

## Association of Fimbriae with the Hydrophobicity of *Streptococcus sanguis* FC-1 and Adherence to Salivary Pellicles

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Received 17 March 1983/Accepted 26 April 1983

A nonhydrophobic mutant of *Streptococcus sanguis* FC-1 was isolated which has a greatly diminished capacity for attaching to experimental salivary pellicles on hydroxyapatite surfaces and for aggregating with salivary components. The mutant appears to be defective in the synthesis of fimbriae, as judged by electron microscopic observations and by its inability to exhibit twitching motility.

Hydrophobic forces have been suggested to be involved in the adherence of a variety of bacteria to host tissues (8, 11, 15, 17). Recently, Nesbitt and co-workers (10) reported that strains of *Streptococcus sanguis* have hydrophobic surface properties as judged from their ability to adsorb to hydrocarbons such as hexadecane. They also showed that agents which disrupt hydrophobic bonds inhibit the attachment of *S. sanguis* cells to saliva-treated hydroxyapatite (S-HA) surfaces which mimic those of the teeth. They suggested, therefore, that the adherence of *S. sanguis* cells to experimental salivary pellicles was at least partially dependent upon the formation of hydrophobic bonds between the streptococci and the adsorbed salivary proteins comprising the pellicle. We now report the isolation of a nonhydrophobic mutant of *S. sanguis* FC-1 which is defective in the synthesis of surface fimbriae and which exhibits a greatly diminished capacity for attaching to experimental pellicles.

*S. sanguis* FC-1 was originally isolated from a sample of human dental plaque. Cultures of the organism were stored in 50% glycerol at  $-20^{\circ}\text{C}$ . The streptococci were propagated in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and on Trypticase soy agar plates containing 5% sheep blood (Scott Laboratories, Fiskeville, R.I.). A nonhydrophobic mutant of strain FC-1 was selected by procedures described previously by Rosenberg and Rosenberg (16). Briefly, cells of *S. sanguis* FC-1 were harvested from overnight Todd-Hewitt broth cultures. The organisms were suspended in 2.0 ml of sterile PUM buffer which contained (per liter):  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ , 22.2 g;  $\text{KH}_2\text{PO}_4$ , 7.3 g; urea, 1.8 g; and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 g. Sterile hexadecane (1 ml) was added, and the suspension was mixed on a Vortex mixer for 1 min at room temperature. Strongly hydrophobic organ-

isms attach to hexadecane droplets, whereas less hydrophobic cells remain in the aqueous phase. Samples of the lower aqueous phase were then removed and inoculated into fresh tubes of Todd-Hewitt broth. This enrichment procedure was repeated six times. Samples of the enriched Todd-Hewitt broth culture were then spread on Trypticase soy agar (BBL) plates, and after incubation, approximately 50 colonies were picked and transferred as spots onto fresh plates. Colonies of nonhydrophobic mutants were detected by their inability to adhere to polystyrene petri dishes as described previously by Rosenberg (12).

*S. sanguis* nhm was isolated in this manner. It formed firm hard colonies on mitis salivarius agar which were indistinguishable from those of parental strain FC-1. In addition, strain nhm had fermentation characteristics similar to those of the parent strain FC-1, and both formed alcohol-precipitable polysaccharide when grown in 5% sucrose broth. The relative hydrophobicity of strains FC-1 and nhm was determined by comparing their ability to adsorb to hexadecane as previously described (14). The parent strain FC-1 adsorbed avidly to hexadecane, whereas only a small percentage of nhm cells attached to this hydrocarbon (Fig. 1). Thus, the mutant strain nhm is much less hydrophobic than the parent strain FC-1. The mutant strain nhm also attached in much lower numbers to S-HA surfaces than did strain FC-1 when assayed as previously described (1, 2) (Table 1). Nesbitt et al. (10) previously showed that hydrophobic bond-disrupting agents such as NaSCN strongly inhibited the adsorption of *S. sanguis* to S-HA surfaces whereas equal molar solutions of NaCl had little effect. Attachment of parental strain FC-1 to S-HA was also found to be strongly inhibited by 0.1 or 0.5 M NaSCN, whereas the weak adher-

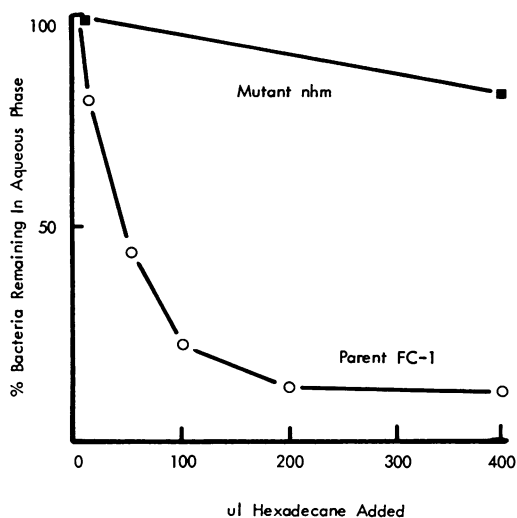


FIG. 1. Adsorption of *S. sanguis* FC-1 and mutant strain nhm to hexadecane. Reaction mixtures contained 3.0 ml of washed streptococcal cells in PUM buffer and various amounts of hexadecane.

ence of mutant strain nhm was less affected (Table 1).

That strain nhm was defective in its ability to interact with salivary components was also investigated by comparing its ability to agglutinate when mixed with clarified whole human saliva as previously described (4). Parent strain FC-1 agglutinated strongly in the presence of saliva, whereas mutant strain nhm did not.

The nature of the defect in mutant strain nhm was studied by electron microscopy. Molybdenum-stained preparations of parental FC-1 cells harvested from overnight Todd-Hewitt broth cultures exhibited very thin, densely distributed fibrils on their surface (Fig. 2A). The majority of FC-1 cells possessed these fibrils, which were most evident at the poles and division planes of the organism. In contrast, cells of mutant strain nhm were not observed to contain such fibrils (Fig. 2B).

The fibrils observed appear to be comparable to the fimbriae described previously on *S. sanguis* cells which are associated with "twitching motility" (6). Therefore, to substantiate the lack of fimbriae on mutant nhm cells, colonies of strain nhm and its parent were examined for twitching motility as previously described (6). The organisms were streaked on heart infusion blood agar plates which were incubated in Brewer jars containing a dish of water to provide a moist atmosphere. Under these conditions, cells which possess polar fimbriae tend to collect at the water-air interface and spread to give rise to irregular fried egg-shaped colonies, whereas nonfimbriated cells do not (5, 6). Colonies of parental strain FC-1 were large and exhibited a fried egg appearance after 5 to 8 days typical of organisms exhibiting twitching motility (Fig. 3A). In contrast, colonies of mutant strain nhm were smaller and much more regular (Fig. 3B). Thus, the apparent absence of twitching motility in mutant strain nhm and also the electron microscopic observations indicate that the defect in nhm cells results in an impaired synthesis of fimbriae. These findings are comparable with those of earlier studies which reported that nonhydrophobic mutants of *Acinetobacter calcoaceticus* also have an impaired synthesis of thin fimbriae (13).

If the adherence of *S. sanguis* to S-HA is dependent upon specific interactions between the fimbriae of the organism and immobilized salivary macromolecules in the pellicle which serve as specific receptors, one would expect that there would be fewer potential binding sites present in experimental pellicles for mutant strain nhm. Therefore, we carried out adsorption isotherms to estimate the numbers of binding sites and the affinities of strain FC-1 and its mutant on S-HA surfaces as previously described (1, 2). The streptococcal concentrations used for the isotherms ranged between  $1 \times 10^7$  and  $100 \times 10^7$  cells per ml. Analyses of the isotherms indicated that the experimental pellicles contained much lower numbers of binding

TABLE 1. Attachment of *S. sanguis* FC-1 and mutant strain nhm to S-HA in the presence or absence of thiocyanate

Addition to reaction mixture	<i>S. sanguis</i> FC-1		<i>S. sanguis</i> nhm	
	No. of cells $\pm$ SE ( $\times 10^3$ ) attached per 5 mg of S-HA <sup>a</sup>	% of control	No. of cells $\pm$ SE ( $\times 10^3$ ) attached per 5 mg of S-HA <sup>a</sup>	% of control
None	94.1 $\pm$ 3.3	100	3.6 $\pm$ 0.2	100
0.1 M NaSCN	18.8 $\pm$ 1.3	20	2.0 $\pm$ 0.1	56
0.5 M NaSCN	2.0 $\pm$ 0.2	2	1.2 $\pm$ 0.1	32

<sup>a</sup> Reaction mixtures (125  $\mu$ l) contained 5 mg of S-HA and  $2.5 \times 10^7$  [<sup>3</sup>H]thymidine-labeled streptococci in 0.05 M KCl containing 1 mM PO<sub>4</sub> (pH 6.0), 1 mM CaCl<sub>2</sub>, and 0.1 mM MgCl<sub>2</sub>.

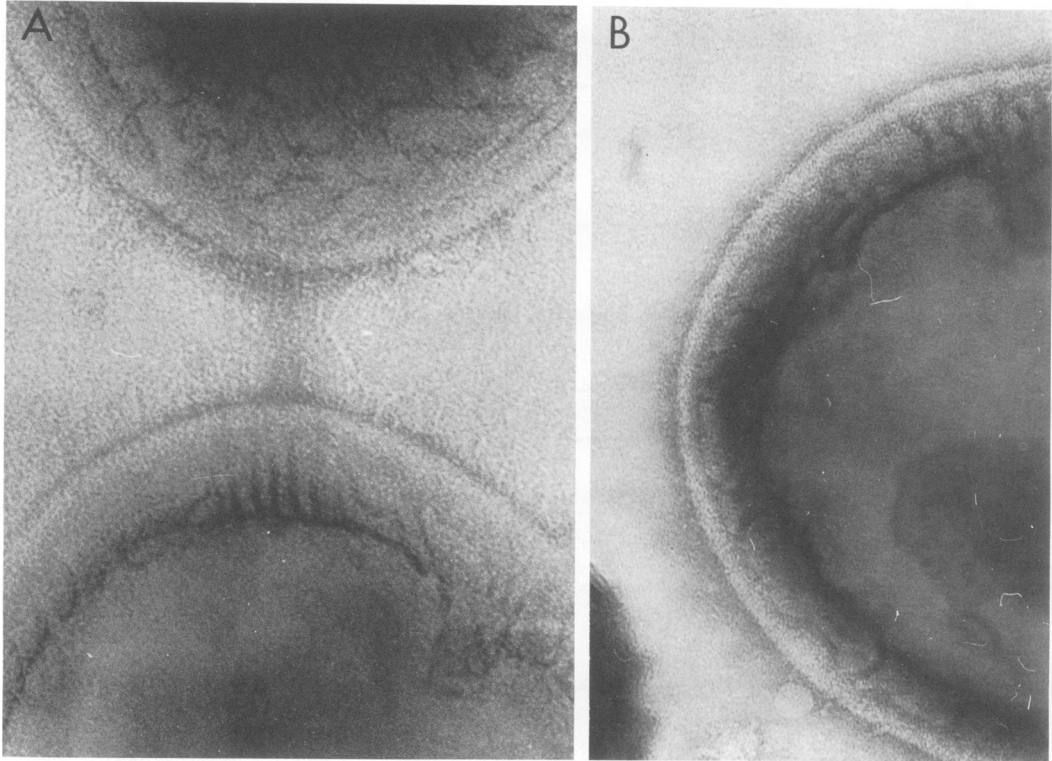


FIG. 2. Electron micrographs of molybdenum-stained cells of *S. sanguis* FC-1 (A) and mutant strain nhm (B). Note the presence of short, thin fibrils (fimbriae) on the surface of FC-1 cells which are absent on mutant strain nhm cells.

sites for mutant strain nhm than for parental strain FC-1 (Table 2). Also, the sites available for strain nhm were of lower affinity.

The data obtained support the suggestion of

Nesbitt et al. (10) that hydrophobic interactions play an important role in the adherence of *S. sanguis* cells to salivary pellicles similar to those on teeth. Nonhydrophobic mutant strain nhm

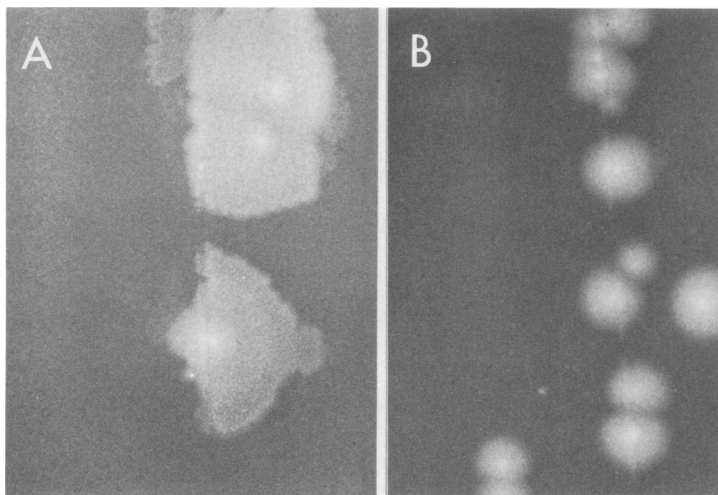


FIG. 3. Colonies of *S. sanguis* FC-1 (A) and mutant strain nhm (B) developing on blood agar plates incubated under a moist atmosphere for 5 days. Note the spreading tendency of FC-1 colonies due to twitching motility, and the lack of spreading of nhm colonies.

TABLE 2. Parameters for adsorption of *S. sanguis* FC-1 and mutant strain nhm to S-HA

Strain	Correlation coefficient	No. of binding sites $\pm$ SE ( $\times 10^6$ ) per 5 mg of S-HA	Affinity (ml/cell) $\pm$ SE ( $\times 10^{-10}$ )
FC-1	0.95	35.0 $\pm$ 1.6	21.2 $\pm$ 1.2
nhm	0.86	1.7 $\pm$ 0.2	12.3 $\pm$ 1.7

exhibited greatly diminished surface hydrophobicity as judged from its weak adherence to hexadecane, and it had a greatly impaired capacity for attaching to S-HA surfaces; it also no longer aggregated with components present in clarified whole human saliva. These observations support the notion that there is a relationship between salivary aggregating factors and adherence of *S. sanguis* cells to S-HA (3). The nature of the defect in nhm cells appears to be an impaired synthesis of fimbriae, as determined by electron microscopic observations of negatively-stained preparations and also by the loss of twitching motility. The data, therefore, suggest that the fimbriae of *S. sanguis* cells are largely responsible for its hydrophobic properties and also for its adsorption to specific salivary receptors in experimental pellicles on hydroxyapatite surfaces. The latter observation is consistent with the report of Fives-Taylor (1a), who previously observed that certain nonadherent mutants of *S. sanguis* exhibited defects in fimbriation. It seems likely that these fimbriae also contain the protein adhesin (7) or sialic-binding lectin (9) (or both) previously described for this organism.

This investigation was supported in part by Public Health Service grant DE-02847 from the National Institute of Dental Research and in part by a grant from the Colgate-Palmolive Co.

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