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Coaggregation Properties of Human Oral *Veillonella* spp.: Relationship to Colonization Site and Oral Ecology

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The primary habitats of oral veillonellae are the tongue, dental plaque, and the buccal mucosa. Isolates were obtained from each habitat and tested for coaggregation with a battery of other oral bacterial strains. All 59 tongue isolates tested for coaggregation were *Veillonella atypica* or *Veillonella dispar*. All but one of them coaggregated with strains of *Streptococcus salivarius*, a predominant inhabitant of the tongue surface but not subgingival dental plaque. These tongue isolates were unable to coaggregate with most normal members of the subgingival flora such as *Actinomyces viscosus*, *Actinomyces naeslundii*, *Actinomyces israelii*, and *Streptococcus sanguis*. In contrast, 24 of 29 *Veillonella* isolates, of which 20 were *Veillonella parvula* from subgingival dental plaque samples, coaggregated strongly with the three species of *Actinomyces*, *S. sanguis*, and other bacteria usually present in subgingival plaque, but they did not coaggregate with *S. salivarius*. The majority of isolates from the buccal mucosa (42 of 55) has coaggregation properties like those from the tongue. These results indicate that the three human oral *Veillonella* species are distributed on oral surfaces that are also occupied by their coaggregation partners and thus provide strong evidence that coaggregation plays a critical role in the bacterial ecology of the oral cavity.

As additional information regarding cell-to-cell recognition among oral bacteria is discovered, it is becoming increasingly clear that a dynamic but organized microbial community exists in the oral cavity. Intergeneric coaggregation, a phenomenon of cell-to-cell recognition, has been described for numerous pairs of oral bacteria from many genera and has been proposed as an important factor in bacterial colonization of the oral cavity (7). Veillonellae frequently are isolated from dental plaque, the tongue, and buccal mucosa (9) and have been shown to participate in coaggregations with many oral bacteria including *Streptococcus salivarius* (39), *Eubacterium saburreum* (21), *Streptococcus mutans* (23), and *Actinomyces viscosus* (1, 7). However, the focus of these reports was not on the veillonellae, but rather on their partners (39, 40), their relationship in a coccal filament cell arrangement (21), or the possible role of coaggregation in colonization of gnotobiotic animals (23).

With regard to potential interdependence of oral bacteria with their coaggregation partners, veillonellae are a particularly interesting group of organisms to consider. First, for metabolic energy they utilize pyruvate and lactate, the intermediate or end products of fermentation by other heterotrophs. This has led some researchers to propose that they participate in food chains within the oral cavity (22, 25). Nutritional relationships between veillonellae and other oral bacteria both in vitro (5, 6, 25) and in vivo have been demonstrated (26, 36). Second, veillonellae appear to have a limited ability to adhere to host tissue. They adhere weakly to saliva-coated hydroxyapatite and are isolated in small numbers from the teeth of monoinfected gnotobiotic animals (23). Also, only small numbers are found on the teeth of human volunteers 1 h after cleaning (18). Although they have been isolated in large numbers from the tongue, the ability of veillonellae to bind directly to epithelial cells has not been investigated (18). However, the known physiologic and adherence properties suggest that veillonellae may derive particular benefit from both adherence to and interaction with other bacterial species.

Researchers in our laboratory previously have described a network of coaggregations involving oral bacteria including the actino-myces and streptococci (3, 11, 14–16). During these investigations three parameters of coaggregation were monitored: (i) coaggregation between a bacterial pair, (ii) inhibition of coaggregation by addition of lactose, and (iii) inhibition of coaggregation by heat or protease treatment of one or both partners. On the basis of these parameters, highly specific coaggregation groups for both actinomyces and streptococci emerged. The present study describes the coaggregation properties of oral *Veillonella* isolates with a variety of oral bacteria including members of the six actinomyces coaggregation groups (groups A to F) and the six streptococcal coaggregation groups (groups 1 to 6). On the basis of these properties, four veillonella coaggregation groups are described. In addition, we tested the hypothesis that different sites in the oral cavity are colonized by organisms with specific surface recognition properties by identifying the coaggregation properties of veillonellae and their coaggregation partners isolated from the same site.

MATERIALS AND METHODS

Isolation of veillonellae from the oral cavity. *Veillonella* isolates were obtained from the tongue, buccal mucosa, saliva, and subgingival dental plaque of adult human volunteers. The subgingival isolates were obtained from a collection of freshly isolated strains from individuals who were participating in studies of the bacteriology of experimental gingivitis and periodontitis in children and adults (29–33). The veillonellae were from 20 subjects, and the 29 strains chosen for this study represented *Veillonella atypica*, *Veillonella dispar*, and *Veillonella parvula*, the three *Veillonella* species found in subgingival dental plaque (29–33). The

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isolates from the tongue, buccal mucosa, and saliva were obtained by the following method. From each of 27 subjects, a 1-ml sample of whole saliva was obtained. The oral cavity was then rinsed vigorously with sterile water for 10 s twice. Samples were obtained with sterile cotton-tipped applicators applied firmly to the dorsal surface of the tongue or buccal mucosa. All samples were immediately streaked on veillonella agar (Difco Laboratories, Detroit, Mich.) containing 7.5 μg of vancomycin (Sigma Chemical Co., St. Louis, Mo.) per ml. The plates were incubated anaerobically (80% N_2, 10% CO_2, 10% H_2) in GasPaks (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 48 h. Three colonies were selected from the 30 to 40 colonies usually present on the plate. Thus, from each subject nine isolates were initially selected, three each from the tongue, buccal mucosa, and saliva. Growth from each original single-colony isolate was streaked and replated two more times on veillonella agar. For final transfer, a colony was suspended in 1 ml of a 5-mg/ml tryptone (Difco) broth and equal portions were inoculated into 10 ml of modified Schaedler medium (2) either with 0.2% glucose or without glucose but supplemented with 0.1 M sodium lactate. All isolates which grew only in media supplemented with lactate and appeared as spherical gram-negative cells were subsequently identified as *Veillonella* spp. by the method described below and by analysis of cellular fatty acids (27).

Veillonella strains from subgingival plaque were isolated from dilutions of plaque cultured in supplemented brain heart infusion agar as described previously (31). Strains were identified by morphology, Gram reaction, inability to ferment carbohydrates but ability to utilize lactate and pyruvate, reduction of nitrate (10), and polyacrylamide gel electrophoresis of soluble cellular proteins (28).

Culture conditions. All *Veillonella* strains were grown in modified Schaedler medium without glucose but supplemented with 0.1 M sodium lactate. Test strains of other oral bacteria were grown in a modified Schaedler broth with 0.2% glucose or in an enriched medium containing (per liter of water) tryptone (5.0 g), yeast extract (5.0 g), K_2HPO_4 (5.0 g), Tween 80 (Sigma) (0.5 ml), and glucose (2.0 g) (20). All cells were grown at 37°C under anaerobic conditions, harvested in the late exponential to early stationary phase of growth, washed three times, and suspended in coaggregation buffer (3).

Coaggregation assay. Cell suspensions of each strain were adjusted to a cell density of about 10^9 cells per ml (260 Klett Units at 660 nm [red filter]; Klett-Summerson, Inc., New York, N.Y.). A visual assay (3) was used to determine coaggregation with potential partner strains (identified in Table 2). This assay involves a scoring system of 0 for no visible coaggregation to 4 for maximum coaggregation. The score of 4 was assigned when large coaggregates were observed immediately upon mixing equal-volume suspensions of the two partner strains, leaving a clear supernatant. The score of 0 was assigned when the mixture of cell suspensions remained unchanged and appeared as a homogeneous suspension. Reversal of coaggregation was monitored by adding lactose to a final concentration of 0.06 M and rescoring each coaggregating pair. The effect of temperature was determined by heating a cell suspension at 85°C for 30 min before mixing it with heated or unheated cells of the partner. If heating of both partners failed to abolish coaggregation, the experiment was repeated by heating at 99°C for 45 min. Most protease digestions were done with 0.45 mg of Protease K (P-0390; Sigma) per ml at 50°C for 1 h. Other proteases tested were pronase (Calbiochem-Behring, La Jolla, Calif.), papain (Sigma), subtilisin (Sigma), trypsin (Sigma), and chymotrypsin (Sigma), and the digestions were done as specified by the supplier. Protease-treated cells were washed with coaggregation buffer three times by centrifugation at 13,180 × *g* for 10 min before being tested for coaggregation.

TABLE 1. Distribution of *Veillonella* species isolated from buccal mucosa, saliva, and the dorsum of the tongue

Species	No. of isolates from:		
	Buccal mucosa	Saliva	Tongue dorsum
V. atypica	13	7	18
V. dispar	43	41	50
V. parvula	6	9	0

RESULTS

Distribution of *Veillonella* isolates obtained from saliva and three colonization sites in the human oral cavity. *V. parvula* constitutes 93 to 98% of the *Veillonella* flora of subgingival plaque, whereas the percentage of *V. atypica* and *V. dispar* ranges from 1 to 2 and 1 to 5, respectively (29–33). For the current study we chose 5 strains of *V. atypica* and 4 strains of *V. dispar*, along with 20 strains of *V. parvula* from the collection of subgingival *Veillonella* isolates from earlier investigations of the bacteriology of experimental gingivitis and periodontitis of adults and children (29–33). These 29 strains were from 20 individuals and were used to represent subgingival veillonellae. The same three *Veillonella* species were found in samples taken from saliva, buccal mucosa, and the dorsal surface of the tongue (Table 1). Although significant numbers of *V. parvula* were present in saliva and buccal mucosa samples, none were found in samples from the tongue. The most common isolate observed from these three sample areas was *V. dispar*. The ratio of *V. atypica* to *V. dispar* was about 1:2, 1:5, and 1:4 in samples from the tongue, saliva, and buccal mucosa, respectively. Collectively these results indicate that *V. atypica* and *V. dispar* predominate on the tongue (Table 1), whereas earlier investigations have shown that *V. parvula* is markedly more numerous than the other two species in subgingival plaque (29–33).

Coaggregation of *Veillonella* isolates from subgingival dental plaque. Initially 29 isolates from subgingival dental plaque were tested for coaggregation with a variety of oral bacteria (Table 2). Of these isolates, 24 exhibited similar coaggregation properties. They coaggregated with most of the reference strains for the actinomyces and streptococcal coaggregation groups, *Fusobacterium nucleatum*, and *Actinomyces israelii*. Few of these isolates coaggregated with strains of *Actinomyces odontolyticus*, *Rothia dentocariosa*, and *Propionibacterium acnes*. None of the 24 isolates coaggregated with the strains of *S. salivarius* tested. In contrast, the five remaining isolates coaggregated with *S. salivarius* and *F. nucleatum* strains tested, yet failed to coaggregate with any of the other reference strains in Table 2. None of the isolates coaggregated with any of eight strains of mutans streptococci.

On the basis of this preliminary survey of the 29 isolates from subgingival plaque, 11 strains representing the coaggregation patterns observed were selected for further testing. Individual strains were tested for coaggregation with reference strains from each of the actinomyces and streptococcal

TABLE 2. Coaggregation of 29 *Veillonella* isolates from subgingival dental plaque with other oral bacteria

Partner strain	No. of isolates that coaggregated with at least one strain[a]	
	24 isolates[b]	5 isolates[c]
F. nucleatum (13 strains)	24	5
Reference streptococci		
S. sanguis DL1 (group 1)	24	0
S. sanguis H1 (group 2)	0	0
S. sanguis 34 (group 3)	24	0
S. sanguis J22 (group 4)	24	0
S. morbillorum PK509 (group 5)	24	0
S. sanguis PK488 (group 6)	24	0
Reference actinomyces		
A. viscosus T14V (group A)	24	0
A. naeslundii PK19 (group B)	24	0
A. naeslundii PK947 (group C)	13	0
A. naeslundii PK606 (group D)	24	0
A. naeslundii PK984 (group E)	24	0
Actinomyces serovar WVa963 PK1259 (group F)	24	0
A. israelii (5 strains)	24	0
A. odontolyticus PK48	4	0
R. dentocariosa PK44	7	0
P. acnes PK93	6	0
S. salivarius (6 strains)	0	5
Mutans streptococci (8 strains)[d]	0	0

[a] No strain coaggregated with *B. denticola* PK1277, *B. intermedius* PK1511, *B. loescheii* PK1295, *C. gingivalis* DR2001, DR2002, and DR2021, *C. ochracea* ATCC 33596, *C. sputigena* ATCC 33612, *Actinobacillus actinomycetemcomitans* N27 and Y4, *Peptostreptococcus anaerobius* PK1415 (ATCC 27337; kindly provided by M. Curtis), or 46 *Selenomonas* strains.

[b] These 24 isolates included 3 strains of *V. atypica*, 4 strains of *V. dispar*, and 17 strains of *V. parvula*.

[c] These five isolates included two strains of *V. atypica* and three strains of *V. parvula*.

[d] The strains used were LM-7, C67-1, FA-1, 10449, AHT, JC-2, BHT, and 6715-10 and were kindly provided by J. Donkersloot.

coaggregation groups, as well as the six strains of *S. salivarius*. By determining partner specificity, lactose reversibility, and heat and protease sensitivity of the interactions, we found that three coaggregation groups emerged (Table 3). The bottom line in each group (identified as PV in Table 3) indicates the coaggregation score for untreated cells of each cell type. In ascending order, the lines above these control values show the coaggregation scores when (i) only the *Veillonella* cell type is heated, (ii) only the partner cell type is heated, and (iii) both cell types are heated.

The first group (group I), represented by *V. atypica* PK1910, coaggregated with all actinomyces coaggregation groups except group C (reference strain, *Actinomyces naeslundii* PK947) and all streptococcal coaggregation groups except group 2 (reference strain, *Streptococcus sangius* H1). Lactose-reversible coaggregations were seen with streptococcal coaggregation groups 3 and 5 (reference strains, *S. sanguis* C104 and PK488, respectively). This group failed to coaggregate with any of the *S. salivarius* strains tested.

The second group (group II), represented by *V. parvula* PK1915, participated in all the coaggregations described for group I and, in addition, coaggregated with the actinomyces coaggregation group C reference strain (*A. naeslundii* PK947) in a lactose-nonreversible manner. Like group I, this group failed to coaggregate with any of the *S. salivarius* test strains.

The third group of isolates (group III) failed to coaggregate with any of the actinomyces and streptococcal coaggregation groups. In contrast, they coaggregated with all six *S. salivarius* strains tested, and these interactions were not lactose reversible. Heating the veillonellae of all three groups prevented coaggregation with all partners (except the coaggregation of group II veillonellae with streptococcal coaggregation group 4), whereas similar treatment of the partners had no observable effect on coaggregation.

In previous studies (3, 11, 14, 15) heating of either or both partners at 85°C for 30 min was sufficient to inactivate coaggregation, and protease treatment of cells produced corresponding results. In the present study several coaggregations required more vigorous heating (99°C for 45 min) to inactivate coaggregation, and heating or protease digestion of a cell produced different effects on coaggregation (Table 4). For example, the coaggregation of *V. atypica* PK1910 (veillonella coaggregation group I) and *S. sanguis* J22 (streptococcal coaggregation group 4) required that the *Veillonella* partner be heated at 99°C for 45 min to inactivate the reaction. In addition, heating of J22 had no effect. In contrast, protease digestion of PK1910 has no effect on the interaction, whereas protease digestion of J22 inactivated the coaggregation. Identical results were obtained with Protease K, pronase, papain, subtilisin, chymotrypsin, and trypsin. Even when the enzymatic digestions were performed at 10-fold-higher concentrations, the *Veillonella* strains were unaffected. These types of interactions were seen only with veillonella coaggregation groups I and II and streptococcal coaggregation groups 1, 4, and 6 (reference strains, *S. sanguis* DL1, J22, and PK488, respectively).

Coaggregation of *Veillonella* isolates from the tongue, buccal mucosa, and saliva. A total of 187 isolates from 27 subjects were obtained. Of these isolates 68 were from the tongue, 57 were from whole saliva, and 62 were from buccal mucosa; 164 strains were tested for coaggregation with the same battery of partner strains used for the subgingival veillonellae (Table 2). The remaining 23 isolates were not tested for coaggregation because they self-aggregated. Each of the fresh isolates (59 from tongue, 50 from saliva, and 55 from buccal mucosa) was tested to determine whether any of the three coaggregation patterns of subgingival plaque isolates could be observed. Most isolates exhibited one of these three coaggregation patterns (Table 5). However, a fourth group (group IV) was observed which, like group III from plaque, coaggregated with all the *S. salivarius* strains, but also coaggregated by a lactose-reversible reaction with *S. sanguis* J22 (streptococcal coaggregation group 4). The *Veillonella* strain, but not the *Streptococcus* strain, was inactivated by heat (85°C for 30 min) or protease treatment. Several isolates from each of the veillonella coaggregation groups were tested for their ability to coaggregate with mutans streptococci, and in all cases the results were negative.

Nearly all (98%) of the isolates from the tongue were from veillonella coaggregation groups III and IV (i.e., groups which coaggregate with *S. salivarius*), whereas only 17% of isolates from subgingival plaque displayed these coaggregation patterns. In contrast, 83% of isolates from subgingival plaque were from coaggregation groups I and II (the groups coaggregating with subgingival bacteria such as *S. sanguis* and *A. naeslundii*), and only 1 of 59 isolates from the tongue exhibited these coaggregation patterns. Saliva and buccal mucosa isolates were predominantly from coaggregation

TABLE 3. Coaggregation properties of *Veillonella* strains from subgingival plaque[a]

Veillonella test strain[b]	Heat treatment[c]	Coaggregation with:												
		Actinomyces coaggregation group[d]						Streptococcal coaggregation group[e]						*S. salivarius*[f]
		A	B	C	D	E	F	1	2	3	4	5	6	
V. atypica PK1910 (group I)	P*V*	0	0	0	0[g]	0	0	0	0	0	0[g]	0	0	0
	P*V	3	3	0	4[g]	3	2	3	0	3[h]	4[g]	3[h]	2	0
	PV*	0	0	0	0[g]	0	0	0	0	0	0[g]	0	0	0
	PV	3	3	0	4	3	2	3	0	3[h]	4	3[h]	2	0
V. parvula PK1915 (group II)	P*V*	0	0	0	0[g]	0	0	0	0	0	0[g]	0	0	0
	P*V	3	3	3	4[g]	3	2	3	0	3[h]	4[g]	3[h]	2	0
	PV*	0	0	0	0[g]	0	0	0	0	0	4[g]	0	0	0
	PV	3	3	3	4	3	2	3	0	3[h]	4	3[h]	3	0
V. atypica PK1955 (group III)	P*V*	0	0	0	0	0	0	0	0	0	0	0	0	0
	P*V	0	0	0	0	0	0	0	0	0	0	0	0	4
	PV*	0	0	0	0	0	0	0	0	0	0	0	0	0
	PV	0	0	0	0	0	0	0	0	0	0	0	0	4

[a] Method for assigning coaggregation scores is as described in Materials and Methods.
[b] *V. atypica* PK1910 (VPI E6L-5) was used to represent veillonella coaggregation group I, which also included two additional strains of *V. atypica*, three strains of *V. dispar*, and three strains of *V. parvula*. *V. parvula* PK1915 (VPI E8K-5) was used to represent veillonella coaggregation group II, which also included 13 other strains of *V. parvula* and one strain of *V. dispar*. *V. atypica* PK1955 (VPI D72A-3) was used to represent veillonella coaggregation group III, which also included another strain of *V. atypica* and three strains of *V. parvula*.
[c] P, Partner strain (either *Actinomyces* or *Streptococcus* sp.); V, *Veillonella* strain; * indicates that the strain was heated at 85°C for 30 min before being mixed with its paired strain.
[d] *A. viscosus* T14V, *A. naeslundii* PK19, PK947, PK606, and PK984, and *Actinomyces* serovar WVa963 strain PK1259, which represented actinomyces coaggregation groups A, B, C, D, E, and F, respectively, were tested.
[e] *S. sanguis* DL1, H1, 34, C104, and J22, *S. morbillorum* PK509, and *S. sanguis* PK488, which represented streptococcal coaggregation groups 1, 2, 3, 3, 4, 5, and 6, respectively, were tested.
[f] *S. salivarius* HB was kindly provided by B. McBride and strains 25975, 29945, 9222, 13419, and 7073 were kindly provided by J. Donkersloot and originally obtained from the American Type Culture Collection, Rockville, Md.
[g] The strain indicated by the asterisk was heated at 99°C for 45 min before being mixed with its paired strain.
[h] Coaggregation was reversed by addition of lactose to a final concentration of 60 mM.

groups III and IV, although a few of the isolates from these sites exhibited properties of coaggregation groups I and II.

DISCUSSION

Several observations reported here support the notion that coaggregation (interbacterial adherence) plays a functional role in bacterial colonization of different habitats within the oral cavity: (i) the three species *V. atypica*, *V. dispar*, and *S. salivarius*, which are the most numerous members of their respective genera on the tongue, form coaggregates with each other (veillonella coaggregation groups III and IV), but only infrequently do isolates of these species coaggregate with subgingival bacteria such as *S. sanguis*, *A. viscosus*, *A. naeslundii*, and *A. israelii*; (ii) *V. parvula*, which constitutes between 93 and 98% of the veillonellae in subgingival dental plaque, coaggregates with strains of the four species of subgingival bacteria cited above (veillonella coaggregation groups I and II); (iii) *V. dispar* is the predominant *Veillonella* sp. found outside of subgingival dental plaque, and of the 126 isolates tested for coaggregation, 119 coaggregated with *S. salivarius* (veillonella coaggregation groups III and IV); (iv)

TABLE 4. Comparison of the effects on coaggregation of heating at 85 or 99°C or protease treatment of veillonellae or their streptococcal partners

Veillonella coaggregation group[a]	Strain treated[b]	Coaggregation score[c] for following streptococcal group[d] and treatment:								
		Group 1			Group 4			Group 6		
		85°C	99°C	Protease[e]	85°C	99°C	Protease	85°C	99°C	Protease
I	P*V*	0	0	0	4	0	0	0	0	0
	P*V	3	3	0	4	4	0	2	2	0
	PV*	0	0	3	4	0	4	0	0	2
	PV	3	3	3	4	4	4	2	2	2
II	P*V*	0	0	0	4	0	0	0	0	0
	P*V	3	3	0	4	4	0	2	2	0
	PV*	0	0	3	4	4	4	0	0	2
	PV	3	3	3	4	4	4	2	2	2

[a] *V. atypica* PK1910 (VPI E6L-5) and *V. dispar* PK1950 (VPI E7F-26B) were used to represent veillonella coaggregation groups I and II, respectively.
[b] P, Partner strain; V, *Veillonella* strain; *, treated strain.
[c] Coaggregation scores are as described in Materials and Methods.
[d] The streptococcal reference strains representing coaggregation groups 1, 4, and 6 were *S. sanguis* DL1, J22, and PK488, respectively.
[e] Protease digestion with 0.45 mg of Protease K per ml at 50°C for 1 h.

TABLE 5. Distribution of veillonella isolates from different sources among the four veillonella coaggregation groups

Veillonella coaggregation group[a]	No. (%) of *Veillonella* isolates from following source:			
	Tongue (59 strains)	Saliva (50 strains)	Buccal mucosa (55 strains)	Plaque (29 strains)
I	1 (2%)	5 (10%)	11 (20%)	9 (31%)
II	0 (0%)	0 (0%)	2 (4%)	15 (52%)
III	31 (52%)	24 (48%)	29 (53%)	5 (17%)
IV	27 (46%)	21 (42%)	13 (24%)	0 (0%)

[a] The reference strains for veillonella coaggregation groups I and II were *V. atypica* PK1910 (VPI E6L-5) and *V. parvula* PK1915 (VPI E8K-5), respectively, and were obtained from subgingival plaque. *V. dispar* PK2503 and *V. dispar* PK2502 were designated as the reference strains for coaggregation groups III and IV, respectively, and were obtained from the dorsal surface of the tongue. The 193 *Veillonella* isolates examined for coaggregation in this study were distributed as follows: coaggregation group I consisted of 9, 5, and 12 strains of *V. atypica*, *V. dispar*, and *V. parvula*, respectively; coaggregation group II consisted of 1, 2, and 14 strains of *V. atypica*, *V. dispar*, and *V. parvula*, respectively; coaggregation group III consisted of 16, 69, and 4 strains of *V. atypica*, *V. dispar*, and *V. parvula*, respectively; coaggregation group IV consisted of 10, 50, and 1 strains of *V. atypica*, *V. dispar*, and *V. parvula*, respectively.

all six strains of *S. salivarius* tested coaggregated with *V. atypica* and *V. dispar*, but not with *V. parvula*; and (v) although more than 200 *Veillonella* isolates from several econiches of more than 40 subjects were examined for the ability to coaggregate with a variety of partners, only four coaggregation patterns were observed, indicating the high degree of specificity of coaggregation. Other evidence which supports this concept is that similar highly specific coaggregation patterns have been found for most oral bacteria examined to date (3, 4, 12, 13, 17, 19, 35, 41). For example, more than 400 isolates of *S. sanguis*, *A. viscosus*, and *A. naeslundii* of subgingival plaque origin have been tested and only six actinomyces coaggregation groups and six streptococcal groups were delineated (3, 14–16). Representatives of those 12 groups coaggregated with most of the veillonellae from subgingival plaque, but only infrequently (1 of 59 isolates) with the veillonellae from the tongue. Although the possibility exists that other minor coaggregation groups of oral veillonellae exist and failed to be detected within our sample, it appears that the veillonellae are yet another example of a taxonomic group of oral bacteria that has a specific but limited number of coaggregation partners. These observations extend those made with oral streptococci by Gibbons and van Houte (8), who proposed that adherence of bacteria to host tissue was a critical ecological determinant. The data presented here extend that proposal to include bacterial accretion and strengthen the hypothesis outlined above.

With further evidence that coaggregation is an important determinant of oral bacterial colonization, the mechanisms of such interactions continue to be of considerable interest. Previous studies (3, 15) described coaggregations which were inactivated by heating one or both partners and also frequently reversed by the addition of sugars, particularly lactose, to the cell suspension. These properties provided initial evidence that coaggregations involve lectin-carbohydrate recognition (24), as has been described for a number of other cell-to-cell interactions (34). Many of the coaggregations seen in the present study are similar to those described previously between other oral bacteria. These interactions are abolished by heating or protease digestion of one or both partners and frequently reversed by the addition of lactose to the cell suspension. One group of interactions described in the present study displayed unusual properties (Table 4). This group of interactions was inactivated by heating the veillonella partner, whereas protease digestion of the same cell type had no effect. In contrast, heating the streptococcal partner did not inactivate coaggregation, whereas protease digestion of this cell type inactivated the interaction. A similar observation was made in a preliminary report of the coaggregation between *S. sanguis* and a *Fusobacterium* sp. (B. C. McBride, T. King, T. Edwards, and M. Gisslow, J. Dent. Res. [Spec. Issue] **56**:A156, 1977). These results may simply reflect variations in sensitivity of different surface proteins to heat or protease treatments. On the other hand, the possibility that novel surface structures are mediating these interactions is an exciting one and is currently being investigated in our laboratory.

A major focus of the present study was to test the hypothesis that surface recognition phenomena such as coaggregations among oral bacteria are important determinants in bacterial colonization of different surfaces. If so, then the residents of the same econiche should coaggregate, whereas inhabitants of different surfaces would be less likely to recognize each other. Accordingly, in a lotic environment such as the oral cavity, coaggregation would be a mechanism by which suspended bacteria might adhere to bacteria already attached to a particular surface. Likely candidates for these attached bacteria are the oral streptococci. It has been reported that *S. sanguis* adheres much better to the tooth surface and *S. salivarius* prefers the tongue (8, 37, 38). This hypothesis is supported by the data presented here, since veillonellae from subgingival plaque coaggregated frequently with other subgingival plaque bacteria (e.g., *S. sanguis*) but infrequently with bacteria from the tongue (e.g., *S. salivarius*), whereas veillonellae from the tongue coaggregated almost exclusively with *S. salivarius*.

These results also demonstrate the obvious importance of using an appropriate battery of potential coaggregation partner strains when testing for coaggregation of a given fresh isolate. Bacteria from the same site or econiche are the most likely coaggregation partners (14). Although *S. salivarius* coaggregates strongly with veillonellae from the tongue, it does not coaggregate with any of more than 30 strains of subgingival bacteria including *A. israelii*, *A. viscosus*, *Bacteroides denticola*, *Bacteroides intermedius*, *Bacteroides loescheii*, *Capnocytophaga gingivalis*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Actinobacillus actinomycetemcomitans*, *Peptostreptococcus anaerobius*, *Propionibacterium acnes*, *R. dentocariosa*, *Streptococcus morbillorum*, or *S. sanguis* (C. Hughes and P. Kolenbrander, unpublished results). The same was true of most of the veillonellae isolated from the tongue (see above), whereas those from subgingival plaque coaggregated with many of the subgingival strains, especially those thought to be primary colonizers of the tooth surface, such as *S. sanguis* and *A. viscosus*. On the basis of the results reported here and fully supported by the data in numerous previous studies of the coaggregation properties of subgingival bacteria and of the adherence properties of oral bacteria in gnotobiotic animals (23, 35), we propose that bacterial coaggregation plays a critical role in determining initial attachment and subsequent colonization of many indigenous bacteria in the human oral cavity.

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