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Coaggregation of *Fusobacterium nucleatum*, *Selenomonas flueggei*, *Selenomonas infelix*, *Selenomonas noxia*, and *Selenomonas sputigena* with Strains from 11 Genera of Oral Bacteria

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Twenty-eight strains of *Fusobacterium nucleatum* and 41 *Selenomonas* strains, including *S. sputigena* (24 strains), *S. flueggei* (10 strains), *S. infelix* (5 strains), and *S. noxia* (2 strains), were tested for their ability to coaggregate with each other and with 49 other strains of oral bacteria representing *Actinobacillus*, *Actinomyces*, *Bacteroides*, *Capnocytophaga*, *Gemella*, *Peptostreptococcus*, *Porphyromonas*, *Propionibacterium*, *Rothia*, *Streptococcus*, and *Veillonella* species. Selenomonads coaggregated with fusobacteria and with *Actinomyces naeslundii* PK984 but not with any of the other bacteria, including other selenomonads. In contrast, fusobacteria coaggregated with members of all genera, although not with all strains of each species tested. Each fusobacterium strain appeared to have its own set of partners and coaggregation properties, unlike their partners, whose coaggregation properties in earlier surveys delineated distinct coaggregation groups. Coaggregations of fusobacteria with the 63 gram-negative strains were usually inhibited by EDTA, whereas those with the 27 gram-positive strains were usually not inhibited. Likewise, lactose-inhibitable coaggregations were common among some strains of fusobacteria and some strains from each of the genera containing gram-negative partners but were rarely observed with gram-positive partners. Heating the fusobacteria at 85°C for 30 min completely prevented coaggregation with most partners, suggesting the involvement of a protein on the fusobacteria. Heat treatment of many of the gram-negative partners not only enhanced their coaggregation with the fusobacteria but also changed lactose-sensitive coaggregations to lactose-insensitive coaggregations. Although fusobacteria coaggregated with a broader variety of oral partner strains than any other group of oral bacteria tested to date, each fusobacterium exhibited coaggregation with only a certain set of partner strains, and none of the fusobacteria adhered to other strains of fusobacteria, indicating that recognition of partner cell surfaces is selective. The strains of *F. nucleatum* are heterogeneous and cannot be clustered into distinct coaggregation groups. Collectively, these results indicate that coaggregation between fusobacteria and many gram-negative partners is significantly different from their coaggregation with gram-positive partners. The contrasting variety of partners for fusobacteria and selenomonads supports the concept of coaggregation partner specificity that has been observed with every genus of oral bacteria so far examined.

Fusobacterium nucleatum and *Selenomonas sputigena* progressively increase in number along with deteriorating periodontal health from simple gingivitis to severe periodontitis (40, 41). Both species have been identified as significantly associated with active periodontal diseased sites (10). Although both are gram negative, they are very different morphologically and physiologically (14). In the current study, fusobacteria and selenomonads were used to represent the increasing population of gram-negative bacteria known to replace the gram-positive bacteria in human experimental gingivitis studies (32, 49) and periodontitis (10, 41) and to examine the extent of coaggregation among gram-negative bacteria that comprise these populations. Such a survey has not been done, although extensive surveys of coaggregations among gram-positive bacteria and between gram-positive and gram-negative bacteria are well known (for reviews, see references 6, 19, 19a, 34).

Coaggregation of *S. sputigena* with other oral bacteria has not been reported, whereas cell-to-cell interactions of *F. nucleatum* with certain streptococci were among the early coaggregations noted (18). Some coaggregations were prevented by heating or trypsin digestion of one cell type, whereas others were inhibited by treating their partner. Some pairs were inhibited by 0.02 M EDTA, but others were not. A brief report of the properties of coaggregation of *F. nucleatum* with *Streptococcus sanguis*, *Streptococcus mitis*, *Bacteroides melaninogenicus*, or *Staphylococcus aureus* indicated that the ability of fusobacteria to coaggregate was destroyed by heat (90°C for 10 min) or protease, whereas the ability of *S. sanguis* to coaggregate with fusobacteria was affected by protease but not by heat (B. C. McBride, J. King, T. Edwards, and M. Gisslow, J. Dent. Res. **56**:A156, 1977). These experimental results suggested that a protein(s) on both partner cells is responsible for coaggregation. In agreement with this idea, a 41-kilodalton protein firmly anchored in the fusobacterium outer membrane was proposed to be involved in a protein-protein interaction in the corncob formation with *S. sanguis* (8, 9). Recently, a single polypeptide (M_r, 39,500) was isolated from the cell envelope fraction of *F. nucleatum* ATCC 10953 by trypsin digestion, delipidation, ion-exchange chromatography, and finally extraction with dodecyltrimethylammonium bromide to remove contaminating lipopolysaccharide (17). This polypeptide inhibited corncob formation with *S. sanguis* CC5A, and antiserum made against the polypeptide blocked coaggregation.

A galactose-inhibitable interaction between sonicated

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fragments of *F. nucleatum* and several species of oral bacteria including *Porphyromonas* (*Bacteroides*) *gingivalis* (47), *Gemella*, (*Streptococcus*) *morbillorum* (33), and *S. sanguis* was reported (11). Cells of both gram-negative and gram-positive cell types absorbed the hemagglutination-active component present in the sonicated fragments. Hemagglutination of human and sheep erythrocytes by sonicated fragments or whole cells of *F. nucleatum* (12) was inhibited by D-galactose and *N*-acetyl-D-galactosamine (35). Recently, a galactose-binding lectin from *F. nucleatum* was identified by its ability to bind and elute from asialofetuin covalently coupled to Sepharose beads (42). These results indicate that fusobacteria probably have multiple kinds of surface interactions, which may include different combinations of sugar-inhibitable lectin-carbohydrate and protein-protein interactions.

Results from previous surveys indicated that the coaggregation properties of fresh isolates of certain oral actinomyces (7, 24, 25), streptococci (7, 24, 26), and veillonellae (15) can be categorized to identify six, six, and four coaggregation groups, respectively. In the current survey, reference strains of each of the above 16 groups of actinomyces, streptococci, and veillonellae were used to represent the potential for coaggregations by all of the members of each group with the fusobacteria and selenomonads. These reference strains represent more than 200 strains of veillonellae and 100 strains each of streptococci and actinomyces (15, 19, 19a). In sharp contrast to the coaggregation properties exhibited by members of coaggregation groups of other oral bacteria, the results of this survey strongly suggest that the coaggregation properties of fusobacteria cannot be segregated into definitive coaggregation groups.

MATERIALS AND METHODS

Bacterial strains and culture conditions. All strains used in this study were human oral isolates. The 28 *Fusobacterium* isolates and 41 *Selenomonas* isolates were from subgingival sites and were obtained as previously described (40, 41). The original strain number given by The Anaerobe Laboratory at the Virginia Polytechnic Institute and State University is identified within parentheses in the footnotes of the appropriate tables. The isolates that were examined in this survey but not included in the tables, because their coaggregation properties were similar to the properties of the strains presented in the tables were as follows: *S. sputigena* PK1959 (D19B-28), PK1960 (D8B-25), PK1962 (D51A-1), PK1963 (D14A-11), PK1964 (D86A-9), PK1965 (E4T-7), PK1966 (D48B-10), PK1968 (D72A-15), PK1969 (E1C-6), PK1972 (D80D-20), PK1973 (D13B-23B), PK1974 (D66D-27), PK1975 (D28E-29), PK1976 (D22B-7), PK1977 (D55B-28A), PK1978 (D68A-25A), PK1979 (D77C-20A), PK1980 (D75B-2), PK1981 (D83A-14), PK1982 (D49B-8), PK1983 (D97B-25), and PK1989 (E4M-27B); *Selenomonas infelix* PK1961 (D81D-13), PK1987 (E8F-17), and PK1993 (D33A-25); *Selenomonas flueggei* PK1984 (D72A-17), PK1985 (D83N-15), PK1986 (D71D-1), PK1988 (E4γ-20), PK1990 (D9A-12), PK1991 (D36F-24), PK1992 (D28J-13), and PK1994 (D71A-10); and *S. noxia* PK1971 (D26C-20). Several fusobacteria were less thoroughly examined than the ones whose coaggregation properties are given in this survey; and they are *F. nucleatum* PK1593 (D21B-13), PK1596 (D98D-24), PK1598 (D79A-14), PK1599 (D10B-5), PK1902 (E3C-22), and PK1906 (D28B-8). Isolates were characterized and identified by morphological, biochemical, chromatographic, and electrophoretic methods already described in detail (14, 36, 38). The partner strains tested included reference strains representing the four veillonella, six streptococcus, and six actinomyces coaggregation groups, which are identified in the legends in Tables 6, 3, and 4, respectively. *Bacteroides loeschei* PK1295, *B. intermedius* PK1511, and *B. denticola* PK1277 were chosen to represent their respective species because the coaggregation properties of the other eight strains of *B. loeschei*, seven strains of *B. intermedius*, and six strains of *B. denticola* were very similar to those of the reference strains (22). *Capnocytophaga sputigena* ATCC 33612, *C. ochracea* ATCC 33596, and *C. gingivalis* DR2001 represented the three species, and their coaggregation properties have been described (20). *Actinobacillus actinomycetemcomitans* Y4 and N27, *Peptostreptococcus anaerobius* ATCC 27337, *Propionibacterium acnes* PK93, *Rothia dentocariosa* PK44, *Actinomyces odontolyticus* PK48, 6 strains of *Actinomyces israelii*, and 10 strains of *Porphyromonas* (*Bacteroides*) *gingivalis* were used as potential partners. *P. gingivalis* 381 was kindly supplied by R. Ellen.

The *Selenomonas* isolates were grown in complex broth medium consisting of brain heart infusion broth supplemented with yeast extract, vitamin K_1, cysteine, and hemin (14). *P. gingivalis* was grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.). All other strains were grown in modified Schaedler medium with glucose (5) or lactate (15) as the source of energy. Cells were grown at 37°C under anaerobic conditions with the GasPak system (BBL Microbiology Systems), harvested in the late-exponential or early-stationary phase of growth, washed three times, and suspended in coaggregation buffer (7).

Coaggregation assay. Cell suspensions 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 (7) was used to determine coaggregation with potential partner strains. Briefly, the assay involves a scoring system of 0 for no visible coaggregation to 4 for maximum coaggregation. In the current survey, reversal of coaggregation was determined by the addition of lactose to a final concentration of 0.06 M and rescoring each coaggregating pair. If visible coaggregates remained, EDTA was then added to a final concentration of 1.0 mM. Previous studies have shown that coaggregations reversed by lactose are also inhibited by EDTA (7). 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.

RESULTS

Coaggregation properties of *Selenomonas* species. Of the 41 *Selenomonas* strains tested, 24 were *S. sputigena*, 10 were *S. flueggei*, 5 were *S. infelix*, and 2 were *S. noxia*. Each of the isolates was tested for its ability to coaggregate with the other 40 selenomonad isolates, 28 strains of *F. nucleatum*, and 49 other strains of oral bacteria. None of the selenomonads coaggregated with other selenomonads. All but three of them coaggregated with 1 or more of the 28 fusobacteria. Some examples of the more reactive selenomonads from each of the four species tested are given in Table 1. Many of the coaggregations were inhibited by lactose (60 mM) and EDTA (1 mM). A common property of these coaggregations was that they formed visible coaggregates only after gentle rocking of the test tube containing suspensions of the two cell types. By microscopy, these intergeneric coaggregates appeared as small, loosely arranged cellular networks (Fig. 1A) or as selenomonads adherent to sites along the length of the fusobacterium cell (Fig. 1B). The only other partner of

TABLE 1. Coaggregation of *F. nucleatum* and *Selenomonas* strains[a]

F. nucleatum strain	Coaggregation score with:						
	S. sputigena		*S. flueggei*		*S. infelix*		*S. noxia* PK1967
	PK1559	PK1568	PK1957	PK1958	PK1970	PK1956	
PK1909	0	0	0	0	0	2^0	0
PK1908	$3^{2,0}$	3^2	$2^{2,2}$	$3^{2,0}$	1^0	$4^{2,0}$	2^0
PK1907	0	0	0	$3^{2,0}$	0	1^0	0
PK1905	3^0	2^1	$3^{2,2}$	$3^{3,3}$	2^0	$3^{2,0}$	2^0
PK1904	0	0	0	0	0	0	0
PK1903	2^1	2^0	$3^{2,0}$	$3^{3,0}$	$2^{2,0}$	$3^{2,0}$	2^0
PK1901	0	0	0	0	0	0	0
PK1597	0	0	1^0	2^0	0	2^0	0
PK1595	0	0	1^0	1^0	0	3^0	2^0
PK1594	$2^{2,0}$	$2^{2,1}$	1^0	3^0	$2^{1,0}$	2^0	0
PK1592	0	0	1^0	2^0	0	1^0	0
PK1591	0	0	0	0	0	0	0
PK1590	2^0	2^0	$3^{1,1}$	$3^{3,2}$	1^0	3^0	2^0
PK1589	2^0	0	2^0	$3^{1,0}$	0	$3^{2,1}$	0
PK1588	0	0	1^0	$2^{1,1}$	0	$1^{1,0}$	0

[a] The method for assigning coaggregation scores was as described in Materials and Methods; coaggregation scores are given in three parts: the first score is that given after the two strains were mixed together, the second score is the first superscript and is the value after the addition of lactose to a final concentration of 60 mM, and the third score is the value after the addition of EDTA to a final concentration of 1 mM. The *Selenomonas* strains used here were *S. sputigena* PK1559 (E7C-17) and PK1568 (D27A-3), *S. flueggei* PK1957 (D82G-1A) and PK1958 (D32B-1), *S. infelix* PK1970 (D69C-1) and PK1956 (D82E-18), and *S. noxia* PK1967 (D104C-8). The *F. nucleatum* strains used in this study were PK1588 (D75B-20), PK1589 (E8A-19), PK1590 (D83F-55), PK1591 (D83B-27), PK1592 (D14D-4), PK1594 (E2S-11A), PK1595 (E3M-7A), PK1597 (D69B-21), PK1901 (E1A-3), PK1903 (D9A-4), PK1904 (D75A-9), PK1905 (D49B-9), PK1907 (D84B-26), PK1908 (D96B-2), and PK1909 (D53A-10).

TABLE 2. Effect of heating cells of *Selenomonas* sp. and *F. nucleatum* on their ability to coaggregate[a]

Selenomonas strain and treatment[b]	Coaggregation score with *F. nucleatum*:		
	PK1590	PK1905	PK1594
S. infelix PK1956			
F*S*	0	0	0
F*S	3^3	3^3	0
FS*	3^3	3^3	3^0
FS	3^0	3^2	2^0
S. flueggei PK1957			
F*S*	0	0	0
F*S	3^3	3^3	0
FS*	3^2	3^3	3^2
FS	3^1	3^2	1^0
S. flueggei PK1958			
F*S*	0	0	0
F*S	3^3	3^3	0
FS*	3^3	3^3	4^2
FS	3^3	3^3	3^0

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.

[b] F, *Fusobacterium* strain; S, *Selenomonas* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.

any selenomonad (three strains of *S. sputigena*, three strains of *S. flueggei*, and one strain of *S. noxia*) was *A. naeslundii* PK984, the reference strain for actinomyces coaggregation group E. These coaggregates were compact and phase bright (Fig. 1C) and often filled the entire microscope field. For comparison, the selenomonads, fusobacteria, and actinomyces are shown separately in Fig. 1D, E, and F, respectively.

Heat treatment of the selenomonads prevented coaggregation with the actinomyces strain, but heating the actinomyces had no effect on the coaggregation, which also was not abolished by the addition of lactose (data not shown). However, heating the selenomonads did not prevent coaggregation with any of the fusobacteria (Table 2). The nine coaggregation patterns presented here represent the range of those observed with other selenomonad-fusobacterium pairs. Some of the interactions were unimodal (heating of one cell type prevents coaggregation, whereas heating of the other cell type has no effect) and lactose inhibitable (e.g., *S. infelix* PK1956 and *F. nucleatum* PK1594). Others were bimodal coaggregations (heating both cell types is required to completely block coaggregation) that were either lactose inhibitable (*S. infelix* PK1956 with *F. nucleatum* PK1590) or insensitive to lactose (*S. flueggei* PK1958 with *F. nucleatum* PK1590 or PK1905).

An unusual effect of heat treatment was observed with both unimodal and bimodal lactose-inhibitable coaggregations. In unimodal coaggregations, heating the selenomon-

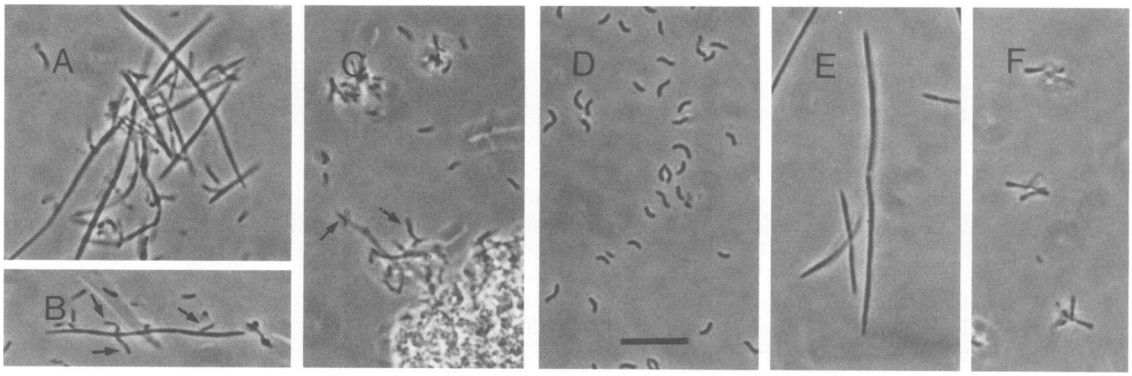

FIG. 1. Phase-contrast microscopy of coaggregates formed between *S. flueggei* PK1958 and *F. nucleatum* PK1594 at a 1:1 ratio of partners (A) or with an excess of selenomonads (B, arrows) attached to a fusobacterial filament and coaggregates of *S. flueggei* PK1958 and *A. naeslundii* PK984 (C). The edge of a large coaggregate that extends below the panel is shown where selenomonads (arrows) are shown to be attached to the actinomyces (filamentous and lighter grey). Two small coaggregates are visible at the top of the panel. Approximate cell densities of the partners before mixing together are shown in D, E, and F for *S. flueggei* PK1958, *F. nucleatum* PK1594, and *A. naeslundii* PK984, respectively. Bar, 10 μm.

TABLE 3. Coaggregation of *F. nucleatum* and reference strains of *S. sanguis* and *G. morbillorum* that represent streptococcal coaggregation groups 1 through 6[a]

F. nucleatum strain	Coaggregation score with partner strain[b]:						
	S. sanguis					*G. morbillorum*	*S. sanguis*
	DL1	H1	34	C104	J22	PK509	PK488
PK1909	$2^{1,0}$	0	$3^{3,3}$	$3^{2,1}$	$3^{3,3}$	$3^{3,0}$	$2^{2,1}$
PK1908	$3^{3,3}$	$2^{2,0}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$
PK1907	$3^{3,2}$	0	$3^{3,3}$	$3^{3,2}$	$3^{3,3}$	$3^{3,3}$	$3^{3,2}$
PK1905	$3^{3,3}$	$3^{2,0}$	$3^{3,2}$	$3^{3,2}$	$3^{3,2}$	$3^{3,3}$	$3^{3,2}$
PK1904	$3^{3,2}$	$2^{1,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$
PK1903	$3^{3,0}$	$3^{2,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$
PK1901	$3^{3,3}$	0	$3^{3,0}$	2^{0}	$3^{2,0}$	2^{0}	$2^{2,0}$
PK1597	$3^{2,0}$	0	$3^{3,3}$	$3^{3,0}$	$3^{3,0}$	$3^{3,1}$	$3^{2,0}$
PK1595	$1^{1,0}$	0	2^{0}	0	1^{0}	3^{0}	$3^{2,2}$
PK1594	$3^{3,0}$	$2^{1,0}$	$3^{3,0}$	$3^{2,0}$	$3^{3,0}$	4^{2}	$4^{3,0}$
PK1592	$3^{3,0}$	$2^{2,2}$	$3^{3,2}$	$2^{2,0}$	$3^{3,0}$	$1^{1,0}$	$3^{3,0}$
PK1591	$3^{3,2}$	$3^{2,0}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$
PK1590	$3^{3,3}$	$2^{1,0}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$
PK1589	$3^{3,3}$	$3^{2,0}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$
PK1588	$3^{3,0}$	0	$3^{3,2}$	$3^{2,0}$	$3^{3,1}$	$3^{2,0}$	$3^{3,2}$

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.

[b] *S. sanguis* DL1, H1, 34, C104, and J22; *G. morbillorum* PK509; and *S. sanguis* PK488, which represented streptococcal coaggregation groups 1, 2, 3, 3, 4, 5, and 6, respectively, were tested.

ads resulted in an increased coaggregation score, and often the coaggregation was less sensitive to lactose (e.g., *F. nucleatum* PK1594 with either *S. flueggei* PK1957 or *S. flueggei* PK1958). In lactose-inhibitable bimodal coaggregations, heat treatment of either cell type reduced or abolished the lactose sensitivity, but heating of both cell types was necessary to block coaggregation completely (e.g., *S. infelix* PK1956 and *F. nucleatum* PK1590).

Coaggregation properties of *F. nucleatum*. In contrast to *Selenomonas* strains, *F. nucleatum* coaggregated with many kinds of bacteria. Although all 28 *Fusobacterium* strains were tested, the results of only 15 strains are presented. The partner cell types examined included *Streptococcus* and *Gemella* (Table 3); *Actinomyces* (Tables 4 and 5); *Peptostreptococcus*, *Propionibacterium*, and *Rothia* (Table 5); *Actinobacillus*, *Veillonella*, *Bacteroides*, and *Capnocytophaga* (Table 6); and *Porphyromonas* (Table 7) strains. Inspection of the data presented in Tables 1 and 3 to 7 revealed no definitive clusters of strains of fusobacteria with identical coaggregation properties that would delineate coaggregation groups, as has been observed for oral streptococci (7, 24, 25), actinomyces (7, 24, 26), and veillonellae (15).

Unlike the strong +4 coaggregations between several fusobacteria and bacteroides (Tables 6 and 7) that were completely inhibited by lactose, the completely lactose-inhibitable coaggregations between fusobacteria and gram-positive partners usually involved weaker interactions such as +1 or +2 coaggregations (Tables 3, 4, and 5). Three exceptions were noted, which involved lactose-inhibitable +3 coaggregation scores between *F. nucleatum* PK1595 and *Gemella morbillorum* PK509 (Table 3) or *Actinomyces naeslundii* PK91 (Table 5) as well as *F. nucleatum* PK1589 and *P. anaerobius* ATCC 27337 (Table 5).

No other group of bacteria tested to date coaggregates with as many different kinds of cell types as do the fusobacteria examined here. The possibility that these coaggregations were random with this wide variety of partners was examined by growing 21 of the 28 strains again but in a different broth medium (modified Schaedler broth) than before (brain heart infusion based broth; see Materials and Methods) and at a different place (National Institute of Dental Research) than before (Anaerobe Laboratory, Virginia Polytechnic Institute and State University). Fourteen partners were chosen and included three streptococci, five actinomyces, three veillonellae, and one each bacteroides, capnocytophaga, and actinobacillus. To determine repeatability of these potentially random coaggregating pairs, three scores were considered: (i) the original score after the two strains were mixed, (ii) the score after 60 mM lactose was added, and (iii) the score after 1 mM EDTA was added. Although most of the scores were the same in both experiments, some were positive in one experiment and negative in the other. Of the 735 scores tallied (data not shown), 26 differed in the two experiments, giving a variation of 3.5%.

TABLE 4. Coaggregation of *F. nucleatum* and reference strains of *A. naeslundii*, *Actinomyces* serovar WVa963, and *Actinomyces viscosus* that represent actinomyces coaggregation groups A through F[a]

F. nucleatum strain	Coaggregation score with *Actinomyces* strain[b]:								
	MG-1	T14V	PK19	PK29	PK947	PK602	PK606	PK984	PK1259
PK1909	$3^{3,3}$	$3^{3,3}$	$3^{3,1}$	$3^{3,2}$	$3^{3,0}$	$3^{3,2}$	$3^{3,2}$	$3^{3,2}$	1^{0}
PK1908	$3^{3,3}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$
PK1907	$3^{3,3}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$
PK1905	$3^{3,3}$	$3^{3,2}$	$3^{3,3}$	$3^{3,3}$	$3^{3,2}$	$3^{3,1}$	$3^{3,0}$	$3^{3,0}$	$3^{3,1}$
PK1904	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,1}$	$3^{3,0}$
PK1903	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,1}$	$3^{3,0}$
PK1901	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$
PK1597	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,3}$	$3^{3,2}$	$3^{3,0}$	1^{0}
PK1595	2^{0}	$2^{2,2}$	1^{0}	1^{0}	2^{0}	2^{0}	2^{0}	$3^{3,3}$	$3^{2,0}$
PK1594	$4^{4,0}$	$3^{3,0}$	$3^{3,0}$	$4^{3,0}$	$3^{3,0}$	$3^{3,0}$	$4^{4,0}$	$3^{3,2}$	$4^{4,0}$
PK1592	$4^{3,2}$	$3^{3,3}$	$3^{3,3}$	$3^{3,0}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{2,0}$
PK1591	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,2}$	$3^{3,3}$
PK1590	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,2}$
PK1589	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,0}$
PK1588	$3^{3,2}$	$3^{3,3}$	$3^{3,0}$	$3^{3,0}$	$3^{3,0}$	$3^{3,2}$	$3^{3,3}$	$3^{3,3}$	$2^{1,0}$

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.

[b] *A. viscosus* MG-1 and T14V; *A. naeslundii* PK19, PK29, PK947, PK602, PK606, and PK984; and *Actinomyces* serovar WVa963 strain PK1259, which represented actinomyces coaggregation groups A, A, B, B, C, C, D, E, and F, respectively, were tested.

TABLE 5. Coaggregation of *F. nucleatum* with *A. israelii*, *A. naeslundii*, *A. odontolyticus*, *P. anaerobius*, *P. acnes*, and *R. dentocariosa*[a]

F. nucleatum strain	Coaggregation score with:										
	Actinomyces strain[b]:								*P. anaerobius* ATTCC 27337	*P. acnes* PK93	*R. dentocariosa* PK44
	PK13	PK14	PK16	PK39	PK81	ATCC 12103	PK91	PK48			
PK1909	$3^{3,3}$	$3^{2,0}$	$3^{3,2}$	$3^{3,3}$	0	$3^{3,2}$	$3^{3,3}$	1^{0}	0	$4^{3,2}$	$3^{3,3}$
PK1908	$3^{2,0}$	$4^{3,2}$	$3^{3,1}$	$4^{3,3}$	$4^{4,0}$	$4^{3,3}$	$3^{3,3}$	$4^{4,0}$	3^{1}	$3^{1,0}$	$3^{3,3}$
PK1907	$4^{3,3}$	$3^{3,1}$	$3^{3,0}$	$3^{3,2}$	$2^{1,0}$	$3^{3,2}$	$3^{3,3}$	$3^{3,2}$	0	$3^{3,1}$	$3^{3,3}$
PK1905	$2^{2,0}$	$4^{3,0}$	$3^{3,3}$	$3^{3,3}$	$3^{2,2}$	$3^{3,3}$	$3^{3,2}$	$4^{3,3}$	2^{0}	$1^{1,0}$	$3^{2,2}$
PK1904	$4^{4,3}$	$3^{3,1}$	$3^{3,0}$	$4^{3,0}$	$4^{3,1}$	$4^{3,0}$	$4^{4,0}$	$4^{3,0}$	0	$4^{3,3}$	$4^{3,0}$
PK1903	$4^{4,0}$	$3^{3,2}$	$4^{4,0}$	$3^{3,2}$	$3^{3,0}$	$4^{3,1}$	$3^{3,0}$	$4^{4,0}$	3^{1}	$4^{3,3}$	$4^{3,3}$
PK1901	2^{0}	$3^{3,2}$	$2^{1,0}$	$3^{3,0}$	0	$3^{3,0}$	1^{0}	$3^{3,0}$	0	$3^{3,3}$	0
PK1597	$4^{3,3}$	$3^{3,3}$	$2^{1,0}$	$3^{3,3}$	0	$3^{3,3}$	$4^{3,3}$	$2^{1,0}$	3^{1}	$4^{3,3}$	$4^{3,3}$
PK1595	2^{0}	$3^{3,3}$	$4^{3,3}$	$4^{3,3}$	$3^{3,0}$	$4^{3,3}$	3^{0}	$4^{4,0}$	0	1^{0}	$3^{2,0}$
PK1594	$4^{4,0}$	$3^{3,0}$	$4^{3,0}$	$4^{3,0}$	ND	$4^{3,0}$	$4^{3,0}$	ND	ND	$3^{3,0}$	$4^{3,1}$
PK1592	$4^{4,3}$	$2^{2,2}$	$4^{4,3}$	$4^{4,4}$	$3^{3,0}$	$4^{3,3}$	$4^{3,1}$	$4^{3,0}$	0	$4^{4,3}$	$3^{3,3}$
PK1591	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$4^{3,3}$	ND	$3^{3,3}$	$3^{3,3}$	ND	ND	$3^{2,2}$	$3^{3,3}$
PK1590	$4^{4,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	0	$3^{3,3}$	$3^{3,3}$	$4^{3,2}$	0	$4^{3,3}$	$4^{3,3}$
PK1589	$4^{4,3}$	$3^{3,3}$	$3^{3,3}$	$3^{3,3}$	$4^{3,2}$	$3^{3,3}$	$3^{3,3}$	$4^{3,3}$	3^{0}	$4^{3,3}$	$3^{3,3}$
PK1588	$4^{3,3}$	$3^{2,2}$	$3^{2,0}$	$3^{3,3}$	$3^{2,0}$	$3^{3,2}$	$3^{3,1}$	$3^{3,0}$	0	$4^{3,3}$	$3^{3,3}$

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1. ND, Not done.
[b] *A. israelii* PK13, PK14, PK16, PK39, PK81, and ATCC 12103; *A. naeslundii* PK91; and *A. odontolyticus* PK48.

Considering only the original score given after mixing the two cell types, again a variation in scores of only 3.3% was observed. All but one of these changes were in scores of 0, +1, or +2, whereas the strong coaggregations of +3 and +4 scores did not change. Thus, it appears that the coaggregation properties of a single strain of fusobacterium are quite definitive even under two different growth conditions. Furthermore, individual strains of fusobacteria appeared to specifically recognize only certain partners, whereas as a group they coaggregated with nearly all of the strains tested. Interestingly, none of the fusobacteria coaggregated with the other fusobacteria, which further demonstrates the specificity of their coaggregation with other oral partners.

Effect of heat treatment (85°C, 30 min) of cells on ability to coaggregate. Each cell type of selected pairs of fusobacteria and partner strains was subjected to heating before mixing. Results that are representative of these experiments with the various partners are given for two to four strains of each partner cell type and four fusobacteria. Most coaggregations with both gram-negative (Tables 8, 9, 10, and 11) and gram-positive (Tables 12 and 13) partners were unimodal. Forty-two of the partnerships between two gram-negative cell types were unimodal, such as the coaggregation of *Veillonella atypica* PK1910 and *F. nucleatum* PK1590 (Table 8); only 11 were bimodal coaggregations, as seen in *B. intermedius* PK1511 and *F. nucleatum* PK1905 (Table 11).

TABLE 6. Coaggregation of *F. nucleatum* and strains of *A. actinomycetemcomitans*, *Veillonella* sp., *Bacteroides* sp., and *Capnocytophaga* sp.[a]

F. nucleatum strain	Coaggregation score with:											
	A. actinomycetemcomitans		*Veillonella* sp.[b]				*Bacteroides* sp.[c]			*Capnocytophaga* sp.[d]		
	Y4	N27	PK1910	PK1950	PK2503	PK2502	PK1295	PK1277	PK1511	ATCC 33612	ATCC 33596	ATCC 33624
PK1909	3^{0}	1^{0}	2^{0}	0	1^{0}	0	0	0	0	0	$3^{3,3}$	1^{0}
PK1908	1^{0}	$3^{3,2}$	ND	ND	ND	ND	$2^{1,0}$	0	$3^{1,0}$	1^{0}	$4^{1,0}$	$3^{3,0}$
PK1907	0	$2^{2,2}$	1^{0}	2^{0}	$3^{2,0}$	2^{0}	3^{0}	0	$3^{2,0}$	3^{0}	$4^{3,1}$	$3^{1,0}$
PK1905	$2^{1,0}$	$3^{3,2}$	$3^{2,2}$	$3^{3,3}$	$3^{1,0}$	$3^{3,2}$	2^{0}	1^{0}	$3^{2,1}$	3^{0}	$3^{1,0}$	$3^{3,0}$
PK1904	2^{0}	3^{0}	$3^{3,0}$	$2^{2,0}$	0	$3^{3,0}$	1^{0}	$3^{2,0}$	3^{0}	2^{0}	$4^{3,3}$	$3^{3,0}$
PK1903	0	$3^{1,0}$	$4^{4,0}$	$4^{3,0}$	$3^{2,0}$	$4^{3,0}$	$3^{3,0}$	0	$2^{1,0}$	2^{0}	$4^{3,3}$	$3^{3,0}$
PK1901	0	$2^{1,0}$	$3^{3,0}$	$4^{4,0}$	3^{0}	$3^{2,0}$	0	0	0	1^{0}	1^{0}	3^{0}
PK1597	0	$2^{1,0}$	0	0	0	0	$3^{2,0}$	0	0	0	$4^{2,0}$	$3^{2,0}$
PK1595	3^{0}	2^{0}	0	0	0	0	0	0	0	0	2^{0}	1^{0}
PK1594	3^{0}	$3^{3,0}$	$3^{2,0}$	$4^{3,0}$	3^{0}	$4^{3,0}$	3^{0}	4^{0}	4^{0}	3^{0}	$4^{3,0}$	3^{0}
PK1592	0	0	0	0	0	1^{0}	$3^{2,0}$	0	0	1^{0}	$3^{3,3}$	3^{0}
PK1591	$3^{2,0}$	$2^{2,2}$	$3^{2,0}$	$3^{2,2}$	$4^{3,3}$	$4^{3,3}$	$1^{1,0}$	1^{0}	$2^{2,0}$	$3^{2,2}$	$3^{2,2}$	$3^{2,0}$
PK1590	2^{0}	$2^{1,1}$	1^{0}	0	0	0	0	0	0	3^{0}	$3^{3,3}$	$3^{2,1}$
PK1589	3^{0}	$3^{3,3}$	$4^{4,3}$	$4^{3,2}$	$3^{2,1}$	$4^{4,4}$	1^{0}	0	0	3^{0}	$3^{3,3}$	$3^{2,0}$
PK1588	0	1^{0}	$4^{3,0}$	$3^{2,0}$	0	$4^{3,3}$	0	0	0	1^{0}	$3^{3,3}$	$2^{1,0}$

[a] The method for assigning coaggregation scores is given in footnote *a* Table 1. ND, Not done.
[b] *V. atypica* PK1910 and *V. dispar* PK1950, PK2503, and PK2502 represent veillonella coaggregation groups 1, 2, 3, and 4, respectively.
[c] *B. loeschei* PK1295, *B. denticola* PK1277, and *B. intermedius* PK1511.
[d] *C. sputigena* ATCC 33612, *C. ochracea* ATCC 33596, and *C. gingivalis* ATCC 33624.

TABLE 7. Coaggregation of *F. nucleatum* with *P. gingivalis*[a]

F. nucleatum strain	Coaggregation score with *P. gingivalis* strain[b]:									
	PK1918	PK1919	PK1921	PK1922	PK1923	PK1924	PK1925	PK1932	PK1933	PK1491
PK1909	3^0	2^0	3^2	3^0	2^0	3^0	3^0	0	0	0
PK1908		$4^{3,0}$			4^0			0		
PK1907		$4^{4,3}$	3^3	1^0	0	1^0	0	0	0	
PK1905				4^0	4^0	4^0	4^0			
PK1904	4^0	4^0	3^0	4^0	3^0	4^0	4^0	0	0	
PK1903		$4^{4,3}$	3^3	4^0	4^0	4^0	4^0	4^4	$4^{4,0}$	3^3
PK1901		$3^{3,0}$	2^0	0	2^0	2^0	1^0	1^1	2^0	
PK1597		$4^{3,2}$	3^3	0	0	0	0	0	0	
PK1595		3^0	2^0	4^0	4^0	4^0	4^0	0		4^0
PK1594		$4^{3,2}$	3^3	4^0	4^0	4^0	4^0	3^0	3^0	3^3
PK1592		$4^{4,4}$	4^4	0	0	0	0	3^3	0	
PK1591			3^3	3^0	3^0	3^0	3^0	0		
PK1590						2^0				
PK1589		$4^{4,1}$	4^3	4^0	4^0	4^0	4^0	2^2	0	
PK1588		$4^{4,4}$	4^4	4^0	3^0	4^0	4^0	0	0	

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] The strains of *P* gingivalis used were PK1918 (D13B-11), PK1919 (D67D-9), PK1921 (D83T-3), PK1922 (VPI14018), PK1923 (VPI14019), PK1924 (VPI14020), PK1925 (VPI14021), PK1932 (VPI12505, also called W50), PK1933 (VPI1332, also called W83), and PK1491 (381).

All but one coaggregation examined between a gram-positive and a gram-negative cell type (Table 13; *A. naeslundii* PK606 and *F. nucleatum* PK1590) was unimodal, with the fusobacterium being heat inactivated. In every pairing, heat treatment of both cell types prevented coaggregation, which implicates a protein(s) as a coaggregation mediator(s).

Effect of lactose on coaggregation. None of the coaggregations with gram-positive partners was inhibited completely by lactose (Tables 12 and 13). In contrast, 22 of 43 gram-negative–gram-negative pairs that coaggregated (Tables 8, 9, 10, and 11) were completely inhibited by 60 mM lactose, and another 14 pairs were partially inhibited by lactose. As indicated above for coaggregations with selenomonads (Table 2), the lactose-inhibitable coaggregations between fusobacteria and their gram-negative partners were also less affected by the sugar when either one or the other partner was heated. For example, in unimodal coaggregations, heating the veillonellae (Table 8; *V. atypica* PK1910 with *F. nucleatum* PK1907), capnocytophagae (Table 10; *C. sputigena* ATCC 33612 with *F. nucleatum* PK1590), or bacteroides (Table 11; *B. loeschei* PK1295 with *F. nucleatum* PK1905) prevented lactose from inhibiting these coaggregations. The bimodal coaggregations between *A. actinomycetemcomitans* Y4 and *F. nucleatum* PK1590 (Table 9) or *P. gingivalis* PK1924 and *F. nucleatum* PK1905 (Table 11) exhibited similar properties. Since heat inactivation and lactose inhibition of many of the coaggregations was observed, we suggest that many of the coaggregations of fusobacteria with other gram-negative partners are mediated by lectin-carbohydrate interactions.

TABLE 8. Effect of heating cells of *Veillonella* spp. and *F. nucleatum* on their ability to coaggregate[a]

Veillonella strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
V. atypica PK1910				
F*V*[d]	0	0	0	0
F*V	0	0	0	0
FV*	3^0	4^4	4^3	4^4
FV	1^0	3^2	1^0	3^2
V. dispar PK1950				
F*V*	0	0	0	0
F*V	0	0	0	0
FV*	0	4^4	4^3	4^4
FV	0	3^3	2^0	4^3
V. dispar PK2503				
F*V*	0	0	0	0
F*V	0	0	0	0
FV*	0	3^3	3^2	4^3
FV	0	3^1	3^2	3^0
V. dispar PK2502				
F*V*	0	0	0	0
F*V	0	0	0	0
FV*	0	4^3	3^3	4^4
FV	0	3^3	2^0	4^3

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] Veillonellae are identified in footnote *b* of Table 6.
[c] Fusobacteria are identified in footnote *a* of Table 1.
[d] F, *Fusobacterium* strain; V, *Veillonella* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.

TABLE 9. Effect of heating cells of *A. actinomycetemcomitans* and *F. nucleatum* on their ability to coaggregate[a]

A. actinomycetem comitans strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
Y4				
F*A*	0	0	0	0
F*A	3^3	0	0	0
FA*	1^0	3^2	2^0	4^3
FA	2^0	2^1	0	3^0
N27				
F*A*	0	0	0	0
F*A	0	0	0	0
FA*	2^2	3^3	3^3	3^3
FA	2^1	3^3	2^2	3^3

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] F, *Fusobacterium* strain; A, *Actinobacillus* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.
[c] Fusobacteria are identified in footnote *a* of Table 1.

TABLE 10. Effect of heating cells of *Capnocytophaga* sp. and *F. nucleatum* on their ability to coaggregate[a]

Capnocytophaga strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
ATCC 33612				
F*C*	0	0	0	0
F*C	0	0	0	0
FC*	4^4	4^4	4^4	3^2
FC	3^0	3^0	3^0	3^0
ATCC 33596				
F*C*	0	0	0	0
F*C	0	0	0	0
FC*	3^3	3^3	4^3	4^4
FC	3^3	3^1	4^3	4^3
DR2001				
F*C*	0	0	0	0
F*C	0	0	0	0
FC*	0	3^3	4^4	4^4
FC	0	2^0	3^3	3^1

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] *C. gingivalis* DR2001; other capnocytophagae are identified in footnote *d* of Table 6. F, *Fusobacterium* strain; C, *Capnocytophaga* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.
[c] Fusobacteria are identified in footnote *a* of Table 1.

TABLE 11. Effect of heating cells of *Bacteroides* sp. or *P. gingivalis* PK1924 and *F. nucleatum* on their ability to coaggregate[a]

Bacteroides or *Porphyromonas* strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
PK1295				
F*B*	0	0	0	0
F*B	0	0	0	0
FB*	0	3^3	4^3	4^4
FB	0	2^0	3^0	3^0
PK1277				
F*B*	0	0	0	0
F*B	0	0	0	0
FB*	0	3^2	0	4^3
FB	0	1^0	0	4^0
PK1511				
F*B*	0	0	0	0
F*B	0	3^2	1^0	0
FB*	0	4^4	0	4^2
FB	0	3^2	3^2	4^0
PK1924				
F*P*	0	0	0	0
F*P	2^0	2^2	2^2	0
FP*	1^0	4^4	1^0	4^2
FP	2^0	4^0	1^0	4^0

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] Bacteroides are identified in footnote *c* of Table 6, and the porphyromonad is identified in footnote *b* of Table 7. F, *Fusobacterium* strain; B, *Bacteroides* strain; P, *Porphyromonas* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.
[c] Fusobacteria are identified in footnote *a* of Table 1.

TABLE 12. Effect of heating cells of *S. sanguis* or *G. morbillorum* and *F. nucleatum* on their ability to coaggregate[a]

Streptococcus or *Gemella* strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
PK488				
F*S*	0	0	0	0
F*S	0	0	0	0
FS*	3^3	3^3	3^3	4^4
FS	3^3	3^3	3^3	4^3
PK509				
F*G*	0	0	0	0
F*G	0	0	0	0
FG*	3^3	3^3	3^3	4^3
FG	3^3	3^3	3^3	4^2

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] The *Streptococcus* and *Gemella* strains are identified in footnote *b* of Table 3. F, *Fusobacterium* strain; S, *Streptococcus* strain; G, *Gemella* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.
[c] Fusobacteria are identified in footnote *a* of Table 1.

DISCUSSION

Selenomonas isolates coaggregate with the most limited array of partners observed to date compared with *Actinomyces* (7, 24, 25), *Bacteroides* (22), *Capnocytophaga* (20, 23), *Haemophilus* (29, 30), *Streptococcus* (7, 18, 24, 26), and *Veillonella* (15, 19, 19a) species. In contrast, *Fusobacterium* isolates coaggregate with the widest variety of oral bacteria.

TABLE 13. Effect of heating cells of *Actinomyces* sp. and *F. nucleatum* on their ability to coaggregate[a]

Actinomyces strain and treatment[b]	Coaggregation score with *F. nucleatum*[c]:			
	PK1590	PK1905	PK1907	PK1594
PK13				
F*A*	0	0	0	0
F*A	0	0	0	0
FA*	4^4	3^2	3^3	4^4
FA	4^4	2^2	4^3	4^4
PK91				
F*A*	0	0	0	0
F*A	0	0	0	0
FA*	3^3	3^3	3^3	4^3
FA	3^3	3^3	3^3	4^3
PK606				
F*A*	0	0	0	0
F*A	2^1	0	0	0
FA*	3^3	3^3	3^3	4^4
FA	3^3	3^3	3^3	4^4
PK1259				
F*A*	0	0	0	0
F*A	0	0	0	0
FA*	3^3	3^3	3^3	4^4
FA	3^3	3^3	3^3	4^4

[a] The method for assigning coaggregation scores is given in footnote *a* of Table 1.
[b] *Actinomyces* strains are identified in footnote *b* of Table 4 and footnote *b* of Table 5. F, *Fusobacterium* strain; A, *Actinomyces* strain; *, strain was heated at 85°C for 30 min before being mixed with its paired strain.
[c] Fusobacteria are identified in footnote *a* of Table 1.

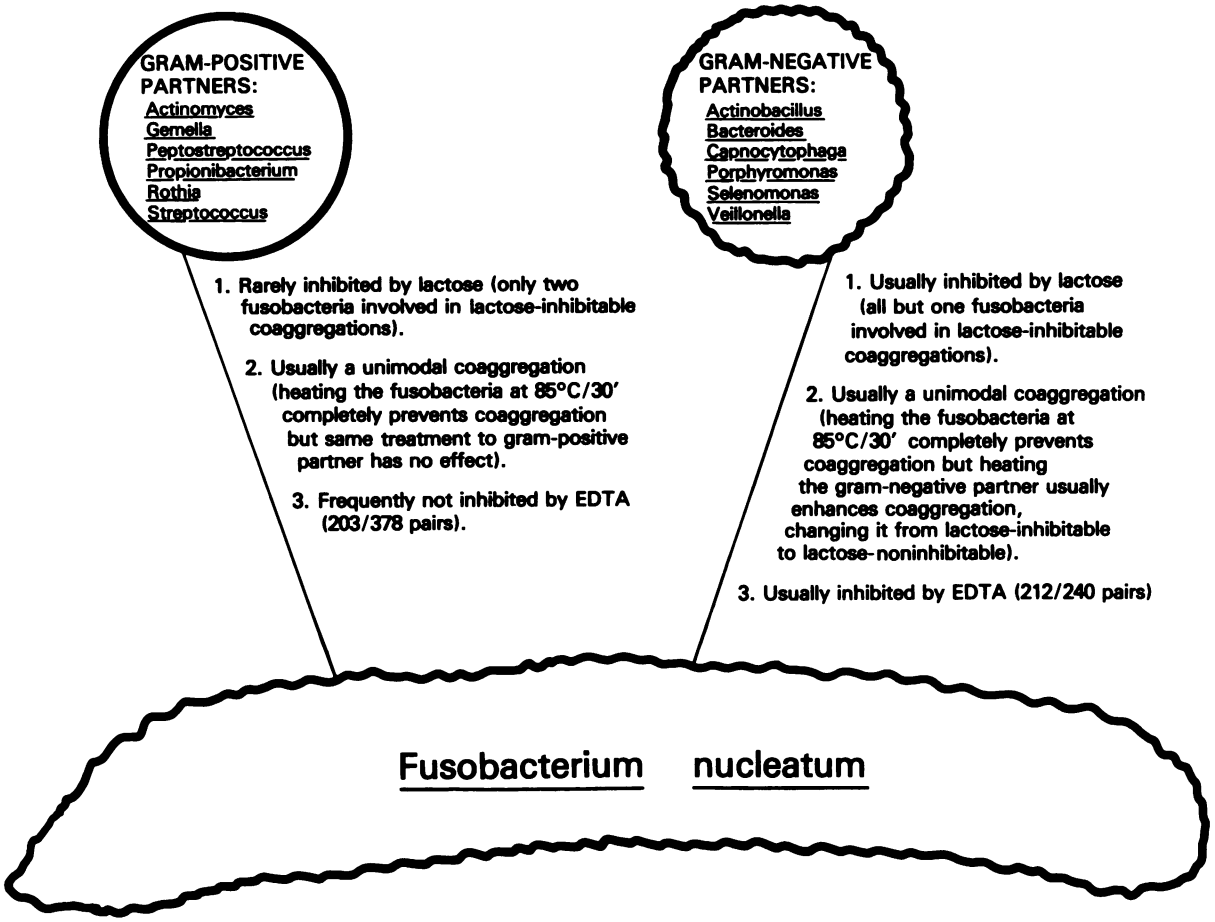

FIG. 2. Diagram summarizing the differences in the basic properties of coaggregations between strains of *F. nucleatum* and gram-positive or gram-negative partners. Most fusobacteria coaggregated with some gram-positive and some gram-negative cell types, but the adherence mechanisms appeared to be quite different.

In fact, every potential partner strain so far tested, with the exception of some selenomonads, coaggregated with at least one of the fusobacteria examined in this study.

Two previously unencountered properties of coaggregations were observed in this investigation of coaggregations among gram-negative human oral bacteria. First, some lactose-inhibitable coaggregations between fusobacteria and members of all the tested genera comprising gram-negative bacteria such as *Selenomonas*, *Veillonella*, *Actinobacillus*, *Capnocytophaga*, and *Bacteroides* or *Porphyromonas* species (Tables 2, 8, 9, 10, and 11, respectively) became less sensitive to lactose when the partner was heated before being mixed with unheated fusobacteria. These results suggest that lactose-sensitive coaggregation may become masked by a newly exposed, heat-activated, lactose-insensitive site on the gram-negative partner (21). Second, in one instance, coaggregation between untreated *A. actinomycetemcomitans* Y4 and *F. nucleatum* PK1907 (Table 9) was undetectable, but when the actinobacillus was heated, a weak lactose-inhibitable interaction was observed. This may be another case of heat-treatment-related uncovering of coaggregation mediator, or it may be that weak interactions such as +1 coaggregation scores occasionally are negative when retested. In previous surveys with gram-positive bacteria, it infrequently happened that a weak coaggregation score observed with both cell types untreated was stronger when one of the partners was treated, as was seen with *N*-acetylsuccinimide-treated *A. naeslundii* W752 and untreated *S. sanguis* M5 (7). In the current survey of partnerships among gram-negative cells, this enhanced coaggregation score occurred with all intergeneric pairings between gram-negative cell types. These results suggest that intergeneric coaggregations between gram-negative cells are very different from those between gram-positive–gram-positive or gram-positive–gram-negative pairs.

The differences in the basic properties of coaggregations between fusobacteria and both their gram-positive and gram-negative partners are depicted in a simplified diagram (Fig. 2). Although more than 600 pairs are represented here, the intent of the figure is to present only a general distinction between the coaggregations with the two kinds of partners. Three properties of the coaggregations are listed, but other properties such as a complete analysis of inhibiting sugars, the effect of protease digestion of cells on the ability of cells to coaggregate, competing cell types for coaggregation with a common partner, and the coaggregation profile of coaggregation-defective mutants will also be useful in distinguishing the basic properties of coaggregations with these two kinds of partners and in solidifying the hypothesis that gram-negative partners can bear a heat-modifiable coaggregation mediator.

Because of this widespread array of partners, it was of interest to determine whether individual strains of fusobacteria had specific partners or random interactions with other

bacteria. Three points support the idea that fusobacteria have specific partners. First, each strain coaggregated with only some of the 90 potential partner cell types tested here, and its array of partners was not the same as another strain of fusobacterium. Second, none of the fusobacteria coaggregated with other isolates of fusobacteria. Third, 21 of the 28 fusobacteria were grown again after the results of the initial survey indicated this widespread coaggregation, and the second time they were grown in a different medium at a different geographical location. A comparison of the results of the two surveys and more than 700 reactions revealed that the set of partner strains for each representative fusobacterium was nearly the same in both surveys. A variation of 3.5% was noted, but all of these differences were in the weaker coaggregation scores, which probably is a reflection of the properties of the interaction rather than of the partnership. Thus, it appears that fusobacteria as a group coaggregate with all or nearly all oral bacteria but that individual strains of fusobacteria recognize a specific set of partner strains.

Reference strains representing specific coaggregation groups of fusobacteria cannot be designated on the basis of the results presented here. These results are in full agreement with earlier DNA-DNA hybridization results with fresh isolates of *F. nucleatum* (44). The fusobacteria comprised a heterogeneous group of organisms that, by S1 nuclease assay, had DNA homology values around 60%, which is the lower limit by this assay for members of the same species (1). *Fusobacterium* isolates from the same mouth are often genotypically different (Y. Selin and J. L. Johnson, J. Dent. Res. **60**:A420, 1981), and phenotypic heterogeneity among oral fusobacteria has been reported in the soluble protein profiles as determined by polyacrylamide gel electrophoresis (2). Recent studies have confirmed and expanded the observation that *F. nucleatum* consists of at least four and probably more genotypes by DNA-DNA hybridization analyses of fresh isolates (J. L. Johnson, personal communication).

F. nucleatum is one of the most numerous bacteria found in subgingival samples taken from both healthy and diseased sites (37, 39). As the number of total bacteria increases about 10-fold, *F. nucleatum* numbers increase about 4-fold (37, 39) in samples obtained from diseased sites in adult periodontitis patients as compared with healthy sites in the same patient. *S. sputigena* increases about fivefold under similar conditions (10), and other unnamed *Selenomonas* species also increase from undetectable to about 1% of the flora when healthy and diseased sites are compared (41). Being motile, selenomonads could locate a favorable environment through taxis. They may develop a strong association with fusobacteria, both through adherence and for environmental needs, which may explain their increase in numbers along with the fusobacteria. Fusobacteria, on the other hand, are not motile, so they may rely on cell-to-cell contact to provide the necessary metabolic environment. It is well known that fusobacteria coaggregate with streptococci (18), and where the central rod-shaped fusobacteria are surrounded by the spherical streptococci, they form special morphological arrangements that resemble corncobs (27, 28). Corncobs are frequently observed in dental plaque samples (16, 31), are often found distal to the tooth surface, and are thus likely to be active in the dynamic and rapidly changing surface of dental plaque.

Accretion of cells and ensuing cell growth of the new bacterial inhabitants of developing dental plaque would be consistent with early observations in experimental gingivitis patients that bacterial populations change from mainly gram-positive cells to gram-negative cells (32, 49). Fusobacteria possess an unusual metabolic capability of glutamate-stimulated glucose uptake (45, 46), which would favor their growth if a ready supply of amino acids and glucose were consistently present. An amino acid supply can occur naturally through protease action, which is well known for several oral spirochetes (43, 50) and bacteroides (4, 48), including *P. gingivalis* (3, 13). Thus, it is plausable that fusobacteria can accomplish their numerical predominance under all conditions by integrating metabolic communication and coaggregation with bacteria that populate the econiche in health (e.g., streptococci and actinomyces) and in disease (*P. gingivalis*).

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