A Comparison of the Adhesion, Coaggregation and Cell-surface Hydrophobicity Properties of Fibrillar and Fimbriate Strains of *Streptococcus salivarius*

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Fibrillar and fimbriate strains of *Streptococcus salivarius* were compared for their ability to adhere to buccal epithelial cells and saliva-coated hydroxyapatite beads, and for their ability to coaggregate with *Veillonella* strains. The fibrillar Lancefield group K strains adhered statistically significantly better to both buccal epithelial cells and saliva-coated hydroxyapatite beads than the fimbriate strains, which lacked the Lancefield group K antigen. After 1 h the fibrillar strains coaggregated statistically significantly better than the fimbriate strains with *V. parvula* strain V1, but after 24 h, coaggregation both of fibrillar and of fimbriate strains reached approximately 90%. Freshly isolated *Veillonella* strains all coaggregated with the *S. salivarius* strains, but the percentage coaggregation varied considerably after 1 h depending on the *Veillonella* strain. Coaggregation was independent of the presence of Ca2+. *S. salivarius* strain HB-V5, a mutant of strain HB that had lost the Veillonella-binding protein, coaggregated weakly with *V. parvula* strain V1, but coaggregated very well with other wild-type veillonellae, suggesting the presence of an alternative mechanism for Veillonella-binding for strain HB. Fibrillar strains were, therefore, more adhesive to oral surfaces and coaggregated with veillonellae after 1 h better than the fimbriate *S. salivarius* strains. Both fibrillar and fimbriate strains were highly hydrophobic in the hexadecane–buffer partition assay.

INTRODUCTION

Adhesins are often located on the surface of bacteria in the form of fimbriae. These appendages, which are very common in Gram-negative genera, are approximately 4.0–10.0 nm in width, 0.5–4.0 μm long, and are usually evenly distributed over the cell surface (Ottow, 1975; Pearce & Buchanan, 1980). So far only a very few Gram-positive genera have been found to carry structures which fit this description and which are responsible for adhesion. *Corynebacterium renale* strains (serotypes I, II and III) have prominent fimbriae which are probably responsible for haemagglutination of trypsinized sheep erythrocytes (Honda & Yanagawa, 1974) and adhesion to tissue culture cells (Honda & Yanagawa, 1975). Serotypes II and III have subsequently been renamed as *Corynebacterium pilosum* and *Corynebacterium cystitidis* respectively (Yanagawa & Honda, 1978). *Actinomyces viscosus* carries two distinct types of fimbriae, one type responsible for adhesion to saliva-coated hydroxyapatite beads (Clark et al., 1984) and the other responsible for coaggregation with *Streptococcus sanguis* (Cisar et al., 1983).

Recently, a new type of fibrillar surface appendage, morphologically distinct from fimbriae, has been described on strains of *Streptococcus salivarius* (Handley et al., 1984) and *S. sanguis*.

Abbreviations: BEC, buccal epithelial cells; CG, coaggregation buffer; CM, coaggregation medium; S-SHA, saliva-coated spheroidal hydroxyapatite; VBP, Veillonella-binding protein

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(Handley et al., 1985). Fibrils are structurally distinct from fimbriae for a number of reasons. Fibrils have no measurable width, tapering towards the ends or clumping together, and they are much shorter than fimbriae. Fibril lengths vary from 40–400 nm but have a consistent length for each strain. Fibrils may be arranged peritrichously or localized in tufts in a lateral or polar position.

Strains of *S. salivarius*, a commensal found on the dorsal surface of the tongue (Krasse, 1964), may be divided into two groups on the basis of cell surface appendages and the possession of the Lancefield K antigen. Approximately half of the fresh isolates from saliva react with Lancefield group K antiserum and carry two types of peritrichous fibrils: long protease-resistant fibrils and much shorter protease-sensitive fibrils (Handley et al., 1984). The latter can be further subdivided into three structurally and functionally different classes of fibrils (Weerkamp et al., 1986). The wild-type strain HB carries fibrils (Handley et al., 1984) and also carries antigen C (AgC) as a cell-surface adhesin, which is responsible for adhesion to buccal epithelial cells (BEC), salivary aggregation and haemagglutination (Weerkamp & Jacobs, 1982), properties known as host-associated adhesion factors. Strain HB also carries antigen B (AgB), synonym Veillonella-binding protein (VBP), responsible for coaggregation reactions with *Veillonella parvula* strain V1, previously known as *Veillonella alcalescens* V1 (Weerkamp & Jacobs, 1982). Surface structure analysis of mutants of strain HB deficient in AgB and AgC enabled us to show that AgB is associated with a class of 91 nm fibrils and that AgC is associated with a class of 72 nm fibrils (Weerkamp et al., 1986). We also detected a class of shorter (63 nm) fibrils on mutant strain HB-V51, which has no known function.

The other, approximately 50% of isolates carried peritrichous fimbriae, which were 0.5–1.0 μm long and 4 nm wide and fitted the description of fimbriae given by Ottow (1975) and others. The fimbriate strains did not carry the Lancefield group K antigen. The fibrillar Lancefield K+ strains and the fimbriate Lancefield K- strains are probably synonymous with serotypes I and I1 (Horsfall, 1951; Montague & Knox, 1968) since only type I strains react with the Lancefield group K antiserum (Stewart & McKeever, 1963; Williams, 1956).

It has been reported that Lancefield K+ strains of *S. salivarius* are more likely to adhere to BEC, to haemagglutinate and to be aggregated by saliva than are the Lancefield K- strains (Weerkamp & McBride, 1980a). The relationship between cell surface hydrophobicity and adhesion for *S. salivarius* is not known. Adhesion of *S. sanguis* is thought to be stabilized by hydrophobic interactions (Nesbitt et al., 1982). Moreover, high values for cell-surface hydrophobicity indicated a greater ability to adhere to some oral surfaces (Nesbitt et al., 1982). However, a lack of correlation between cell-surface hydrophobicity and adhesion has also been recorded for *S. sanguis* strains (Wyatt et al., 1987). The relative cell-surface hydrophobicities of fibrillar and fimbriate strains of *S. salivarius* have not been recorded.

Another adhesive property of *S. salivarius* also demonstrated by Weerkamp & McBride (1980a) was coaggregation with *Veillonella parvula* strain V1, previously called *Veillonella alcalescens* V1. The taxonomy of the genus *Veillonella* has been revised (Mays et al., 1982) so that no distinction is now made between these two strains, which are both included in *V. parvula*. Coaggregation, first described by Gibbons & Nygaard (1970), is thought to facilitate beneficial nutritional interactions between the coaggregation partners and is a common phenomenon between various oral genera. The type of surface structure carried on *S. sanguis* is related to its ability to coaggregate with *Actinomyces viscosus* and *Actinomyces naeslundii* (Handley et al., 1985). However, whether or not surface structures of *S. salivarius* correlate with Veillonella-coaggregating ability has not been investigated. Weerkamp & McBride (1980a) found that up to 91% of *S. salivarius* strains could coaggregate with *V. parvula* strain V1, but the visual scores for this property ranged from 1+ to 4+ and it was not specified which strains were Lancefield K+ or K-. There are no data available quantifying adhesive or coaggregating properties of strains of *S. salivarius* where the fibrillar or fimbriate nature of the cell surface is already known. Therefore, this study had two main aims. One was to quantify the relative adhesive abilities of the fibrillar and fimbriate *S. salivarius* strains in order to look for any correlation between types of surface structure, adhesive ability and cell surface hydrophobicity. The second aim was to compare the coaggregating ability of fibrillar and fimbriate strains of *S. salivarius* with *V.
paraula strain V1 and to investigate the incidence of streptococcal-binding ability in freshly isolated strains of Veillonella spp.

METHODS

Organisms and culture methods. S. salivarius strains were isolated from human saliva on brain heart infusion (BHI) agar (Oxoid) containing 5% (v/v) added horse blood. Plates were incubated at 37 °C under reduced oxygen tension in a candle jar. Identification was done as described by Handley et al. (1984). Presumptive recognition was made on colony morphology and the strains were identified using the short series of tests devised by Hardie & Bowden (1976). S. salivarius strains did not ferment mannitol or sorbitol but they did ferment inulin. Arginine was not hydrolysed but aesculin was. The Voges–Proskauder test was variable and hydrogen peroxide was not produced. Strains were stored at −70 °C in 50% (v/v) glycerol and grown on BHI blood agar when required.

S. salivarius strains SS3, SPED2, DBD, CHR, DC, JC, AM and CH were isolated and identified in this way. Dr A. H. Weerkamp (University of Groningen, The Netherlands) kindly supplied strain HB, and its two mutants HB-7 and HB-V5, which are known to be deficient in host-associated adhesion properties and to be unable to coaggregate with V. paraula strain V1, respectively (Weerkamp & McBride, 1980a). Strain HB-7 has lost the 72 nm fibrils and AgC and strain HB-V5 has lost the 91 nm fibrils and AgB (Weerkamp et al., 1986). The Lancefield group K strains CN 3928 and CN 3410 were obtained from Wellcome Diagnostics, and are the strains used to produce the Wellcome Diagnostics group K streptococcal grouping antiserum. S. salivarius NCTC 8806 was also included in this study. S. salivarius NCTC 8806 and CN 3928 carried long fibrils only, 154 nm and 191 nm, respectively (Handley et al., 1984). All other fibrillar strains used carried both long and short fibrils.

V. paraula strain V1 was provided by Dr A. H. Weerkamp and grown on BHI agar with 5% horse blood anaerobically for 3 d at 37 °C. Fresh Veillonella strains were obtained by swabbing the posterior of the human tongue dorsum and isolated on a selective medium, tryptone lactate (TL) agar (Rogosa, 1964); this contained (g 1−1 tryptone (5), yeast extract (3), sodium thioglycolate (0.75), basic fuchsin (0.002), agar (15), Tween 80 (1) and 70% (w/w) sodium lactate (21 ml−1); the final pH was 7.5, and vancomycin (7.5 μg ml−1) was added after sterilization. Presumptive recognition of Veillonella strains was made on the basis of colony morphology and Gram reaction. The only organisms to grow on the Rogosa selective medium were anaerobic Gram-negative cocci, and the only genus to fit this description is Veillonella (Cowan & Steel, 1965). The genus Veillonella includes the species V. paraula, V. dispar and V. atypica (Mays, 1982), but strains were not identified beyond the genus level in this study. Purified strains were subcultured onto TL agar without vancomycin.

The basic coaggregation medium (CM) used to grow the veillonellae for coaggregation experiments was described by Cisar et al. (1979); it contained (g 1−1 tryptone (5), yeast extract (5), Na2HPO4 (5) and 0.05% (v/v) Tween 80 (0.5 ml 1−1); the final pH was 7.4. Growth of Veillonella strains required the addition of 1 ml of a 1% (v/v) solution of 70% (w/w) sodium lactate to 100 ml CM medium. S. salivarius strains were grown in BHI broth with 0.3% yeast extract. The coaggregation buffer (CG) used for the coaggregation assay contained 0.05 M-Tris/HCl and 0.005 M-CaCl2 at a final pH of 7.0, and was modified from Cisar et al. (1979). The same Tris buffer without CaCl2 was used to investigate the influence of Ca2+ on coaggregation.

BEC adhesion assay. The method used to quantify attachment of S. salivarius to BEC was adapted from the technique used by Lambden et al. (1979). Strains were inoculated into 10 ml BHI broth with 0.3% yeast extract and 2 μCi (74 MBq) [methyl-3H]thymidine ml−1 and incubated in a candle jar at 37 °C for 18 h. The cells were washed three times in phosphate-buffered saline (PBS; Dulbecco ‘A’ tablets, Oxoid) and resuspended to an OD550 of 0.6 (10^6 bacterial cells ml−1 by direct count).

BEC were collected from three subjects and pooled into 2 ml PBS. After three washings in PBS they were resuspended to an OD590 of 0.2 (1–2 × 10^6 BEC ml−1 by direct count). For the assay 0.5 ml each of the standardized BEC and bacterial suspensions were incubated in a Bijou bottle at room temperature on a rotary device (20 r.p.m.) for 30 min. Samples (400 μl) of assay mixture were layered onto 7.0 ml 6% (w/v) dextran (Dextraven 110, Fisons) in glass test-tubes. Six replicate tubes were prepared and centrifuged at 500 g for 2.5 min to sediment the BEC, leaving unattached bacteria about 1 cm down the column. After removal of 6–9 ml of dextran the BEC were agitated on a vortex mixer and the radioactivity associated with six 50 μl samples was determined in an Intertechnique SL 300 scintillation counter. The percentage adherence of each strain attached to BEC was calculated from six replicates as follows.

\[
\text{Percentage adherence} = \frac{\text{c.p.m. sample} - \text{c.p.m. control B}}{\text{c.p.m. control A}} \times 100
\]

Sample = c.p.m. associated with the pellet recovered from a 400 μl sample of BEC plus attached bacteria spun through the dextran cushion.

Control B = c.p.m. associated with the pellet after a 400 μl sample of bacteria was centrifuged into the cushion.

Control A = c.p.m. associated with a 400 μl sample of bacteria filtered onto a 2.5 cm Whatman GF/C disc.
When separate batches of BHI-grown bacteria were tested for adhesion there was no batch-to-batch variation.

**Assay for adhesion to saliva-coated spheroidal hydroxyapatite (S-SHA).** Details of the assay used were published by Wyatt et al. (1987). Strains were radiolabelled and grown as for the BEC adhesion assay. Samples of SHA beads (20 mg) were placed in Bijou bottles and washed overnight in distilled water. The beads were then coated in 0.5 ml parotid saliva (collected from one donor) by turning in a rotary device at 20 r.p.m. for 2 h at about 20 °C. Saliva-coated beads and bacteria were washed in buffered KCl (50 mM-KCl, 1 mM-KH₂PO₄, 1 mM-K₂HPO₄, pH 6.5). Bacteria were resuspended in the buffer to a final concentration of 5–6 × 10⁶ ml⁻¹ (measured by direct counting).

For the assay, 0.5 ml of standardized bacterial suspension was added to each bottle of S-SHA beads and incubated for 2 h with rotation (20 r.p.m.) at about 20 °C. Adhesion was maximal after 2 h and the S-SHA beads were washed three times with buffer. A slurry of the S-SHA beads with 400 μl of water was transferred to a toluene-based scintillation cocktail (4 g PPO l⁻¹ and 50 mg POPOP l⁻¹) containing 33% (v/v) Triton X-100. The radioactivity associated with 12 replicate samples, each from a separate Bijou bottle, was determined in the scintillation counter. The percentage of the initial bacterial suspension adhering to the beads was calculated and a correction factor of 60% was added for quenching due to the opacity of SHA (Wyatt et al., 1987). Duplicate batches of all strains were tested.

**Coaggregation assays** (i) **Visual coaggregation assay.** *S. salivarius* strains were grown for 18 h in BHI + yeast extract and *Veillonella* strains were grown in CM medium for 2 d. All organisms were washed three times in CG buffer with CaCl₂, and resuspended to a final OD₆₆₀ of 1. Samples (0.2 ml) of each bacterial suspension were mixed in a lyophilization tube, blended on a vortex mixer and left to stand at room temperature. Coaggregation scores were read after 1 h and 24 h by agitating the suspensions on a vortex mixer and gently flicking the tubes six times to maximize the flocculation due to interbacterial aggregation (coaggregation). The suspensions were assigned a coaggregation score from 0 to 4+ (Cisar et al., 1979; Handley et al., 1985) according to the following scale: 0, no visible aggregates in suspension; 1+, very small uniform coaggregates in suspension, usually only visible with a hand lens; 2+, definite coaggregates easily seen but suspension remained turbid without settling of coaggregates; 3+, large coaggregates formed with some settling; 4+, large coaggregates which settled very quickly leaving a clear supernatant. Control tubes consisted of 0.2 ml of each bacterial suspension plus 0.2 ml of CG buffer with CaCl₂, to check for autoaggregation. The experiments were repeated to confirm scores.

(ii) **Spectrophotometric coaggregation assay.** In order to quantify the extent of coaggregation, a suspension (0.5 ml) of each *S. salivarius* strain together with a suspension (0.5 ml) of *V. parvula* strain V1 (both at an OD₆₆₀ of 1.0) were placed in a glass test-tube, mixed on a vortex mixer and immediately transferred to a cuvette (1.6 ml volume). The OD₆₆₀ of the bacterial mixture was measured at 1 h and 24 h. Control tubes contained 1.0 ml of either bacterial suspension. The percentage coaggregation was calculated using the following equation.

\[
\frac{OD_{660}(S.\ salivarius\ control + V.\ parvula\ control)/2 - OD_{660}(S.\ salivarius + V.\ parvula)}{OD_{660}(S.\ salivarius\ control + V.\ parvula\ control)/2} \times 100
\]

*HOV* salivarius control and *V. parvula* control represent OD₆₆₀ of control tubes at time t.

(S. salivarius + *V. parvula*), represents OD₆₆₀ of the mixture of both suspensions at time t.

To quantify the time-course of coaggregation, OD₆₆₀ readings were taken at 1, 2, 4, 5, 6, 7 and 24 h.

**Hexadecane partition assay.** The method used by Rosenberg et al. (1980) was modified and used to compare the cell-surface hydrophobicity of the *S. salivarius* strains. Cells were grown in BHI containing 0.3% yeast extract at 37 °C for 18 h. harvested, washed twice in Sörensen’s phosphate buffer (0.66 M; pH 7.2) and resuspended to an OD₄₄₀ of 0.5. Samples of this suspension (3.0 ml) were blended on a vortex mixer for 90 s after 200 μl hexadecane had been layered on top. The tubes were left to stand for 15 min for separation of the two phases and the OD₄₄₀ of the aqueous phase was measured. Hydrophobicity was calculated from six replicates as the percentage decrease in the optical density of the original bacterial suspension due to cells partitioning into the hexadecane layer.

**Statistical analysis.** The relationship of percentage adherence to strain and to surface structure group was analysed using a nested analysis of variance design (Winer, 1971). To test for differences between strains within the two groups, the variance ratio of variability between strains to variability within strains was calculated. There was evidence of differences between strains within the two groups (P < 0.001). Therefore, to test for differences between surface structure groups, the variance ratio of differences between groups to variability between strains was calculated.

**RESULTS**

**BEC adhesion assay**

The fibrillar strains carrying both the long and short fibrils adhered in significantly higher numbers (P < 0.005) to BEC than did the fimbriate strains and the two strains carrying only
Adhesion of *Streptococcus salivarius*

Table 1. Relationship between surface structure type and adhesion to BEC and S-SHA

<table>
<thead>
<tr>
<th>Surface structure and Lancefield group K phenotype</th>
<th>Strain</th>
<th>Percentage adhesion (± SD) to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long and short fibrils Lancefield K⁺</td>
<td>SS3</td>
<td>14.3 ± 1.3 to BEC* 22.0 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>HB</td>
<td>12.4 ± 2.1 to S-SHA 16.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>CDA</td>
<td>10.5 ± 1.2 to BEC*</td>
</tr>
<tr>
<td></td>
<td>SPED2</td>
<td>9.0 ± 0.9 to S-SHA 14.0 ± 1.0</td>
</tr>
<tr>
<td>Long flexible fimbriae Lancefield K⁻</td>
<td>DBD</td>
<td>4.8 ± 1.9 to BEC* 8.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>CHR</td>
<td>4.3 ± 0.7 to S-SHA 4.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>DC</td>
<td>2.0 ± 0.4 to BEC* 7.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>JC</td>
<td>1.8 ± 0.4 to S-SHA</td>
</tr>
<tr>
<td>Mutants of strain HB</td>
<td>HB-V5‡</td>
<td>12.1 ± 1.4 to BEC* 22.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>HB-7§</td>
<td>0.6 ± 0.3 to S-SHA 3.0 ± 0.7</td>
</tr>
<tr>
<td>Long fibrils Lancefield K⁺</td>
<td>CN 3928</td>
<td>2.9 ± 0.3 to BEC* 1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NCTC 8606</td>
<td>2.9 ± 0.3 to S-SHA</td>
</tr>
</tbody>
</table>

ND, Not done.
* Each value is the mean of six replicates.
† Each value is the mean of twelve replicates.
‡ Strain HB-V5 has lost the 90 nm class of short fibrils responsible for *Veillonella* coaggregation.
§ Strain HB-7 has lost the 72 nm class of short fibrils responsible for host-associated adhesion properties.

long fibrils (Table 1). Mutant HB-V5 adhered to the same extent as its wild-type parent strain HB, but mutant HB-7, which had lost the short 72 nm fibrillar component responsible for adhesion to BEC, could not adhere. Strain HB-7 has retained the Lancefield K⁺ antigen (Weerkamp & McBride, 1980a; P. S. Handley, unpublished observation) but it is not known whether strain HB-V5 is still Lancefield K⁺.

The conditions of this assay had been designed to optimize the numbers of bacteria attaching to the BEC, but, at a ratio of 1000 bacteria to 1 BEC, the maximum percentage adhesion found was the apparently low figure of 14.3 ± 1.3% for strain SS3. However, a visual count of the bacteria attached to BEC showed that for the highly adhesive fibrillar strains at least 80% of BEC carried more than 200 bacteria, which were impossible to count visually. The assay mixture therefore contained an excess of radiolabelled bacteria that did not adhere.

Adhesion to S-SHA

The fibrillar strains bearing long and short fibrils adhered significantly better to S-SHA than the fimbriate strains (*P* < 0.02) and strain NCTC 8606 with long fibrils only (Table 1). The adhesion rankings of the strains were very similar to those shown by the same strains in the BEC adhesion assay, with mutants HB-V5 and HB-7 giving high and low adhesion values, respectively. The fibrillar strains tested ranked in the same sequence for both the BEC and the S-SHA assays, and among the fimbriate strains only strains DC and CHR were of opposite rank in the two assays. Some strains were omitted from the S-SHA assay. The conditions of the S-SHA assay had been optimized for maximal adhesion; the highest level of adhesion to S-SHA shown by any strain was 22% for strain SS3. Even strain SS3 did not adhere very well to S-SHA, as in the same assay adhesive *S. sanguis* strains adhere to a level of 50% (Wyatt et al., 1987).

Cell surface hydrophobicity

Values for the cell-surface hydrophobicity of all the *S. salivarius* strains showed a remarkable consistency, irrespective of type(s) of surface structure(s) present (Table 2). All strains of *S. salivarius* used in this study were highly hydrophobic when tested in the partition assay between Sorensen's phosphate buffer and hexadecane. There was no difference between the cell-surface hydrophobicity of *S. salivarius* strain HB and its coaggregation-deficient mutant, strain HB-V5, but there was a very small difference between the value for strain HB and its BEC adhesion-deficient mutant, HB-7, but the difference was not statistically significant.
Fig. 1. Percentage coaggregation of *V. parvula* strain V1 with *S. salivarius* strains. The lower histograms represent percentage coaggregation after 1 h and the higher histograms the value after 20 h. Bars indicate SD.

Table 2. Coaggregation of strains of *S. salivarius* with *V. parvula* strain V1, and the streptococcal hydrophobicity values

<table>
<thead>
<tr>
<th>Surface structure and Lancefield group K phenotype</th>
<th>Strain</th>
<th>Percentage hydrophobicity, % ± SD</th>
<th>Visual score for coaggregation with <em>V. parvula</em> strain V1†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long and short fibrils Lancefield K+</td>
<td>HB</td>
<td>94.2 ± 1.5</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>SPED2</td>
<td>94.5 ± 2.6</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>SS3</td>
<td>93.4 ± 2.1</td>
<td>4+</td>
</tr>
<tr>
<td>Long flexible fimbriae Lancefield K−</td>
<td>DBD</td>
<td>93.4 ± 1.8</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>CHR</td>
<td>94.3 ± 1.8</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>CH</td>
<td>94.2 ± 0.6</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>DC</td>
<td>94.8 ± 0.8</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>94.6 ± 1.9</td>
<td>2+</td>
</tr>
<tr>
<td>Mutants of strain HB</td>
<td>HB-V5‡</td>
<td>94.7 ± 1.2</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>HB-7§</td>
<td>91.7 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>Long fibrils Lancefield K+</td>
<td>NCTC 8606</td>
<td>95.4 ± 1.2</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>CN 3928</td>
<td>95.0 ± 0.8</td>
<td>0</td>
</tr>
</tbody>
</table>

* Each value is the mean of six replicates taken from one batch of cells.
† The scale for the coaggregation scores is given in Methods.
‡, §. See Table 1.

**Coaggregation of strains with *V. parvula* strain V1**

The subjective visual scoring technique for assessing coaggregation showed that the fibrillar strains formed larger coaggregates that settled more quickly (4+) than the fimbriate strains, which formed smaller coaggregates that settled less quickly (2+) (Table 2). The coaggregation scores were the same whether they were read after 1 h or 24 h. Of the two strains with long fibrils, only one (strain NCTC 8606) coaggregated with *V. parvula* strain V1.

The apparent difference in the ability of the two main cell-surface structural types to coaggregate was investigated further by using a quantitative spectrophotometric assay. Over a period of 20 h the abilities of strain HB (fibrillar) and strain CHR (fimbriate) to coaggregate with *V. parvula* strain V1 were monitored. After 1 h the fibrillar strain HB had coaggregated by 33% and the fimbriate strain CHR by only 10%. This was presumably due to the larger
Adhesion of Streptococcus salivarius

Fig. 2. Percentage coaggregation values of freshly isolated Veillonella strains with S. salivarius strains representing each surface structure type. (a) Fibrillar strain HB, (b) fimbriate strain DC, (c) long fibrils only on strain NCTC 8606, (d) mutant strain HB-V5. Each reading is the mean of three determinations for all experiments.

coaggregates of strain HB sedimenting more quickly than the smaller coaggregates of strain CHR. However, by 20 h the decrease in optical density and, therefore, the percentage coaggregation was the same for both surface structural types. All the fibrillar and fimbriate strains had coaggregated to the same extent by 20 h (Fig. 1), but for each fibrillar strain the 1 h value for percentage coaggregation was always 2–3 times greater ($P < 0.001$) than the value for the fimbriate strains, reflecting the difference in visual coaggregation scores.

Coaggregation of freshly isolated Veillonella strains with the structural subgroups of S. salivarius

All freshly isolated strains of Veillonella could coaggregate well with representative strains from the different surface structure types of S. salivarius, and strains HB, DC, NCTC 8606 and HB-V5 were chosen for this study (Fig. 2a–d). The difference in coaggregating ability after 1 h between the fibrillar strain HB and the fimbriate strain DC was further substantiated with these fresh Veillonella strains (Fig. 2a and 2b, respectively) with two exceptions. Coaggregation of the Veillonella strains LMH and LMH2 with S. salivarius strain DC occurred to the same degree or more rapidly at 1 h than with strain HB. Final 20 h percentage coaggregation values for strain DC with the veillonellae were generally higher than the coaggregation values for strain HB (Fig. 2a, b). S. salivarius strain NCTC 8606 gave the highest 1 h and 20 h coaggregation values with the veillonellae (Fig. 2c). Strain HB-V5 had lost the VBP but it could coaggregate well with nearly all of the veillonellae (Fig. 2d); 7 out of 11 Veillonella strains coaggregated to more than 80% after 20 h. Percentage coaggregation values were not significantly altered when Ca$^{2+}$ was omitted from the CG buffer (data not presented).
Fibrillar strains of *S. salivarius* were more able to adhere to oral surfaces and to coaggregate with *Veillonella* strains than were fimbriate strains. Fibrils have only recently been described and established as being structurally distinct from fimbriae. A genus or species can consist of both fimbriate and fibrillar strains, as found in *S. sanguis* (Handley et al., 1985), *S. salivarius* (Handley et al., 1984) and in the Gram-negative genus *Bacteroides* (Handley & Tipler, 1986). *S. salivarius* strain HB is the only Streptococcus where the relationship between the presence of fibrils and adhesion properties is firmly established. Cell walls of strain HB contain at least three different protein components, two of which are present as functionally separate classes of fibrils (Weerkamp et al., 1986). The organism is therefore able to present a mosaic of surface fibrillar antigens to the environment which enables it to adhere to a variety of oral surfaces. The differences in adhesion to BEC between the fibrillar strains may be reflected in relative differences in the amounts of the fibrillar antigen C present in different strains.

The observation that the ranking of adhesion of all strains tested was almost the same for both the BEC and the S-SHA assays, indicates that similar molecules may mediate both adhesion mechanisms. The salivary macromolecules adsorbing to SHA may be the same as those bound to BEC, although this has not been demonstrated. Lancefield K+ strains of *S. salivarius* adhered in similar numbers to teeth in vivo as did *S. sanguis* strains up to 1 h after inoculation (Weerkamp & McBride, 1980b). However, *S. salivarius* was soon lost from the tooth surface whereas it was retained on the tongue dorsum. Factors other than adhesive ability, such as adverse nutritional factors, could therefore contribute to the poor survival of *S. salivarius* Lancefield K+ strains on the pellicle of teeth in vivo.

In in vivo studies of the colonization of the tongue dorsum, Weerkamp & McBride (1980b) observed that *S. salivarius* strain HB-7 was cleared from the mouths of volunteers in an average time of 7 d, whereas strain HB was cleared after 20 d, and in some cases could be recovered after 3 months. These differences between strain HB and its mutant HB-7 are reflected in their relative abilities to adhere to BEC and S-SHA in vitro. A Lancefield K- strain of *S. salivarius*, strain T3, which carries fimbriae (Handley et al., 1984), was found to adhere less well to the tongue dorsum in vivo than strain HB, again reflecting our in vitro observations. *S. salivarius* showed increased adhesion to keratinized buccal mucosa (e.g. tongue cells) over non-keratinized cells (e.g. BEC) (Sklavanou & Germaine, 1980), but since tongue cells have a very high indigenous bacterial population (P. S. Handley, personal observation) they could not be used in this study due to the possibility of interbacterial adhesion influencing the adhesion results.

The relationship between fimbriae and adhesion in the fimbriate Lancefield K- strains had not been previously investigated. In spite of the fact that cells of these strains carried thin, peritrichous fimbriae, the strains adhered only poorly to BEC and S-SHA. Therefore, the fimbriae are not likely to have a marked adhesive function in these systems. Fimbriae on Gram-negative bacteria are generally responsible for haemagglutination (Duguid & Old, 1980), but Weerkamp & McBride (1980a) found that Lancefield K- strains of *S. salivarius* did not haemagglutinate. We have confirmed this observation (P. S. Handley, unpublished data), therefore implying that the *S. salivarius* fimbriae do not play a role in haemagglutination. Protease treatment did not apparently remove the fimbriae from the cell surface of Lancefield K- strains (Handley et al., 1984), but it did reduce adhesion of the fimbriate strain DBD by 85% (C. R. Brown, unpublished data). It is therefore possible that factors responsible for the decreased adhesion to BEC of the fimbriate Lancefield K- strains are located in the cell wall.

Visual coaggregation scores and 1 h spectrophotometric values indicated that fibrillar strains coaggregated better than fimbriate strains: this was a reflection of the size of coaggregate formed. However, in the spectrophotometric assay, coaggregation percentages after 24 h shown by strains of the two surface structural types were the same. VBP is not produced by the Lancefield K- strains (Weerkamp & McBride, 1981) so a separate mechanism for Veillonella-binding must exist in these organisms. It is not known whether this 'early' and 'late' coaggregation shown by fibrillar and fimbriate strains, respectively, influences survival in vivo. Veillonellae and *S. salivarius* occur on the tongue dorsum where deep crypts exist between the papillae of the tongue. In this environment, protected from shear forces, both the adhesive
fibrillar strains and the less-adhesive fimbriate strains of *S. salivarius* could be established in coaggregation consortia, where nutritional factors play a greater role than adhesive ability. The veillonellae are thought to be nutritionally dependent on *S. salivarius* using the lactate they produce (Mikx and van der Hoeven, 1975). The veillonellae themselves are very poorly adhesive to BEC (D. W. S. Hart). No satisfactory hypothesis has been proposed to explain why approximately equal numbers of Lancefield K⁺ and K⁻ strains are recovered from the mouth (Montague & Knox, 1968; Weerkamp & McBride, 1980b; Handley et al., 1984), when the Lancefield K⁻ strains are consistently found to be less adhesive in vitro. However, in vivo the host’s immune system has been shown to modify the expression of surface antigens. Immunosuppression of five antigens expressed by *S. salivarius* strain CM6 has been demonstrated in rats, and organisms recovered after a few days showed reduced adhesion to BEC *in vitro* (Howell et al., 1979). The fibrillar strains may not therefore be able to express fully their adhesive potential *in vivo*.

The ability of the mutant strain HB-V5 to coaggregate well with the freshly isolated veillonellae was unexpected, as this mutant had lost the VBP (Weerkamp & McBride, 1981), and the class of 90 nm fibrils carrying the VBP (Weerkamp et al., 1986), and because coaggregation with *V. parvula* strain V1 was strongly reduced. It is therefore possible that mutant HB-V5 (and therefore its parent, HB) could carry another system responsible for coaggregation, independent of the VBP system. This suggestion is reinforced by the observation that two out of four of our fresh veillonellae isolates (strains CD and VW) could not be coaggregated with purified VBP and yet they could coaggregate with mutant HB-V5, whereas the other two veillonellae tested (strains V1 and JB) were coaggregated by the VBP (A. H. Weerkamp, personal communication). This indicates that at least some of the veillonellae are coaggregated by an alternative mechanism.

*S. salivarius* strains NCTC 8606 and CN 3928 are both atypical Lancefield K⁺ strains. Although strain NCTC 8606 coaggregated very well with the freshly isolated veillonellae, we have not been able to detect short fibrils (Handley et al., 1984). However, antigen B (VBP) is detectable in purified cell walls (A. H. Weerkamp, personal communication). In addition, strain NCTC 8606 also showed a low level of adhesion to BEC for a Lancefield K⁺ strain, which also contributes to its atypical nature. Strain CN 3928, although used by Wellcome Diagnostics to raise the Lancefield K grouping antiserum, should not be considered as a *S. salivarius* strain, as it did not coaggregate with *V. parvula* strain V1, adhered poorly to BEC and was untypeable by the scheme of Hardie & Bowden (1976) as well as by the API 20 Strep identification system.

Cell surface hydrophobicity, measured in the highly polar coaggregation buffer, was very high in all the *S. salivarius* strains tested. The values for percentage adhesion to hexadecane found were very similar to values found for fresh isolates of *S. sanguis* (Wyatt et al., 1987), who also found no correlation between hydrophobicity and adhesion to S-SHA beads for peritrichously fibrillar strains of *S. sanguis*. Although fibrillar and fimbriate strains of *S. salivarius* were equally hydrophobic, it is probable that the presence of short fibrils correlates with overall cell-surface hydrophobicity, as protease treatment removed the short fibrils (Handley et al., 1984) and considerably reduced hydrophobicity of strain HB (J. P. Doran, unpublished observations). Protease treatment removed the fibrillar tufts of some *S. sanguis* strains and reduced hydrophobicity by >90%, also supporting the suggested correlation between fibrils and hydrophobicity in *S. salivarius* (Hesketh et al., 1987). Although mutant HB-7 had lost both the long fibrils and the 72 nm fibrils, it showed only a 2-5% reduction in hydrophobicity as compared to its parent strain HB. Therefore, it appears unlikely that these two fibril classes contribute to this property to any great extent. However, it also seems likely on the basis of our results that partitioning between hexadecane and a highly ionized buffer is an insensitive technique with strains of *S. salivarius* as it was not able to detect differences in cell-surface hydrophobicity between strains with grossly different surface morphology and composition.

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