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Surface Structures (Peritrichous Fibrils and Tufts of Fibrils) Found on *Streptococcus sanguis* Strains May be Related to Their Ability to Coaggregate with Other Oral Genera

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We screened 36 strains of *Streptococcus sanguis* biotype I and 8 strains of *S. sanguis* biotype II for the presence of surface structures and for their ability to coaggregate with *Actinomyces viscosus*, *Actinomyces naeslundii*, and *Fusobacterium nucleatum*. Negative staining under an electron microscope revealed detectable surface structures on all *S. sanguis* strains. The majority of strains (38 of 44) carried peritrichous fibrils, which have an irregular profile and no distinct width. They usually appeared as a fringe with a constant width around the cell. Strains selected for measurement had a fringe with an average length of 72.4 ± 8.5 nm on biotype I strains and 51.6 ± 3.3 nm on biotype II strains. Some fibrillar biotype I strains carried an additional, longer (158.7 ± 33.1 nm) type of fibril projecting through the shorter fibrils. Fibrillar density was characteristic for each strain, ranging from very dense on all cells in a population to very sparse on a few cells in a population. A small group of six strains carried tufts of fibrils in a lateral or polar position on the cell. Either one or two lengths of fibril were present in the tuft depending on the strain. One strain carried both peritrichous fibrils and fimbriae. Fimbriae are flexible structures with a constant width (4.5 to 5.0 nm) all along their length but very variable lengths (≤0.7 μm) on each cell. *S. sanguis* I and II both included strains with peritrichous fibrils and tufts of fibrils, but the mixed morphotype strain was confined to biotype II. Fibrils were present on cells at all stages throughout the growth cycle for the strains tested. Freshly isolated fibrillar strains coaggregated consistently well with *A. viscosus* and *A. naeslundii*, although some fibrillar reference strains lacked the ability. In addition, all tufted strains could not coaggregate, but the strains with the mixed morphotype coaggregated well. Coaggregation with *F. nucleatum* was very strong for the fibrillar strains, but less strong for the tufted strains. We discuss the possible correlation between *S. sanguis* surface structure and ability to coaggregate.

Streptococcus sanguis appears in the human mouth when teeth erupt (3) and subsequently occurs in high numbers in supragingival plaque. Up to 8 h after cleansing, 15 to 35% of plaque isolates are *S. sanguis*, rising to as much as 70% at 1 to 2 days after cleansing (33). In vitro studies show that *S. sanguis* selectively adheres to a cleaned tooth surface very quickly (34, 35), which correlates well with its ability to adsorb to saliva-coated hydroxyapatite beads in vitro (1). Apart from adhering to the glycoprotein pellicle on the tooth surface, *S. sanguis* adheres well to other oral bacteria in vitro, a property known as coaggregation. This was first observed by Gibbons and Nygaard (9), and subsequently, a number of detailed studies have been published on coaggregation between *S. sanguis* and *Fusobacterium nucleatum* (8, 25), *Actinomyces viscosus*, *Actinomyces naeslundii* (4, 18–20), and *Bacterionema matruchotti* (24, 27, 28). The cell surface of *S. sanguis* therefore carries adhesins responsible for adhesion to the acquired pellicle and adhesion to other plaque microorganisms. Although a number of biochemical studies are available implicating various surface-associated chemical components (adhesins) in *S. sanguis* adhesion to the pellicle (2, 26) and adhesion to other bacteria (4, 8), very little attention has been directed at a concise description of the surface structures present on *S. sanguis* strains which may be responsible for coaggregation and adhesion to teeth.

Studies unrelated to adhesion or coaggregation have shown that *S. sanguis* strains can carry surface structures, but the descriptions are either not detailed or only apply to a few strains. In thin sections, *S. sanguis* M5 from dental plaque, carries hair-like filamentous processes extending up to 55 nm from the surface of the cell wall (22). Another study used negative staining to reveal fimbriae on some strains of *S. sanguis* biotype I and II from various sources, although these structures were not measured or clearly described (6). In contrast to these observations of peritrichous structures, *S. sanguis* strains isolated from human throats were found to carry polar tufts of long, thin, 4.0-nm wide fimbriae (14). These fimbriae were only present on a small proportion of cells in a culture. These reports give apparently conflicting descriptions of *S. sanguis* surface structures. The micrographs presented in this study show that within our collection of 46 strains of *S. sanguis* biotypes I and II, two main structural subgroups existed. The majority (38 of 44) of strains carried peritrichous fibrils and a minority (6 of 44) carried asymmetric tufts of fibrils.

There are very few reports of *S. sanguis* surface structures being specifically involved in coaggregation. However some structural observations have been made on the small group of *S. sanguis* biotype I strains that coaggregate with *B. matruchotti*, forming in vitro and in vivo corn-cobs (24, 27, 28). Phase-contrast, immunofluorescence, and scanning electron microscope studies showed a polar fibrillar tuft on the *S. sanguis* adhering to the long *Bacterionema* cells (28). There are no reports available describing the surface structures of *S. sanguis* strains that are able to coaggregate with *A. viscosus*, *A. naeslundii*, and *F. nucleatum*. The micrographs and coaggregation data presented in this study indicate that the type of surface structure present on the *S.*

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TABLE 1. Isolation site and surface structure type of all the fresh isolates of S. sanquis I and II used in this study

Surface structure	*S. sanguis* I strains with the designated structure	*S. sanguis* II strains with the designated structure	Isolation site
Fibrils	SC2, MJ2, KB2, MO1, CLW2, GW2, CW2, TD2, LGR2, FT2, MEH, PAR, SPED3, LMH, CH2, JF2, RA, JB, GEO2, JBR, JCL2, SE, CR2b	KN	Coronal plaque
Tufts of fibrils	CR3		Coronal plaque
Fibrils	430/2, 430/4		Periodontal abscesses
Tufts of fibrils	311		Periodontal abscesses
Fibrils and fimbriae		834	Periodontal abscesses
Fibrils	CR, TS1	EY3	Throat swab
Tufts of fibrils	AK1		Throat swab
Tufts of fibrils		PSH2, PHS1a, PSH1b	Plaque from the gingival margin
Fibrils	CR2a	CR1	Buccal swab

sanguis strain may be related to its ability to coaggregate with these organisms.

MATERIALS AND METHODS

Organisms and cultural methods. *S. sanguis* strains were isolated from the gingival margin, periodontal abscesses, throat swabs, and buccal (cheek) swabs (Table 1). The largest number of samples were taken from coronal plaque. Preliminary isolation was done on brain heart infusion agar (BHI) (Oxoid Ltd., Basingstoke, United Kingdom) with 5% horse blood and incubated at 37°C under reduced oxygen tension in a candle jar. Preliminary identification was made on colony morphology, and the strains were initially identified as belonging to *S. sanguis* (biotype I of Facklam [7]) or *Streptococcus mitior*, using the short series of tests devised by Hardie and Bowden (13), supplemented by inulin and raffinose tests. *S. mitior* strains were subsequently separated into *S. sanguis* biotype II strains and *Streptococcus mitis* strains by the criteria proposed by Facklam (7). Strains were subcultured each week on BHI blood agar and also maintained in Robertson cooked meat medium. Serial subculturing did not alter surface structure or coaggregating ability of these fresh isolates. A total of 31 *S. sanguis* I strains and 7 *S. sanguis* II strains were isolated and maintained in this way (Table 1). In addition six reference strains of *S. sanguis* I were included in the study. These were Blackburn NCTC 10231, Wicky NCTC 9124 and NCTC 7863 (ATCC 10556), *S. sanguis* I strain M5 isolated from dental plaque (B. Rosan, University of Pennsylvania, Philadelphia, Pa.), and the Lancefield group H *S. sanguis* I strain CN 2814 (Burroughs Wellcome Limited, Beckenham, United Kingdom), the strain used to produce the Wellcome group H antiserum. *S. sanguis* NCTC 7864 biotype II was also included in the study. The following human oral strains of *Actinomyces* spp. were obtained from T. Melville, Liverpool Dental School, Liverpool, United Kingdom: *A. viscosus* strains WVU627, BE64, and 10951 and *A. naeslundii* strains EF1006, 12104, and TF11. *A. viscosus* 8A06 was provided by G. Schofield, University of Cologne, Cologne, Federal Republic of Germany. *F. nucleatum* NCTC 10952 was provided by L. Tipler, Liverpool Dental School, Liverpool, United Kingdom).

Serological reactions. Extracts of the strains were prepared by the method of Rantz and Randall (31). Organisms were grown for 2 to 5 days on BHI blood agar plates until confluent growth was obtained. The cells were scraped off into 0.5 ml of phosphate-buffered saline in conical test tubes and autoclaved at 15 lb/in^2 for 20 min. All strains were tested for their ability to react with the Wellcome group H and K antiserum and antiserum against the biotype I strain *S. sanguis* M5 (kindly provided by B. Rosan, University of Pennsylvania; Philadelphia, Pa.) by the following methods. (i) In the precipitin ring technique, capillary precipitin tests were carried out by the standard procedure of Lancefield (23). (ii) For double immunodiffusion 1% Noble agar (Difco) was used, and the technique was performed by the method of Ouchterlony (30). (iii) For counter-current immunoelectrophoresis a modification of the method outlined by Kwapinski (21) was used. Agarose (1%) in sodium barbitone buffer at pH 8.2 was used to prepare thin agar on slides. Paired 3-mm-diameter wells 4 mm apart were made. Extracts of the organisms made by the method of Rantz and Randall were placed in the cathode wells, and antiserum was placed in the anode wells. A current of 4 mA per slide was passed through the agarose for 4 h. The slides were examined for precipitin lines.

Negative staining. All strains were grown overnight in BHI broth at 37°C, and the cells were harvested and washed three times in distilled water. Carbon-coated Formvar grids were ionized in a Nanotech 300S coating unit to produce a hydrophilic surface. A drop of 1% methylamine tungstate (Emscope, Ashford, United Kingdom) was added to a drop of cell suspension on a grid, and the excess liquid was removed with a filter paper strip. Micrographs were taken on an A.E.I. Corinth 500 or an H600 Hitachi electron microscope. Microscope calibration of the Corinth 500 was achieved by using a catalase crystal (Agar Aids, Stanstead, United Kingdom).

Coaggregation assays. A visual assay as described by Cisar et al. (4) was used to study coaggregation. *Actinomyces* strains were grown on a complex medium containing 0.5% tryptone, 0.5% yeast extract, 0.5% K_2HPO_4, and 0.05% Tween 80 (vol/vol) at pH 7.5. *Actinomyces* strains were incubated anaerobically at 37°C for 3 to 5 days. *S. sanguis* strains were grown in the same medium supplemented with 0.5% glucose and incubated in static culture at 37°C for 2 days. The medium for *F. nucleatum* was supplemented with 1% glucose and it was grown at 37°C anaerobically for 5 days. The cultures were all harvested by washing three times

in coaggregation buffer (4). The same buffer was used for coaggregation assays and consisted of 0.25 M potassium phosphate (pH 8.0) containing 0.025 M sodium chloride and 0.02% sodium azide. The washed suspensions were adjusted to an optical density at 660 nm of 1.5. These working suspensions were stored at 4°C and retained their coaggregating ability for several weeks. Equal volumes (0.1 ml) of the *S. sanguis* and *Actinomyces* or *F. nucleatum* suspensions were mixed on a rotamixer, left to stand at room temperature for 1 h, mixed again, and flicked gently with the finger a few times, and the coaggregation score was read. This procedure was repeated, after leaving the tubes to stand overnight, to give a second score. Although there was usually no change in score over 18 h. Control tubes were set up with 0.1 ml of each bacterial suspension plus 0.1 ml of coaggregation buffer. Any score in the control tubes due to the autoaggregation was deducted from the test score. The degree of coaggregation was scored by the method of Cisar et al. (4) on a 0 to 4+ rating as follows: 0, no visible aggregates in suspension; 1+, small uniform coaggregates in suspension; 2+, definite coaggregates easily seen but suspension remains turbid without immediate settling of coaggregates; 3+ large coaggregates formed with some settling, although the majority remained in suspension; 4+, large coaggregates which settle very rapidly leaving a clear supernatant. Selected strains of *S. sanguis* I were used to test whether they could coaggregate with each other.

RESULTS

Biochemical tests. The *S. sanguis* biotype I strains had the characteristics described by Hardie and Bowden (13). The strains did not ferment mannitol or sorbitol; they usually fermented inulin, but most did not ferment raffinose. Esculin and arginine were usually fermented. Acetoin was not produced but hydrogen peroxide was produced. Of the 36 *S. sanguis* biotype I strains, 25 produced glucan. The *S. sanguis* biotype II strains had the characteristics described by Facklam (7) and differed from the biotype I strains in the following ways. Inulin was not fermented but raffinose and melibiose were. Esculin and arginine were not usually hydrolyzed. Of eight strains, three produced glucan.

Serology. Although Lancefield group H and *S. sanguis* are not synonymous (6, 7, 32), our strains were tested against group H antiserum to look for possible correlations between serological reactions and surface structure. The Wellcome group H antiserum reacted with 42% (16 of 36) of the *S. sanguis* I stains and with none of the *S. sanguis* II strains. Sixteen of the 38 fibrillar strains and one tufted strain, AKI, reacted with group H antiserum. However, the mixed morphotype (fibrils and fimbriae) strain and two of the three tufted type I strains did not react. One *S. sanguis* I strain (311) and two *S. sanguis* II strains (PSH2 and PSH1b) gave a positive reaction with the Wellcome group K antiserum. M5 antiserum clearly distinguished between the two biotypes, *S. sanguis* I strains were all positive and *S. sanguis* II strains were all negative, when tested against this antiserum. No correlations were found between surface structure and reaction with H, K, and M5 antisera.

Negative staining. All strains of *S. sanguis* carried detectable surface structures as revealed by methylamine tungstate negative staining. One large group of 38 strains carried peritrichous fibrils, and another much smaller group of 6 strains carried asymmetric tufts of fibrils. One strain carried fibrils together with another type of morphologically distinct structure, described as fimbriae, also arranged peritrichously (for definitions see below).

(i) Peritrichous fibrils. The fibrillar strains can be subdivided into two main groups. The first and largest fibrillar group (34 biotype I strains and 4 biotype II strains) was comprised of strains carrying peritrichous fibrils. We have previously described the presence of fibrils on *S. sanguis* I NCTC 7863 and *S. sanguis* II NCTC 7864 (16). These structures are irregular in outline with no distinct width, appearing as a fringe around the cell periphery. The fibrils are not rigid and give the impression of being flexible macromolecular extensions of some cell wall component. This study measured the surface structures of a much larger number of *S. sanguis* I and II strains, and the micrographs presented here show that the length, density, and cell surface location of fibrils varies between each strain. The density of fibrillar cover of the cell wall varies from very dense on *S. sanguis* I GW2 (Fig. 1a) to a situation in which most cells are bald as shown by *S. sanguis* I MJ2 (Fig. 1e). A total of 34 *S. sanguis* I strains and 4 *S. sanguis* II strains carried peritrichous fibrils of varying density (see Table 2 for the measurements and Fig. 5 for a summary diagram). Accurate measurements of fibril length were done on selected strains (Tables 2 and 3), by drawing a line around the longest fibrils, which usually presented a very obvious margin (Fig. 1a and 1b). *S. sanguis* I GW2 carries two lengths of fibril (Fig. 1a). The shorter more globular, densely packed fibrils are 75.3 ± 22.3 nm long, and the very sparse longer fibrils are 150 ± 39.8 nm long (Fig. 1a and Table 2). The mean fibrillar length was calculated from approximately ten micrographs for each strain. Since there was a very obvious difference in the density of fibrils between strains, an attempt was made to make a subjective ranking of fibrillar density (Table 2), ranging from an arbitrary figure of 6+ for *S. sanguis* I GW2 to 1+ for the *S. sanguis* I strains CH2 and CR2b, strains which represent the extreme ends of the density range. The percentage of fibrillar cells varied between strains. All cells of *S. sanguis* I GW2 carried the shorter fibrils but many completely lacked the longer fibrils. Only three other strains in the group selected for measurement, apart from strain GW2, carried two lengths of fibril. These were *S. sanguis* I strains PAR, CN2814, and JF2 (Table 2), which all carry sparse populations of both long and short fibrils (Fig. 1c). The remaining nine strains of *S. sanguis* I in this peritrichously fibrillar group selected for measurement carried short fibrils only, as did all four strains of peritrichously fibrillar *S. sanguis* II (Tables 2 and 3). Strain NCTC 7864 was not included for measurement. *S. sanguis* I Blackburn (Fig. 1b) represents the 4+ density category, but not all cells carry detectable fibrils. In fact, close examination showed that all strains except strain GW2 showed various proportions of cells that were afibrillar. It was impossible to quantify the numbers of cells that definitely lacked these very fine, sometimes almost indistinguishable, fibrils (Fig. 1d, arrow) mainly because of the varying densities of the negative stain at the cell surface. In the 1+ density category, sparse fibrils could be found on some cells as the measurements indicate (Table 2), but the majority of cells were smooth walled (Fig. 1e). The morphology of the peritrichous fibrils varied. Some fibrils consisted of globular components both at the tips and along the length of the fibril (Fig. 1a and b), whereas other fibrils had more clearcut edges but varied in apparent diameter, sometimes tapering towards the ends (Fig. 1c). Different structural conformations might be reflected in different chemical compositions. Two *S. sanguis* I strains (JBR and SPED3) were screened for fibrils throughout the growth cycle in static and shaking culture in BHI broth, and there was no observable difference in

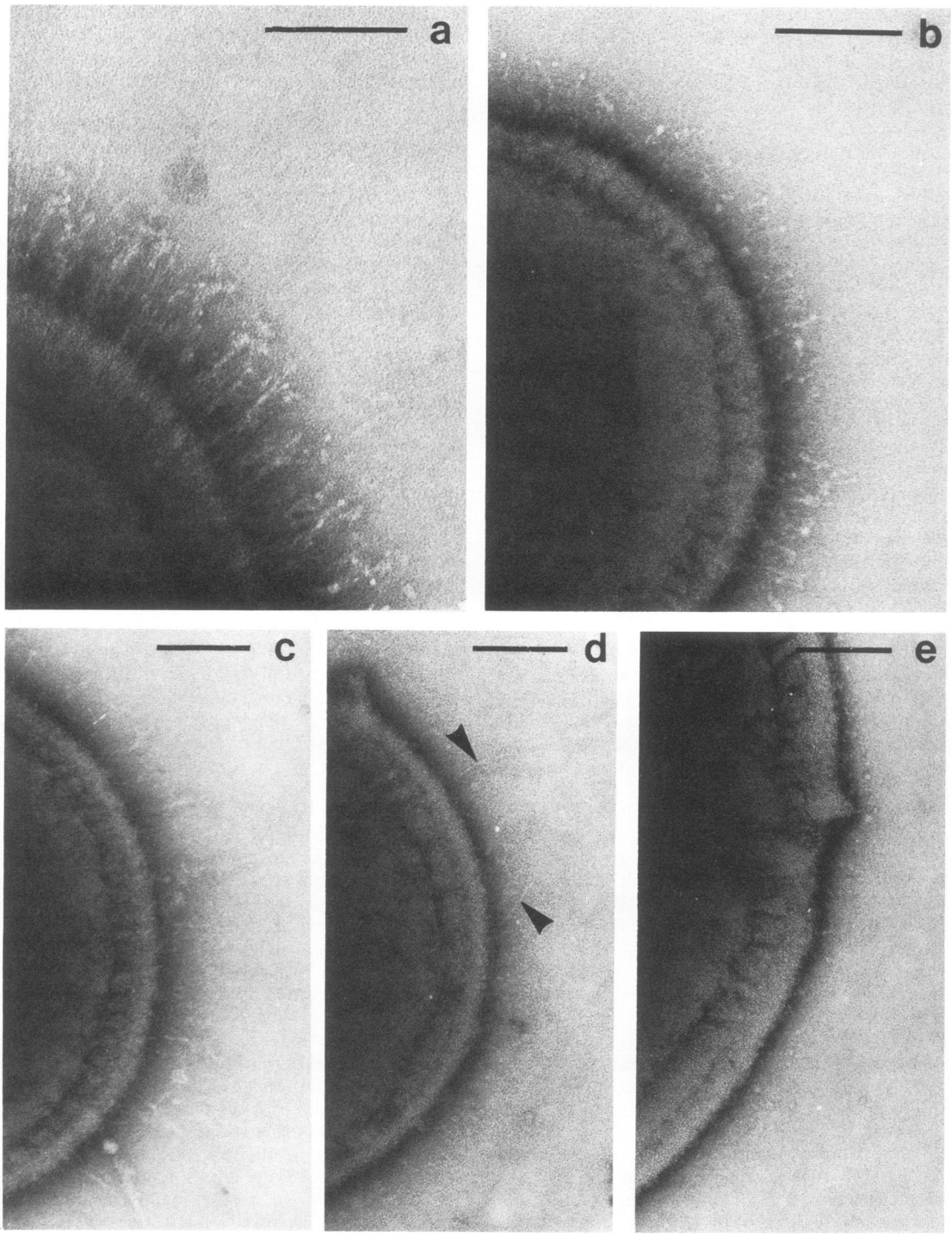

FIG. 1. (a) *S. sanguis* I GW2 carries peritrichous fibrils of two lengths. The longer (150 ± 39.8 nm) fibrils are very fine and project out through the shorter (75.3 ± 22.3 nm) globular fibrils, which have been assigned an arbitrary figure of 6+ to represent density. (b) *S. sanguis* I Blackburn carries peritrichous fibrils of one length only (61.5 ± 7.2 nm) with a 4+ density. (c) *S. sanguis* I JF2 carries fibrils of two lengths (190 ± 6.5 nm and 78.8 ± 10.9 nm) with a 3+ density. (d) *S. sanguis* I LMH carries fibrils of one length (71.0 ± 11.5 nm), but they are very sparse and very fine with a 2+ density (arrows). (e) *S. sanguis* I MJ2 has mostly smooth walled cells. Some cells carry fibrils (69.6 ± 20.4 nm) with a very sparse (1+) density. Note that strains Blackburn, JF2, LMH, and MJ2 have mixed populations of fibrillar and nonfibrillar cells. Bar, 100 nm.

numbers or distribution of fibrils at the different times of sampling. Also, surface ultrastructure was apparently unaltered after growth in the complex coaggregation medium.

Four *S. sanguis* II strains carried fibrils which were apparently structurally similar to the shorter type of fibrils carried on the *S. sanguis* I strains; however, they were all slightly shorter than the *S. sanguis* I fibrils, with an

TABLE 2. Dimensions of surface structures of selected *S. sanquis* biotype I strains

Structural group	Strain	Length of fibrils[a]	
		Long (nm)	Short (nm)
Peritrichous fibrils[b]			
6+	GW2	150 ± 39.8	75.3 ± 22.3
4+	SPED3	Absent	69 ± 14.3
4+	Blackburn = NCTC 10231	Absent	61.5 ± 7.2
3+	PAR	179 ± 59.4	86.7 ± 9.8
3+	CN2814	116 ± 1.7	75.4 ± 5.2
3+	JF2	190 ± 6.5	78.8 ± 10.9
3+	M5	Absent	67.3 ± 13.4
2+	GEO2	Absent	78.3 ± 20.7
2+	LMH	Absent	71.0 ± 11.5
2+	RA	Absent	84.3 ± 6.8
1+	MJ2	Absent	69.6 ± 20.4
1+	CH2	Absent	66.0 ± 8.4
1+	CR2b	Absent	57.8 ± 7.2
Asymmetrical lateral tufts of fibrils	311	420 ± 39.3	242 ± 13.9
	AK1	318 ± 24.1	227 ± 19.8
	CR3	386 ± 40.1	Absent

[a] Numbers are the standard deviation of the length.
[b] Numbers are the relative fibril density.

average length of 51.6 nm for the three fresh isolates (Table 3).

(ii) Tufted fibrils. The second and much smaller fibrillar group consisted of strains carrying tufts of fibrils either in a lateral or polar position on the cell surface. Three *S. sanguis* I strains carried lateral tufts (CR3, 311, and AK1), two *S. sanguis* II strains carried lateral tufts (PSH1a and PSH1b), and one *S. sanguis* II strain (PSH2) carried polar tufts of fibrils. Tables 2 and 3 give measurements of structures and Fig. 5 shows a summary diagram. *S. sanguis* I strains 311 and AK1 carried very prominent lateral tufts consisting of two lengths of fibrils, on either side of the septum, in a dividing cell (Fig. 2a). The shorter fibrils are very densely packed and have a confluent outer edge. A periodicity or banding is usually seen parallel to the outside edge of the fibrils (Fig. 2a). The longer fibrils protrude through the shorter fringe (Fig. 2a, arrow) and often clump together. One of the *S. sanguis* II strains, PSH1b has a similar arrangement of tuft fibrils, but the long and short tufts have different relative lengths (Fig. 3a and Table 3) and the short fibrils do not have a regular periodicity. This strain has an additional fibrillar component around the rest of the cell surface, but as this is usually flattened onto the cell wall, measurements cannot be taken (Fig. 3a). Two other strains, CR3 (biotype I) and PSH1a (biotype II) carry only the longer, clumped fibrillar component and lack the very dense shorter component (Fig. 3b and Tables 2 and 3). Copious clumping of the long fibrils occurs under the negative staining conditions used here and is shown in Fig. 3b. Individual fibrils cannot be distinguished because they are so fine. Only one strain of either biotype was found to have polar tufts of clumped fibrils (Fig. 2b), and this was *S. sanguis* II strain PSH2 (Table 4). This organism formed very long chains, and the tuft was found on the polar cap of the terminal cell in a chain. Sometimes the fibrillar tuft formed to one side of the true cell pole before cell separation took place (Fig. 2b). The tufts are a constant feature of these organisms and were always present at each stage of growth for the strains tested.

Biochemically, the tufted strains, except *S. sanguis* I strain CR3, all lacked the ability to produce glucans from sucrose, whereas 78% of the *S. sanguis* I fibrillar strains were glucan producers as were 37.5% of the *S. sanguis* II fibrillar strains. Facklam (7) included nonglucan producers in his study of *S. sanguis* I and II species.

(iii) Mixed morphotype—peritrichous fibrils and fimbriae in the same strain. *S. sanguis* biotype II 834 carried peritrichous fibrils (76.5 nm long) as well as another ultrastructurally distinct surface appendage, termed fimbriae. The fimbriae were 0.7 μm long, thin (ca. 5.0 nm wide) peritrichous flexible structures, with a constant width all along their length (Fig. 4)., and they never clumped together. These structures conform with the description of fimbriae outlined by Ottow (29). The fimbriae on strain 834 were very obvious, but the fibrils were difficult to detect (Fig. 4, arrow) as the stain was often dense around the cell periphery. This strain did not produce glucan. Morphologically identical fimbriae have been detected on *Streptococcus salivarius* strains which lack the K antigen (13), on *Streptococcus faecalis* strains (12) and on some *Streptococcus milleri* strains (unpublished data). An identical combination of fibrils and fimbriae has only been found on one *S. mitis* strain (unpublished data). Strains with both fibrils and fimbriae are therefore very rare.

(iv) Correlation between surface structure and ability to coaggregate with *A. viscosus* and *A. naeslundii* strains. All strains of *S. sanguis* I and *S. sanguis* II were tested against *Actinomyces* strains. With very few exceptions, the fibrillar strains coaggregated well with the *A. viscosus* strains, although some individual pairs could not coaggregate. The only fibrillar strains, which lacked coaggregating ability were four of six reference strains. *S. sanguis* I strains Blackburn, Wicky, and NCTC 7863 and *S. sanguis* II 7864 all gave 0 and 1+ scores. The coaggregation scores for the *S. sanguis* I strains selected for measurements are presented in Table 4. Generally, coaggregation scores were higher with *A. viscosus* than those with *A. naeslundii*. There was no correlation between fibril density and coaggregation scores; both densely and sparsely covered strains gave high scores in the tests. The tufted strains were consistently unable to coaggregate with the *Actinomyces* species. Strains GW2, SPED3, and CW2 were tested for coaggregation between each other, and the scores were all 0.

A similar coaggregation response was observed when the *S. sanguis* II strains were tested. The three fibrillar type II

TABLE 3. Dimensions of surface structures of *S. sanguis* biotype II strains

Structural group	Strain	Length of fibrils[a]	
		Long	Short
Fibrils[b]			
1+	KN	Absent	53.2 ± 7.7
1+	EY3	Absent	47.8 ± 4.4
3+	CR1	Absent	53.9 ± 5.81
Asymmetrical lateral tuft of fibrils	PSH1a	156.6 ± 71.6	Absent
	PSH1b	461 ± 42.2	111.2 ± 14.2
Polar tufts of fibrils	PSH2	374 ± 65.5	Absent
Peritrichous fibrils and fimbriae	834	4.7 ± 0.3 nm wide ≤0.7 μm long[c]	76.5 ± 11.9[d]

[a] Numbers are the standard deviation of length.
[b] Numbers are the fibril density.
[c] Fimbriae.
[d] Fibrils.

strains showed scores of 4+ and 3+ against *A. viscosus* and *A. naeslundii* strains. However, strains KN and CR1 autoaggregated, reducing their score to 2+. *S. sanguis* II strain 834 with the mixed morphotype showed scores of 4+ and 3+. The three type II tufted strains showed no coaggregation at all against any *A. viscosus* or *A. naeslundii* strain.

The *Actinomyces* strains grown for the coaggregation studies were also screened for the presence of fimbriae. Each strain was found to have a different density of fimbriae per cell and a different proportion of fimbriate cells when compared to each other. Fimbriation ranged from extremely dense on 100% of *A. viscosus* 8AO6 cells to very sparse on

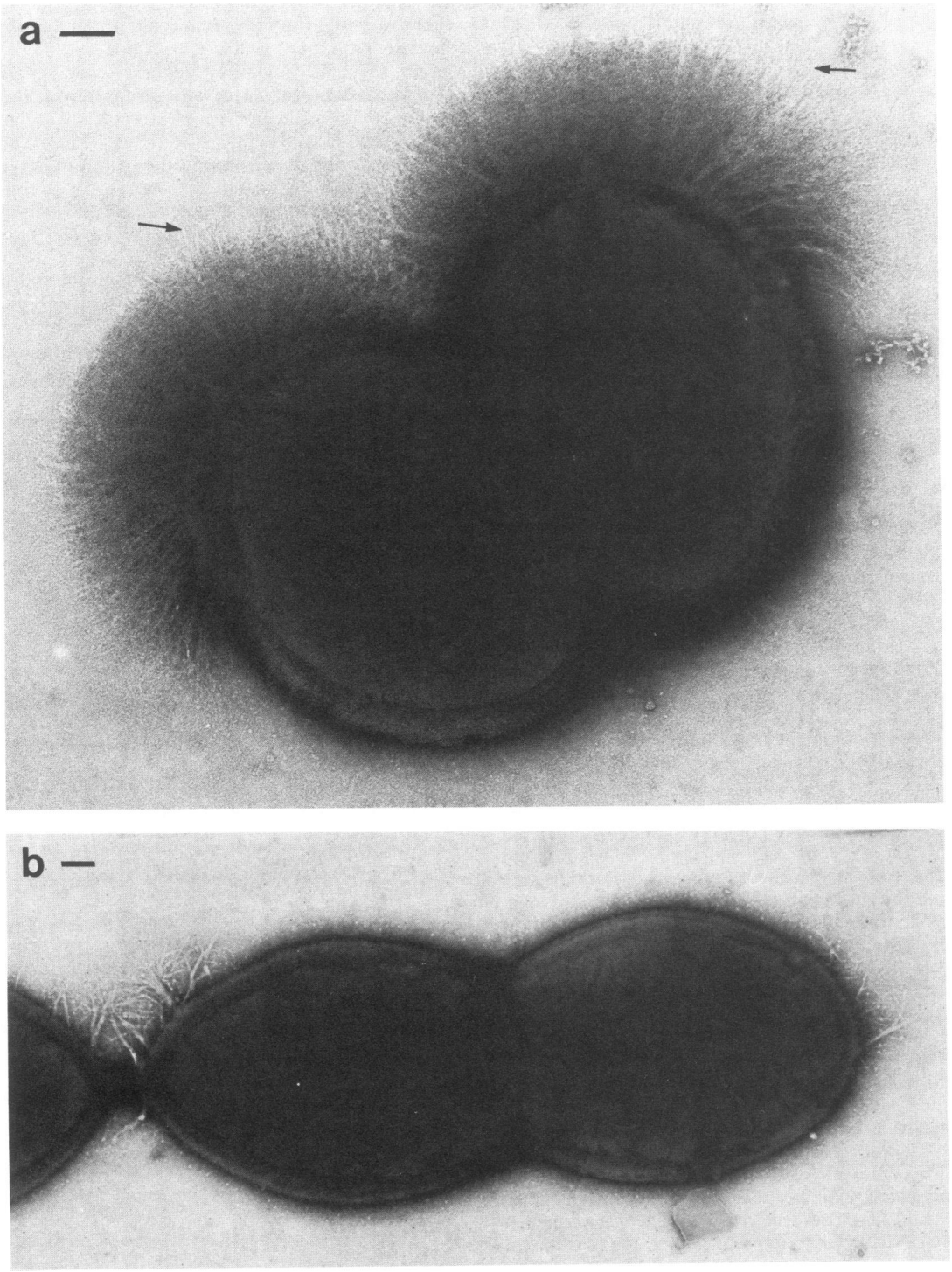

FIG. 2. (a) *S. sanguis* I AK1 carries localized lateral tufts of fibrils of two lengths. The longer fibrils (arrows) are 318 ± 24.1 nm long, and the shorter fibrils are 227 ± 19.8 nm long, with a regular banding parallel to the outer edge. (b) *S. sanguis* II PSH2 carries tufts of fibrils on the polar cap of the end cell in a chain. The fibrils are 374 ± 65.5 nm long. New tufts sometimes grow between cells before division. Bar, 100 nm.

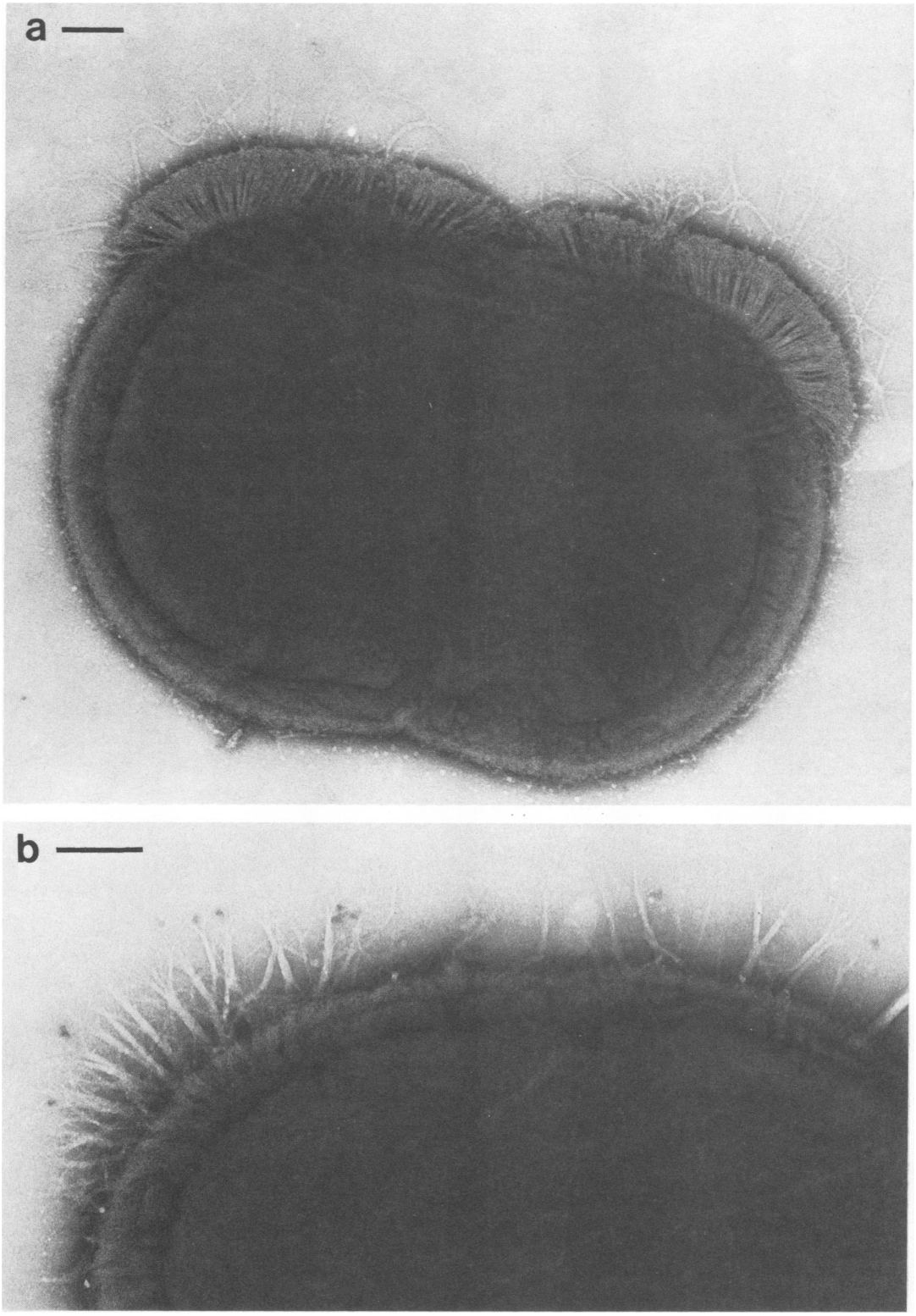

FIG. 3. (a) *S. sanguis* II PSH1b carries lateral tufts of fibrils on either side of the septum. Long fibrils are 461 ± 42.2 nm, and short fibrils are 111.2 ± 14.2 nm long. There are also sparse fibrils around the rest of the cell, but these are flattened onto the cell wall. (b) *S. sanguis* II PSH1a carries lateral tufts of fibrils that clump copiously and are 165.6 ± 71.6 nm long. The other side of the cell is completely bald (not shown). Bar, 100 nm.

TABLE 4. Relationship between surface structures of *S. sanguis* biotype I strains and ability to coaggregate with human isolates of *A. viscosus* and *A. naeslundii*

Structural group	Strain	*A. viscosus*[a]				*A. naeslundii*[a]		
		8A06	WVU627	BE64	10951	EF1006	12104	TF11
Petrichous fibrils (in order of decreasing fibril density	GW2	4	4	3	0	2	3	0
	SPED3	4	4	2	3	2	2	0
	Blackburn	0	1	1	1	1	1	ND
	PAR	3	0	0	1	1	0	0
	CN2814	3	3	3	1	1	1	3
	JF2	3	3	2	3	1	2	2
	GEO2	4	4	4	4	2	2	0
	LMH	3	4	4	4	2	2	0
	RA	4	4	4	3	2	1	2
	MJ2	3	0	0	2	1	2	2
	CH2	4	4	4	4	4	3	0
Assymmetrical lateral tuft of fibrils	311	0	0	0	0	0	0	0
	AK1	0	0	0	0	0	0	0
	CR3	0	0	0	0	0	0	0

[a] Strains of *A. viscosus* and *A. naeslundii* are arranged in order of decreasing fimbriation from left to right.

89% of *A. naeslundii* TF11 cells, which were all taken from 3- to 5-day-old cultures in complex coaggregation medium (micrographs not shown). The *Actinomyces* fimbriae all had an apparently similar ultrastructure, being ca. 5 nm wide and 1.5 μm long. These measurements agree with the description of *A. viscosus* T14V fimbriae reported by Cisar and Vatter (5). *A. naeslundii* strains had fewer fimbriae, but within the two *Actinomyces* species, different densities and numbers of fimbriae did not have any obvious effect on the ability to coaggregate with *S. sanguis* strains.

(v) Correlation between surface structure and ability to coaggregate with *F. nucleatum*. *S. sanguis* I strains were selected on a structural basis for coaggregation with *F. nucleatum* NCTC 10952. Strains GW2 and SPED3, carrying peritrichous fibrils, gave a score of 4+, and strains AK1 and 311, with polar tufts, scored1+ and 2+, respectively. No I strains and *F. nucleatum*, contrary to the observations of Lancy et al. (25). The tufted type II strains all gave a 1+ score with *F. nucleatum*, but the fibrillar type II strains were not tested. *F. nucleatum* NCTC 10952 did not carry any surface structures, although one strain has previously been reported to carry polar fimbriae (17).

DISCUSSION

The micrographs presented here show that *S. sanguis* biotypes I and II are structurally very similar despite biochemical (8) and serological (12) differences between them.

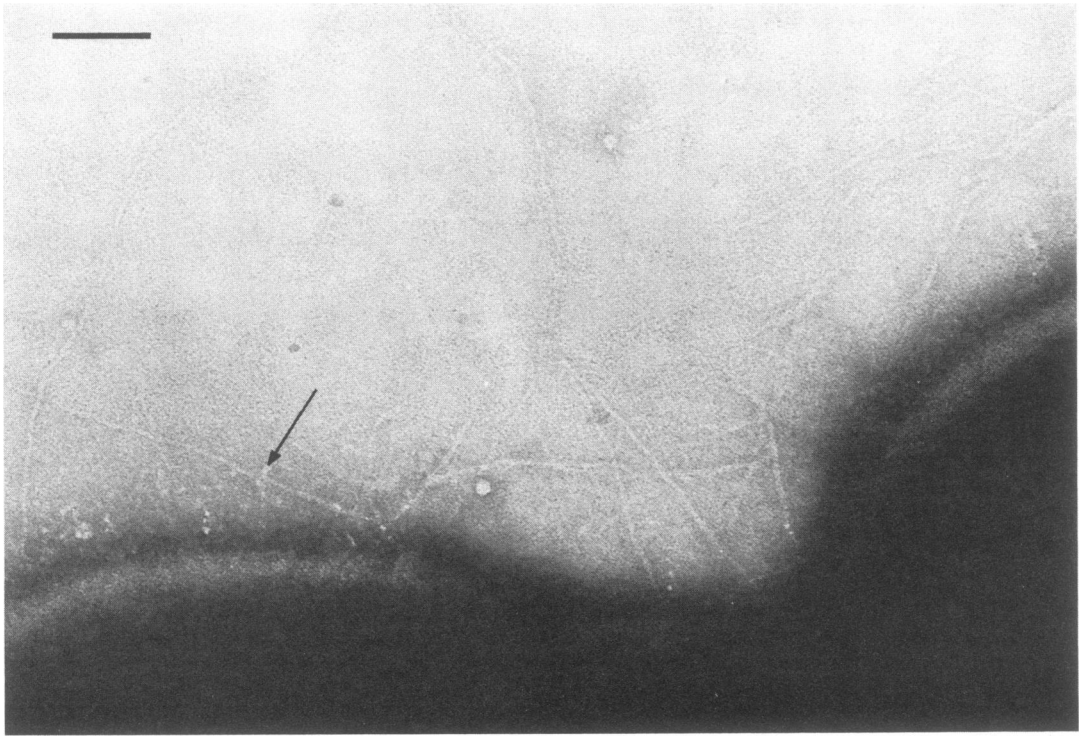

FIG. 4. *S. sanguis* II 834 carries both peritrichous fimbriae and peritrichous fibrils (arrow). Bar, 100 nm.

Each biotype can be subdivided into two structural subgroups, one group carrying peritrichous fibrils and the other group carrying tufts of fibrils. Peritrichous fibrils of one length are the most commonly observed surface structures, but within this category fibrils differ in length (53.9 ± 5.81 nm to 86.7 ± 9.8 nm), density (6+ to 1+), and fine structure. At 72.4 nm the mean length of the biotype I fibrils is longer than the mean length of the biotype II fibrils (51.6 nm), although our sample of biotype II strains was small. The fibrillar length does not apparently correlate with ability to coaggregate, as strains with long or short fibrils coaggregate equally well. The length may be more significant when adhesion to saliva-coated hydroxyapatite beads is considered, as biotype I fibrillar strains adhere in much higher numbers to saliva-coated hydroxyapatite beads than do biotype II fibrillar strains (unpublished data).

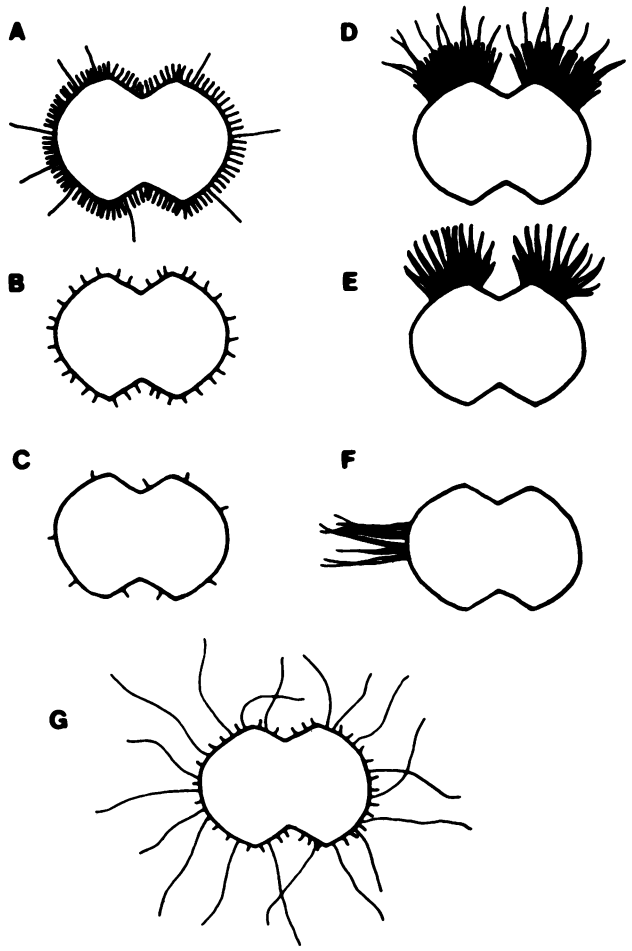

FIG. 5. Diagrammatic representation of the surface structures found on 36 *S. sanguis* biotype I strains and 8 *S. sanguis* biotype II strains. (A, B, and C) Thirty four strains of biotype I and four strains of biotype II show a continuous range of fibrillar density represented by these three diagrams. (A) Two lengths of fibril; the diagram shows the highest density found (6+). (B) Fibrils of one length with an intermediate density (3+). (C) Fibrils of one length, sparsely distributed (1+). Most fibrillar strains have cells with and without fibrils in the population. (D, E, and F) Three strains of biotype I and three strains of biotype II carry localized tufts of fibrils. (D) Lateral tufts of two lengths of fibrils; sparse peritrichous fibrils are sometimes carried as well. (E) Lateral tufts of one length of fibril. (F) Polar tufts of one length of fibril. When tufts are lateral, all cells in a population carry them. Only a small proportion of cells carry the polar tufts. (G) Peritrichous fibrils and fimbriae on one biotype II strain. Fimbriae are not present on all cells in a population.

The density of fibrils is apparently not related to the coaggregating ability of strains, as sparsely fibrillar as well as densely fibrillar strains coaggregate equally well. Also, there is no obvious correlation between fine structure and coaggregating ability, as dense globular fibrils were present on strain GW2 and sparse very fine fibrils were present on strain LMH, with both strains coaggregating well.

Four strains of *S. sanguis* biotype I carry two lengths of peritrichous fibrils, long fibrils with a mean length of 158.7 ± 33.1 nm and short fibrils with a mean length of 79.0 ± 5.3 nm. These strains bear a structural similarity to fibrillar strains of *Streptococcus salivarius*, which also carry two lengths of fibrils (13). In *S. salivarius* it is probable that different adhesive properties are carried on the two distinct structural categories of fibrils (13), but the functions of the *S. sanguis* fibrils are so far unknown.

The vast majority of *S. sanguis* I and II strains isolated from coronal plaque carried peritrichous fibrils (24 of 25 isolates). The small number of tufted strains came mainly from other oral sites (Table 1), the gingival margin, periodontal abscesses, and throat and buccal swabs. These observations on surface structure and site of isolation emerged during this study and further work is necessary to ascertain whether the surface morphology of *S. sanguis* strains is in any way related to the site of growth and therefore isolation.

All six strains carrying tufts of fibrils consistently lacked *Actinomyces* coaggregating activity, indicating a functional difference when the fibrils are localized in this way. The tufted strain, *S. sanguis* I 311 can, however, form in vitro corn-cobs with *B. matruchotti* (24), which is found in mature plaque. Only one tufted strain was recovered from coronal plaque in this study, presumably because the plaque samples were from young plaque. Four of six tufted strains carried two lengths of fibril in the tuft, which suggests that more than one functional category of fibril may exist on these strains. It is not yet known which fibril type functions in coaggregation with *B. matruchotti*.

The subdivision of *S. sanguis* I and II strains into peritrichously fibrillar strains that coaggregate with *Actinomyces* species and tufted strains that cannot coaggregate with *Actinomyces* species indicates that within the species *S. sanguis*, strains have developed specialized structures for coaggregation with different plaque genera.

The ability of *S. sanguis* to coaggregate with *A. viscosus* and *A. naeslundii* is well known. In a number of studies Cisar and Vatter (4), Kolenbrander et al. (19); and Kolenbrander and Williams (20) have been able to identify six different groups of actinomycetes (A through F) and six different groups of streptococci (1 through 6) on the basis of their coaggregation patterns and sensitivity to inhibition by lactose. Their results show that the surface of both coaggregating genera are functionally complex, and the coaggregation adhesins are strain specific. It is very unlikely that the negative-staining technique would be sensitive enough to detect subtle biochemical differences in coaggregation adhesins on the cell surface of the six subgroups of streptococci, particularly if they are all found to have fibrillar surfaces similar to those observed here.

Lactose-reversible coaggregations are very common between *Actinomyces* and *S. sanguis* strains (4), and pilot experiments with D-lactose were carried out to determine whether this property correlated in any way with the presence of a certain type of surface structure. There was no

obvious correlation between inhibition by D-lactose and surface structure type. Lactose inhibited coaggregation involving streptococci with two lengths of peritrichous fibrils as well as those with one length, and some carrying peritrichous fibrils were not inhibited at all.

The mixed morphotype strains coaggregated well with both *A. viscosus* (4+) and *A. naeslundii* (2+) strains. At present it is impossible to assess the relative contribution made by the fibrils and fimbriae to the coaggregation of strain 834. However, three fimbriate strains of *S. milleri* tested were unable to coaggregate with the same *Actinomyces* strains (unpublished data), so it is possible that the properties of the fibrils may dominate in the coaggregation response if both fibrils and fimbriae are present on a strain.

The correlation between *S. sanguis* surface structure and coaggregation with *F. nucleatum* is less clear, and none of the strains tested gave a score of 0. However, the fibrillar strains did give higher scores than the tufted strains. *F. nucleatum* is unusual in that it can coaggregate with both *S. salivarius* strains (36; personal observation) and *S. sanguis* strains (16). Thus, there is no marked strain or species specificity in coaggregation reactions with *F. nucleatum*.

Our results provide a possible explanation for the previously observed strain specificity in coaggregation reactions between *S. sanguis* and *A. viscosus* and *A. naeslundii* (4). Although fibrillar strains coaggregate, there is no evidence as yet that fibrils are responsible for coaggregation. It would be helpful to screen coaggregation negative (COG^-) mutants (19) for possible loss of fibrils. It is probable that in some strains coaggregation adhesins are located on the cell wall; this study shows that in some coaggregating strains of *S. sanguis*, a large proportion of cells do not carry fibrils at all but coaggregation scores are still high.

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