited 60 to 70% phenotypic relatedness to the *A. naeslundii* isolates, each species comprised a cohesive subgroup having 80 to 90% intraspecies relatedness. The *Bifidobacterium* reference strain X573 was placed in the center of the *Bifidobacterium* subgroup. Two *A. israelii* reference strains, ATCC 23860 and ATCC 12103, showed 50 to 60% relatedness with the *A. viscosus* and *A. naeslundii* isolates of cluster 3. Using the ability to grow aerobically in the presence of 10% CO_2 as the primary feature distinguishing *A. naeslundii* from *A. israelii*, we identified no isolates as belonging to the latter species. *A. odontolyticus* W1514 was 70% related to the other *A. odontolyticus* isolates of cluster 6. *A. meyeri* VPI 12031 was related to only one other isolate. Clusters 4 and 7 consisted of all of the *Arachnia propionica* isolates and all of the *Propionibacterium acnes* isolates, respectively. It is clear from this analysis that the actinomycetes tested in this coaggregation study represented a phenotypically diverse group of organisms.

Distribution of coaggregating actinomycetes among plaque samples. One of the aims

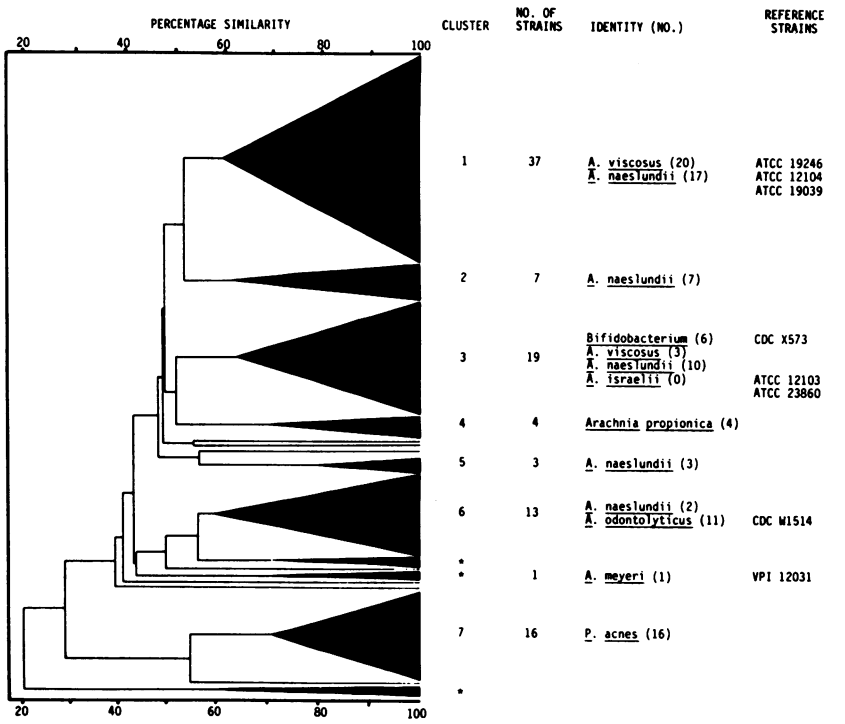


FIG. 1. *Simplified dendrogram constructed by using the Jaccard similarity coefficient and unweighted average linkage clustering. Only clusters containing three or more strains were given a cluster number. ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Disease Control, Atlanta, Ga.; VPI, Virginia Polytechnic Institute, Blacksburg, Va.*

of this study was to determine the prevalence among the plaque samples tested of actinomycetes that were capable of coaggregating with the four reagent strains of oral streptococci. The four reagent strains used represented the four streptococcal coaggregation groups (3). Actinomycete isolates were obtained from all 16 plaque samples. None of the 11 actinomycetes belonging to the genera *Arachnia*, *Bifidobacterium*, and *Bacterionema* coaggregated with the reagent strains. Of 14 *A. odontolyticus* isolates 3 coaggregated with only two of the four reagent strains. These interactions were neither lactose reversible nor similar to the coaggregation patterns found with *A. viscosus* and *A. naeslundii* and were not investigated further. *A. viscosus* and *A. naeslundii* isolates were identified in 15 of the 16 plaque samples, and one or more of the isolates from 14 of these samples coaggregated with streptococci. All of the *A. viscosus* isolates coaggregated, as did 37 of 43 *A. naeslundii* isolates. Thus, coaggregating *A. viscosus* and *A. naeslundii* strains were isolated from 87% of the plaque samples examined, and 91% of the strains coaggregated with oral streptococci.

Description of coaggregation properties of *A. viscosus* and *A. naeslundii* fresh isolates. The following three criteria were used to define the coaggregation properties of freshly isolated oral actinomycetes: (i) the ability to coaggregate in a specific pattern with reagent streptococcal strains; (ii) the reversibility of these coaggregations by lactose; and (iii) the inactivation of the ability of the cells to coaggregate by heat or proteolytic enzyme treatment. On the basis of these criteria, two coaggregation groups (groups A and B) were described in a previous investigation of the coaggregation properties of stock culture strains (3). After a large number of freshly isolated oral actinomycetes were examined, representatives of both of these groups were detected, as well as two new coaggregation groups, groups C and D. In addition, eight freshly isolated strains of *A. naeslundii* exhibited coaggregation reactions identical to the reaction of *A. viscosus* T14AV, a variant of the original group A. Group E as designated here included those actinomycetes which gave this pattern of coaggregation. Therefore, only groups C and D represent new coaggregation patterns

TABLE 1. *Properties of clusters of oral actinomycetes*[a]

Cluster	Species	Coaggregation group	
		Designation	No. of isolates
1	*A. viscosus*	A	20
	A. naeslundii	A	6
		B	3
		C	7
		None	1
2	*A. naeslundii*	E	7
3	*A. viscosus*	A	3
	A. naeslundii	A	5
		B	2
		C	1
		D	1
		E	1
	B. adolescentis	None	6
4	*Arachnia propionica*	None	4
5	*A. naeslundii*	None	3
6	*A. naeslundii*	C	1
		None	1
	A. odontolyticus	None	11
7	*P. acnes*	None	16
X[b]	*A. viscosus*	A	1
	A. naeslundii	A	1
		B	1
		D	1
		None	1
	A. odontolyticus	None	3
	A. meyeri	None	1
	Bacterionema matruchotii	None	1
	Unclassified	None	2

[a] Clusters were determined by numerical methods and computer analysis.
[b] Unclustered strains.

observed in this study.

A summary of the coaggregation reactions between members of the five groups of actinomycetes and the four streptococcal groups is shown in Table 2. In order to compare the properties of the new groups, the salient coaggregation properties of the previously defined groups are as follows: (i) group A actinomycetes do not exhibit lactose-reversible interactions with group 1 streptococci but do show lactose-reversible unimodal (heat inactivation of only one cell type is sufficient to inhibit coaggregation) coaggregation with group 3 streptococci; (ii) group B actinomycetes do not react with group 1 streptococci but show lactose-reversible unimodal coaggregation with both group 3 and group 4 streptococci; and (iii) group E actinomycetes exhibit only unimodal lactose-nonreversible interactions, and they do not coaggregate with group 3 streptococci. The principal distinguishing features of the new coaggregation groups were as follows: (i) group C actinomycetes showed unimodal lactose-reversible interactions with group 1 streptococci and bimodal lactose-reversible coaggregation reactions (heat inactivation of both cell types was necessary to inhibit coaggregation completely) with group 3 streptococci (most of these interactions were completely reversed by lactose, but some were partially reversed to a coaggregation score of 1); and (ii) group D actinomycetes showed bimodal coaggregations with both group 3 and group 4 streptococci. All coaggregation reactions were reversed by 10^{-3} M ethylenediaminetetraacetic acid.

The simplest explanation for these new coaggregation reactions is that two previously undetected interactions were observed during this investigation of fresh isolates. One was lactose reversible, and the other was not. Both involved heat-stable determinants on group C and D actinomycetes that were recognized by heat-inactivated determinants on group 1 and 3 streptococci (see below). With the exception of group E, coaggregation reactions occurred between actinomycetes and heat-inactivated members of

TABLE 2. *Actinomycete coaggregation groups defined by their coaggregation patterns with reagent streptococcal strains*

Actinomycete group	*Streptococcus* heated[a]	Actinomycete heated[a]	Reaction with streptococcal group:[b]			
			1	2	3	4
A	Yes	Yes	0	0	0	0
	Yes	No	0	0	4[c]	4[c]
	No	Yes	3	4	0	3
	No	No	3	4	4[c]	4
B	Yes	Yes	0	0	0	0
	Yes	No	0	0	4[c]	4[c]
	No	Yes	0	3	0	0
	No	No	0	3	4[c]	4[c]
C	Yes	Yes	0	0	0	0
	Yes	No	0	0	4[c]	4[c]
	No	Yes	2[c]	3	2[c]	0
	No	No	2[c]	3	4[c]	4[c]
D	Yes	Yes	0	0	0	0
	Yes	No	0	0	2[c]	2[c]
	No	Yes	3	4	3	4
	No	No	3	4	3	4
E	Yes	Yes	0	0	0	0
	Yes	No	0	0	0	0
	No	Yes	3	4	0	3
	No	No	3	4	0	3

[a] Cell suspension heated at 85°C for 30 min.
[b] Scores were from 0 for no coaggregation to 4 for maximum coaggregation. The representatives of streptococcal groups 1 through 4 used were *S. sanguis* DL1, H1, 34, and J22, respectively.
[c] Coaggregation was reversed by 0.06 M lactose.

streptococcal groups 3 and 4, and in all cases these reactions were reversed completely by 0.06 M lactose (final concentration). In contrast, we detected no reactions between heat-inactivated group 1 or group 2 streptococci and any of the actinomycete groups. The constancy of the lactose reversibility of coaggregations occurring only between oral *S. sanguis* strains and *A. viscosus* or *A. naeslundii* isolates suggests that these interactions are highly specific between these bacteria and are not widespread among other oral actinomycetes.

Distribution of fresh oral isolates among coaggregation groups. Only *A. viscosus* and *A. naeslundii* isolates were represented in the five coaggregation groups (Table 3). None of the other isolates exhibited such coaggregation patterns. Perhaps the most significant feature of the distribution of the isolates was that 100% of the *A. viscosus* isolates fell into group A, whereas *A. naeslundii* isolates were found in all five groups. Thus, not only were the *A. naeslundii* isolates diverse in the numerical taxonomic clustering, but they also exhibited a variety of coaggregation reactions with oral streptococci.

It is evident that the group A pattern of reactions was the most common pattern of coaggregation for oral actinomycetes. Group A contained more than one-half of the isolates (36 of 61 isolates) and was represented by isolates from more plaque samples (12 of 16 samples) than any other group. In fact, new groups C and D collectively contained only 11 isolates (10% of the total actinomycetes) and constituted less than 20% of the coaggregating actinomycetes. Hence, more than 80% of the coaggregations observed with freshly isolated actinomycetes were like those of the group A (including group E) and B stock strains examined previously (3).

TABLE 3. *Relationship of coaggregation groups to Actinomyces species*

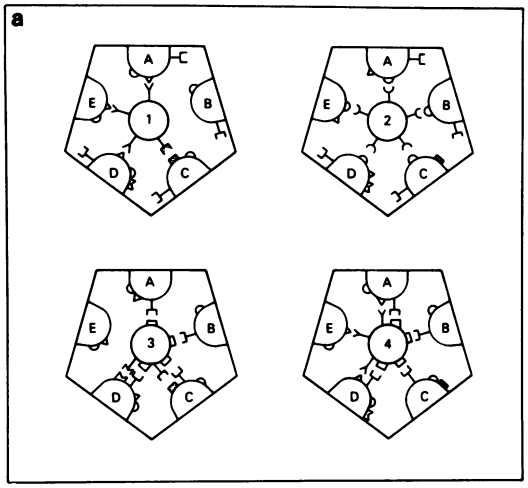
Coaggregation group	No. of plaque samples	Species	% Of total coaggregating strains	Clusters represented	No. of isolates
A	12	*A. viscosus*	39	1	20
				3	3
				X	1
		A. naeslundii	20	1	6
				3	5
				X	1
B	3	*A. naeslundii*	10	1	3
				3	2
				X	1
C	4	*A. naeslundii*	15	1	7
				3	1
				6	1
D	2	*A. naeslundii*	3	3	1
				X	1
E	2	*A. naeslundii*	13	2	7
				3	1

DISCUSSION

The primary aim of this study was to determine whether freshly isolated actinomycetes exhibited coaggregation properties similar to or different from those of the stock cultures of *Actinomyces* species examined in our previous study (3). At least seven laboratories served as original culture sources for one or more of the stock cultures used in the previous study. However, some of the strains, which originated in one laboratory, were supplied to us by a different laboratory. Consequently, culture maintenance was different for each culture, and, more importantly, the lineage of the cultures was impossible to trace, at least in some cases.

From the results presented here, it is clear that the vast majority (82%) (Table 3, groups A, B, and E) of the coaggregating fresh isolates exhibited coaggregation properties identical to those observed previously with stock culture strains. The other coaggregating strains exhibited closely related coaggregation properties in that they involved lactose-reversible coaggregations with oral streptococci. The fact that the coaggregation properties of most isolates were identical to those of stock culture strains strongly suggests that the surface determinants involved in coaggregation are stable structures. The combined observations reported here and previously (3) indicate that only a limited number of coaggregation patterns are likely between oral streptococci and actinomycetes and that most of these involve lactose-reversible interactions.

Figure 2 shows a diagrammatic representation of the interactions between the four *S. sanguis* coaggregation groups and the five *Actinomyces* groups. Most of the freshly isolated actinomycetes fell into groups A, B, and E, which were the groups described previously (3). Two additional patterns of coaggregation were observed in this present study, and these were used to define groups C and D. To account for these new patterns, we show two additional interactions between the actinomycetes of these new groups and the streptococci of groups 1 and 3. Both involve heat-stable components on the actinomycetes which are recognized by heat-inactivated surface components on the streptococcal cells. One interaction is reversed by lactose, and the other is not (Fig. 2b). New interactions were not revealed by coaggregation of the various actinomycetes with group 2 and 4 streptococci.



b Surface Components		Lactose Reversible Interaction
Heat Inactivated	Heat Stable	
		−
		−
		−
		+
		+

FIG. 2. *Diagrammatic representation of the coaggregation patterns delineating the four coaggregation groups of streptococci and five groups of actinomycetes. The circles in the centers of the four pentagons depict streptococcal cells representing the four groups. Each actinomycete group (groups A through E) is shown as a truncated rod pointing toward the center from the sides of each pentagon. The interactions between cells are indicated by the complementary projections, which represent surface components on the actinomycete and streptococcal cells. Representation of interactions by the same set of symbols in the different groups of bacteria does not imply identity of the corresponding components.*

Each of the coaggregation patterns (Fig. 2a) is shown as a set of interactions that is mediated by one or more of the five complementary pairs of surface components. Three of these are not reversed by lactose, and two are lactose reversible. Although other interactions may occur, the distribution of these five complementary pairs is sufficient to distinguish the coaggregation patterns of the various groups. The coaggregation patterns shown here represent the patterns that occur between *S. sanguis* and *A. viscosus* or *A. naeslundii* and, as discussed below, they occur infrequently or not at all with the other actinomycetes examined.

The finding that the sugar lactose reverses the interaction between the group C actinomycetes and group 1 or 3 streptococci supports the hypothesis that certain coaggregations are mediated by lectin-like components on the streptococci which bind carbohydrates on the actinomycetes. This is the first report of a lectin-like component on a streptococcal surface. Besides the reversibility of these coaggregations by lactose, the association of the lectin activity with the *Streptococcus* is also based on the ability of heat and protease treatment to inactivate these cells rather than the actinomycetes. Sugars which reverse the other coaggregations in which streptococci are the heat-inactivated members of coaggregating pairs remain to be identified. The association of a lactose-inhibitable lectin with many stock culture strains of *A. viscosus* and *A. naeslundii* is well established (3, 12). Our results show that a large number of freshly isolated strains also exhibit this activity. Perhaps more surprising is the absence on these bacteria of additional heat-inactivated components which are lactose insensitive. Thus, the specificity of coaggregation reactions depends on a single lectin activity on the actinomycetes and, perhaps, multiple lectins which are distributed on the various groups of streptococci.

In addition to demonstrating that fresh isolates and stock culture strains exhibit similar coaggregation properties, our results also provide information regarding several other questions related to cell-cell interactions between freshly isolated oral actinomycetes and streptococci. First, from an ecological viewpoint, can coaggregating actinomycetes be isolated from all dental plaque samples? Since colonies were picked at random from the initial platings of dilutions of plaque samples, no selective advantage was given to colonies produced by potentially coaggregating actinomycetes. Moreover, the coaggregation experiments were done without prior knowledge of the identity of the isolates and vice versa; i.e., the experiments were double blind. Our results show that most plaque samples (14 of 16 samples) were composed of a population of coaggregating actinomycetes. The reason that coaggregating actinomycetes were not isolated from the other two samples is unknown, but this may have been a result of random picking of colonies.

Second, what is the frequency of lactose-reversible coaggregation reactions in coaggregating actinomycetes? Our results indicated that 90% of the freshly isolated human oral strains of *A. viscosus* and *A. naeslundii* coaggregated with human oral *S. sanguis* strains. Of these, nearly

90% exhibited lactose-reversible coaggregations with certain strains of *S. sanguis*. Thus, the majority of the interactions between these two types of cells involved a lactose-sensitive lectin-like component.

Third, are lactose-reversible interactions between oral bacteria limited to interactions between *S. sanguis* and *A. viscosus* or *A. naeslundii*, or do other actinomycetes participate? Besides the 68 strains of *A. viscosus* and *A. naeslundii* tested, 44 other actinomycetes and strains of *P. acnes* were examined. The *A. meyeri* isolate did not coaggregate, and only 3 of 14 *A. odontolyticus* isolates coaggregated with only two of the reagent streptococcal strains. These interactions were neither lactose reversible nor similar to the coaggregation patterns found with *A. viscosus* and *A. naeslundii*. However, they were identical to the interactions observed previously with *A. odontolyticus* ATCC 17982 (3). Clearly, the *A. odontolyticus* isolates exhibited coaggregation properties different from the properties of the other two *Actinomyces* species described above. None of the other 11 actinomycete isolates, which belonged to the genera *Arachnia*, *Bifidobacterium*, and *Bacterionema*, coaggregated with *S. sanguis*. Thus, the lactose-reversible coaggregations with *S. sanguis* tend to be species specific and seem to be limited to strains of *A. viscosus* and *A. naeslundii*. The only exception found to date is that 3 of the 16 strains of *P. acnes* coaggregated only with group 1 streptococci in a lactose-reversible reaction. This type of coaggregation pattern is distinct from the patterns reported here and will be described fully elsewhere (manuscript in preparation).

Collectively, these three observations indicate that in most cases human plaque is composed of *A. viscosus* and *A. naeslundii* strains that coaggregate with streptococcal cells by lactose-reversible interactions. It appears that these coaggregations involve lectin-like molecules that mediate a network of closely related interactions between cell surface components. A logical extension of this study would be to examine the interactions between the actinomycetes and streptococci obtained from a single plaque sample and to compare the coaggregation patterns obtained with those reported here. Such a study should provide information concerning the in vivo colonization and persistence of these bacteria in dental plaque.

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