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Fimbriae on the Surface of the Gram$^+$ Bacteria *Streptococcus parasanguis*

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Streptococcus parasanguis is one of the primary colonizers of the oral cavity and has been shown to be involved in causing caries and infective endocarditis [1]. Long fibrillar appendages extending from the cell surface are essential for the ability of this organism to adhere to specific host cell receptors and, therefore for the infectious process. Since the initial discovery of fimbriae on the surface of *S. parasanguis* they have been observed in other members of the Streptococcal and Staphylococcal families (*e.g. Streptococcus pneumoniae*, *Staphylococcus aureus* and *Staphylococcus epidermidis)* [2,3]. A wealth of information has been obtained recently from electron microscopy and image analysis studies of Gram-negative bacterial pili [4,5]. This work has shown that although different pilin subunits have high sequence similarity, the pili structures are very different, and that the structural polymorphism correlates well with the environmental requirements of the organisms. However, the structural information available for Gram-positive bacterial fimbriae is negligible. Given the growing number of drug-resistant bacteria, it is crucial to get a thorough knowledge of the adhesion mechanism. Understanding the structural/functional basis of these mechanisms will aid in the development of the best agents to interfere with colonization.

The major constituent of the fimbriae of *S. parasanguis*, Fap1 (fimbriae associated protein 1) was isolated, characterized and cloned aided by monoclonal antibodies that did not bind to afimbriated mutants[6,7]. Fap1 is a 200kDa glycoprotein which belongs to the family of cell wall anchored adhesins. Fap1 belongs to a growing family of serine-rich repeat proteins found in other human pathogens of the Streptococcal and Staphylococcal families. The sequence contains a 50 residues long signal peptide and two regions of (E/V/I)S dipeptide repeats that account for 80% of the sequence. Fap1 forms peritrichous fimbriae of about 0.6μm in length. We have analyzed the feasibility of imaging the bacterial fimbriae using several negative stained preparations(1% uranyl acetate (UA), 2% phosphotunstic acid (PTA), 2% ammonium molybdate, Nanotungstate(NanW) and Nanovan, both from Nanoprobes. We have obtained good results with preparations in UA, PTA and NanW. We have also been able to visualize the *fap1*$^-$and the *gap3*$^-$ strains using similar techniques and have confirmed that the peritrichous fimbriae are not assembled in these strains and that the intermediate fimbriae can easily be visualized.

We are calculating three-dimensional structures of these fimbriae from whole mount preparations of either UA or PTA stained bacteria. Filaments have been windowed from the images, normalized and CTF corrected prior to the image processing analysis using helical-single particle reconstruction algorithms [8] and Radon transform-based alignment algorithms [9]. The fimbriae are very flexible structures that bend easily in all the preparations and have diameters of about 5-6nm. A close look at individual fimbriae reveals an internal substructure with a periodicity of about 6.6nm, which we are currently trying to interpret in correlation with the single Fap1 subunits.

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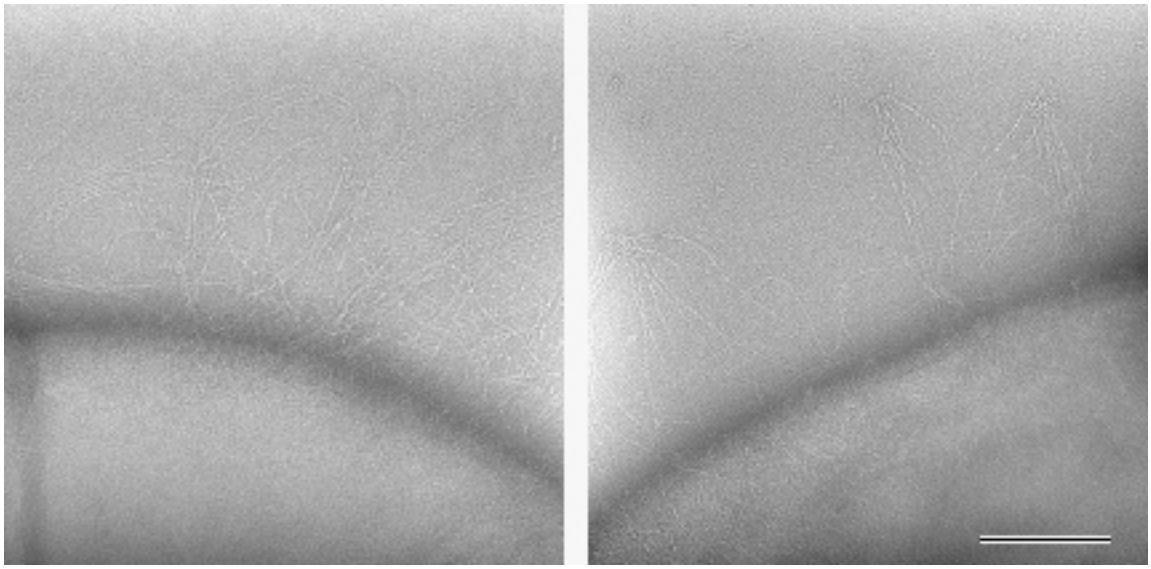

Figure 1 Micrographs of long fimbriae from wild type *S. parasanguis*. Left: Stained with uranyl acetate (UA) Right: Stained with phosphotungstic acid (PTA). Scale=100nm.

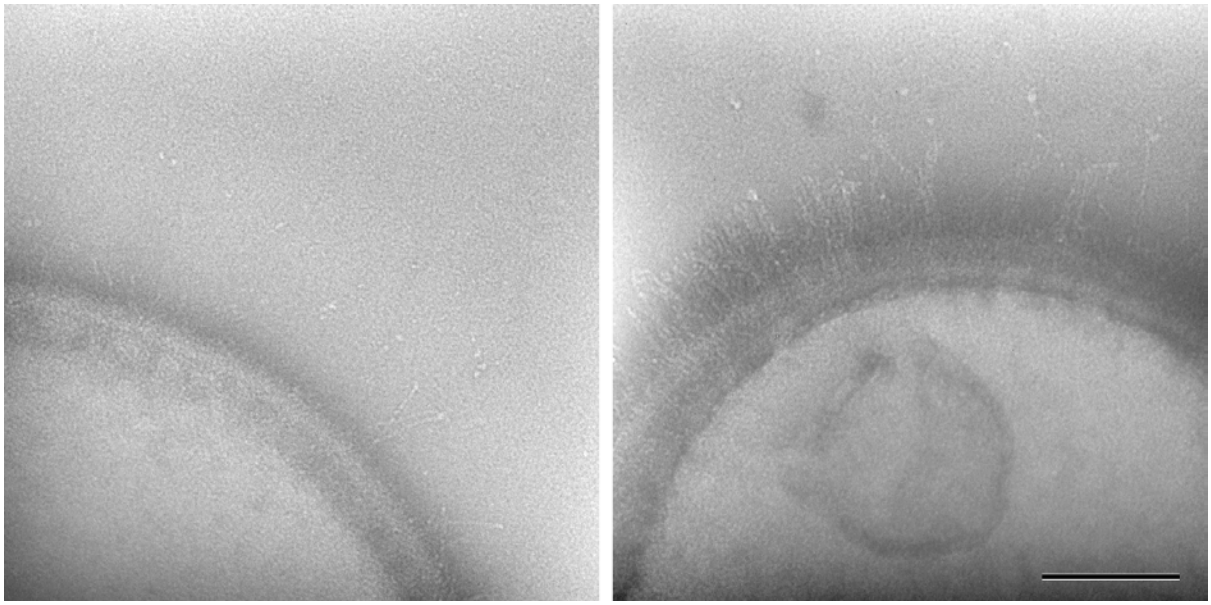

Figure 2 Electron micrographs of whole-mount *S. parasanguis* bacteria in PTA: Left: Gap3- mutant, Right: Fap1- mutant. Scale bar=100nm

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