Highlights:
The Human Oral Microbiome & *Streptococcus sanguinis*

Via 16S rRNA gene-based cloning studies, it's estimated that the oral microbiome harbors approximately 700 different prokaryotic species [4]. *S. sanguinis* is commonly associated with **healthy plaque biofilm** and is occasionally associated with endocarditis (inflammation of the inner lining of the heart) [2,3,4].

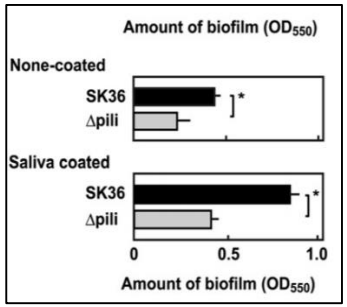
S. sanguinis attributes:

- Gram-positive, nonspore-forming, facultative anaerobe [4]
- Generally regarded as **commensal**; **potentially mutualistic**—documented antagonistic relationship with *S. mutans*, a leading contributor to dental decay [4]
- Like other streptococci, cell division occurs along single axis—chain/paired cocci phenotype [4]
- Common primary colonizer of oral cavity [3]
- Median age of colonization by microbe in infants: 9 months [4]
- In presence of sucrose, main biofilm polymer composed mostly of α-1,6-linked and α-1,3-linked glucose [1]

As a member of *Streptococcus*, a genus identified as key in initial, commensal biofilm colonization of the oral cavity, this microbe is a suitable case study with regards to common mechanisms of pioneer species in this unique microbiome.

Conclusion: Effects of Pili on Biofilm Formation

Figure 6: Wild-type *S. sanguinis* and Δpili mutant were grown in triplicate in 96-well culture plates in THY broth at 37°C for 24 h in 5% CO_2. Plates were either saliva-coated or not. Following crystal violet staining, the OD_{550} was used to quantify biofilm proliferation. Student's *t*-test was used to test for significance. [3]



Summary of both figures *(Fig. 6 & 7)*: In both the saliva-coated and non-coated plates, the pili-deficient mutant was significantly worse at forming biofilms. Confocal microscopic images confirmed this following staining and thickness measurements.

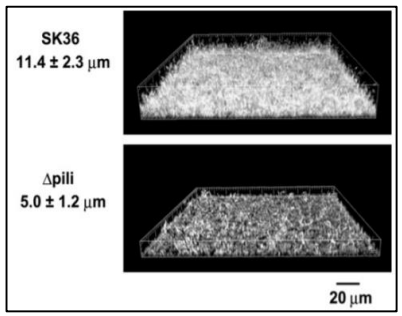


Figure 7: *S. sanguinis* wild-type and Δpili mutant were grown on saliva-coated glass chambers for 48 h at 37°C, stained with Live/Dead BacLight kit, and imaged with a confocal microscope. Biofilm thickness measured at different points of each field and averaged. [3]

Takeaways: Studying *S. sanguinis* provides interesting insights regarding pili-dependent mechanisms that other pioneer species of the oral microbiome might utilize in biofilm colonization. For example:

- Surface adhesin binding to ECM components
- Surface adhesin binding to salivary proteins
- Surface adhesin contributions to biofilm architecture and thickness

References

1. Kopec LK, Vacca Smith AM, Wunder D, Ng-Evans L, Bowen WH. Properties of Streptococcus sanguinis glucans formed under various conditions. Caries Res. 2001 Jan-Feb;35(1):67-74. doi: 10.1159/000047434. PMID: 11125200.
2. Okahashi N, Nakata M, Sakurai A, Terao Y, Hoshino T, Yamaguchi M, Isoda R, Sumitomo T, Nakano K, Kawabata S, Ooshima T. Pili of oral Streptococcus sanguinis bind to fibronectin and contribute to cell adhesion. Biochem Biophys Res Commun. 2010 Jan 8;391(2):1192-6. doi: 10.1016/j.bbrc.2009.12.029. Epub 2009 Dec 14. PMID: 20004645.
3. Okahashi N, Nakata M, Terao Y, Isoda R, Sakurai A, Sumitomo T, Yamaguchi M, Kimura RK, Oiki E, Kawabata S, Ooshima T. Pili of oral Streptococcus sanguinis bind to salivary amylase and promote the biofilm formation. Microb Pathog. 2011 Mar-Apr;50(3-4):148-54. doi: 10.1016/j.micpath.2011.01.005. Epub 2011 Jan 14. PMID: 21238567.
4. Zhu B, Macleod LC, Kitten T, Xu P. Streptococcus sanguinis biofilm formation & interaction with oral pathogens. Future Microbiol. 2018 Jun 1;13(8):915-932. doi: 10.2217/fmb-2018-0043. Epub 2018 Jun 8. PMID: 29882414; PMCID: PMC6060398.

S. sanguinis:

Pili Discovery, Salivary Protein Binding, & Commensal Oral Biofilm Effects

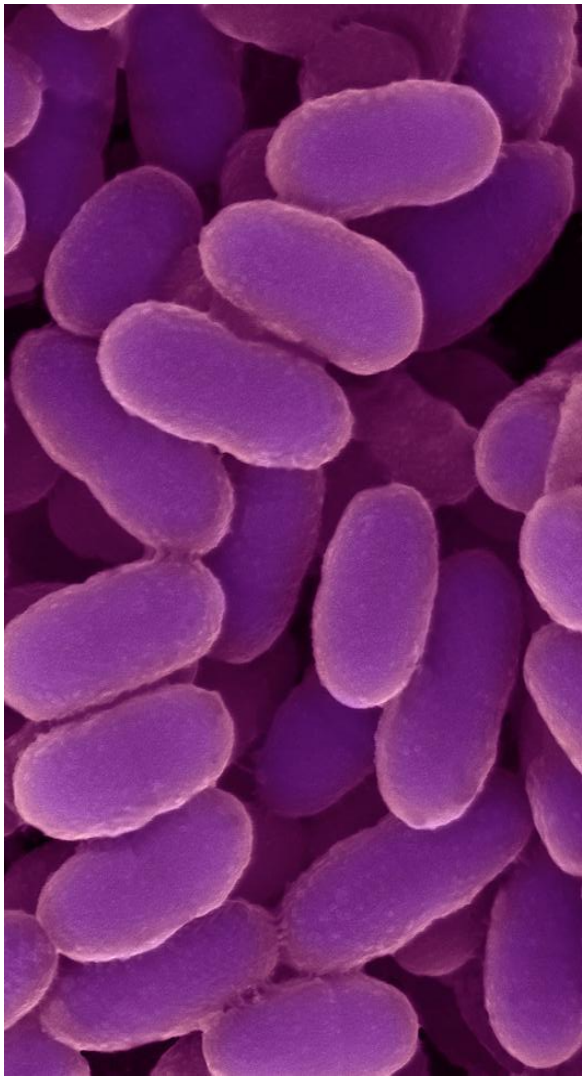


Image credits: https://fineartamerica.com/featured/1-streptococcus-sanguis-dennis-kunkel-microscopyscience-photo-library.html

By: Christopher Hollingsworth

Preceding Research: Discovery of Pili in *S. sanguinis*

In the mid-2000s, a number of papers were published identifying "protruding pilus-like structures" on the surfaces of several species of pathogenic streptococci: "*S. pyogenes, S. agalactiae,* and *S. pneumoniae*" [2]. Oral streptococci were also found to express short fibrils; given, these fibrils presented a differing chemical and visible structure than the pili identified in pathogenic streptococci. The presence of pili on oral streptococci was possible but not yet confirmed.

Complete genome sequence of *S. sanguinis* becomes available—pilus-like locus identified including: *ssa1632, ssa1633, and ssa1634* [2]. Later labeled PilA, PilB, PilC respectively.

- BLAST analysis: 25-30% amino acid identity with *S. pneumoniae* pilus subunits

Identified locus codes for:

- cell wall anchoring LPXTG motif
- "E box" domain commonly observed in other pilus genes

Proposed pilus locus & knockout (Δpili) construction visual:

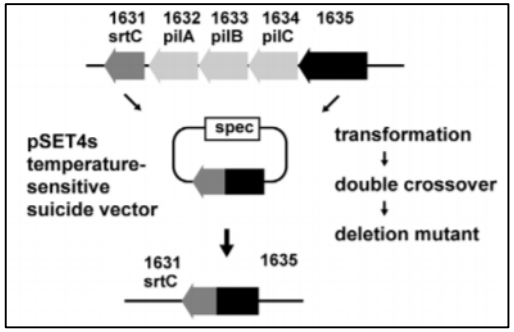


Figure 1: "Gray arrows represent genes coding for PilA, PilB, PilC. Targeted deletion mutant constructed via allelic exchange using the temperature-sensitive suicide vector pSET4s." [2]

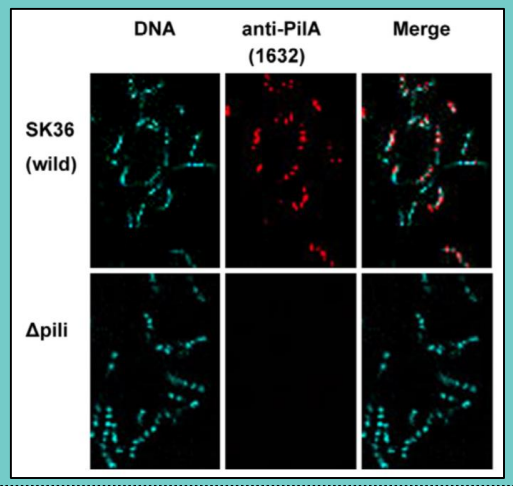


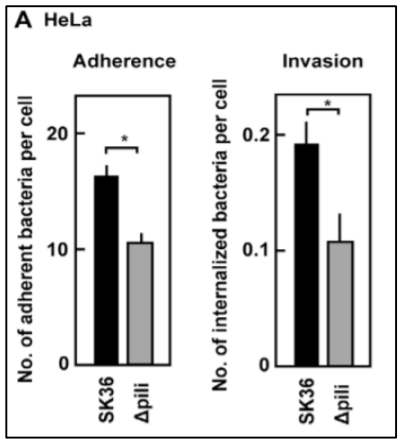
Figure 2: "Immunofluorescence microscopic visualization of pili on cell surface via staining with anti-PilA (SSA1632) (red) and DAPI (blue)." [2]

Pili-presence confirmed

- Presence of pili on cell surface confirmed via confocal microscopic images (*above)* following immunofluorescence staining of wild-type strain and Δpili strain [2]
- Pili expression further confirmed by western blot analysis (*not shown)* using anti-PilA antiserum

Pili: Significant Contributions to Adhesion & Invasion

Figure 3: Adhesion and invasion activity of human epithelial (HeLa) cells of *S. sanguinis* wild-type compared against pili-deficient mutant. Significance determined using student's t-test. [2]



- ***Adherence ability*** to human epithelial cells **decreased by approximately 60%** in Δpili mutant relative to wild-type [2]
- ***Invasion ability*** also reduced in mutant

Conclusion: Pili contribute ***significantly to bacterial colonization activity*** in *S. sanguinis*

Highlights: Mechanisms via which Pili Contribute to Biofilm Formation

1) Fibronectin Adherence Activity of Pili

Well-documented presupposition: adhesins of Gram-positive bacteria often bind ECM proteins [2]

"**Fibronectin** is a glycoprotein found in the extracellular matrix (ECM) that binds to integrins and other components of the ECM."
Source: https://www.novusbio.com/antibody-news/antibodies/fibronectin-organizing-cell-activity-across-the-ecm

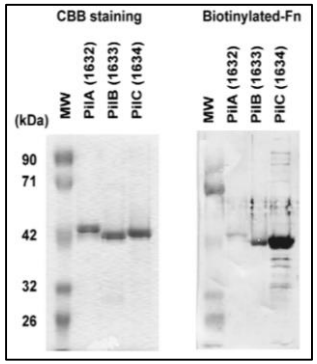


Figure 4: Recombinant pilus proteins subjected to blot analysis using biotinylated fibronectin (Fn) and detected via HRP-streptavidin and TMB reagent system. [2]

- Blot analysis shows strong binding of PilC subunit to fibronectin; PilC is the responsible subunit [2]

2) Saliva & Biofilm Formation Effects

Prior observations [3]:

- **Biofilm formation** shown to be **two** times more efficient in **saliva-coated wells** when compared to noncoated wells
- Recombinant pilus proteins showed binding to whole saliva; strongest with PilB & PilC subunits
- Component in whole saliva being bound identified via biotinylated PilC binding and isolation—**salivary α-amylase**

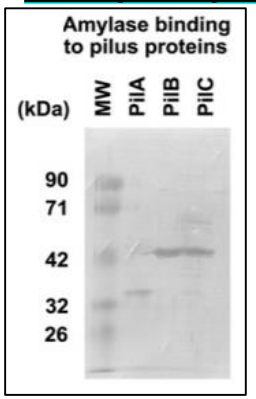


Figure 5: Recombinant pilus protein isolates binding to biotinylated α-amylase detected using HRP-streptavidin and TMB reagent. [3]

- Recombinant protein isolates show similar activity to past whole saliva binding experiments—PilB & PilC exhibit strong binding [3]