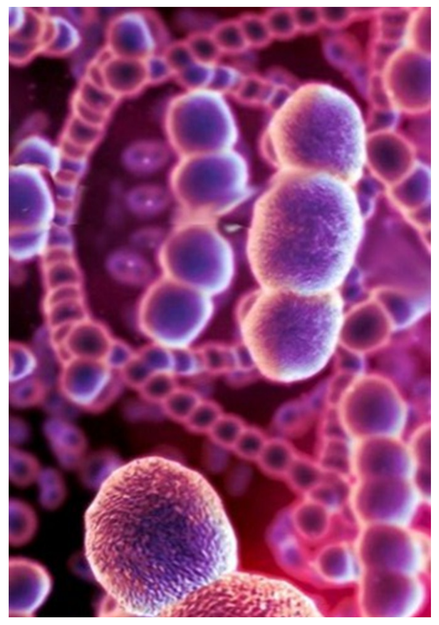
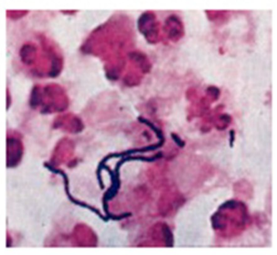
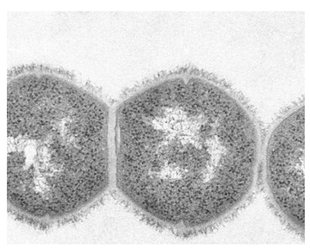
Investigating The Role of *Streptococcus Pyogenes* Pili in Host Tissue Tropism

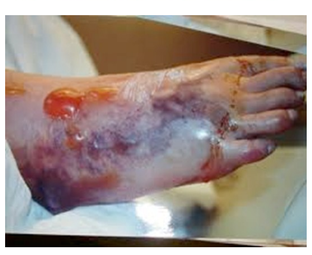
Catherine Jia-Yun Tsai, Jacelyn Loh , and Thomas Proft.

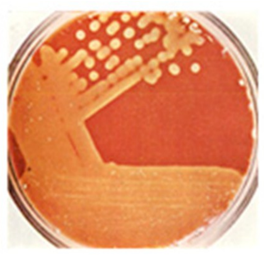
Department of Molecular Medicine & Pathology, School of Medical Sciences,
University of Auckland, Auckland, New Zealand



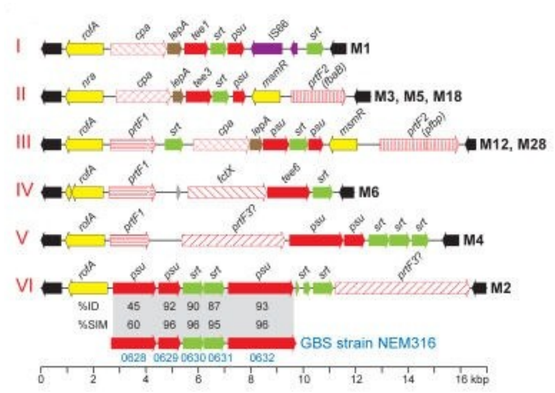








Streptococcus pyogenes (group A streptococcus, GAS) is an important human pathogen that frequently colonises the throat and skin. Pilus structures found recently in GAS have been shown to mediate adhesion to host cells and participate in biofilm formation. Interestingly, the FCT-6 type pilus found in serotype M2 GAS strain shows high homology to the PI-1 pilus found in group B streptococcus (GBS), which colonises the urogenital tract and causes severe neonatal infections. Furthermore, there have been some M2 strains isolated from women with puerperal sepsis. To investigate the importance of streptococcal pili in tissue tropism, and to characterise the function of individual FCT -6 pilus subunits, we generated GAS pilus deletion mutants, *Lactococcus lactis* gain-of function mutants and recombinant forms of individual pilins. These and other GAS M-types, as well as a GBS strain are currently being tested for binding to a variety of human cell lines from pharynx, lung, skin, cervix, and endometrium. To facilitate detection, all bacterial strains were modified to produce firefly luciferase for a strong bioluminescence signal.



Architecture of the FCT region variants shows different gene number and arrangement variations. Serotype M2 shares low homology with other GAS pilus encoding regions, but has a high similarity with GBS pilus.

MATERIALS & METHODOLOGY FRAMEWORK

Cloning, expression and purification of M2 GAS pilus recombinant proteins

6xHis- or GST-tagged recombinant proteins were cloned as soluble forms lacking the N-terminal signal peptide and C-terminal cell wall anchoring motif, expressed in *E. coli* and purified by affinity chromatography.

Construction of deletion & gain-of-function mutants

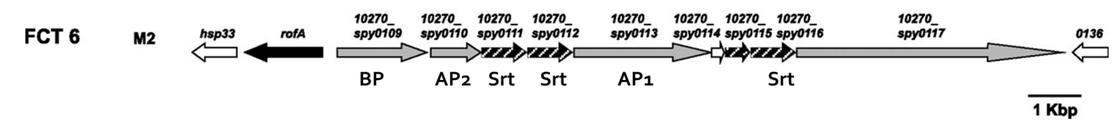
Deletion M2 GAS mutants lacking different pilus components or the whole pilus island were generated by allelic replacement.

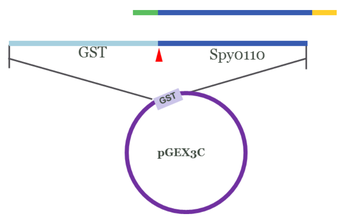
Individual minor pilins were introduced into *L. lactis* by electroporation of the expression constructs.

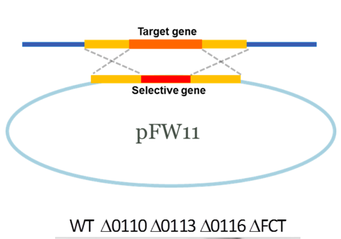
Bioluminescent bacteria

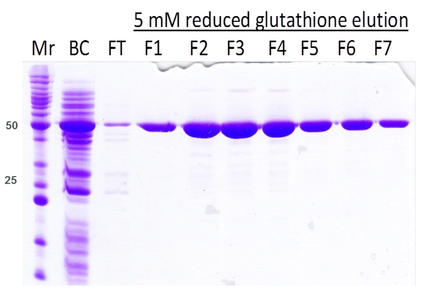
Firefly luciferase gene (FFn) was introduced into different GAS strains by electroporation.

The transformed bacteria could produce strong bioluminescence signal for real-time and live detection.

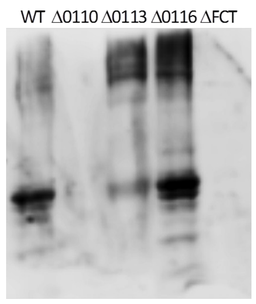




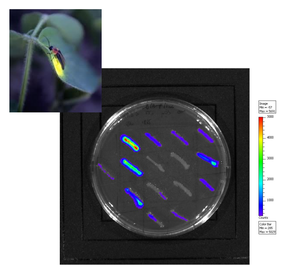




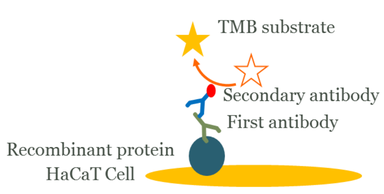
Purification of GST-Spy0110

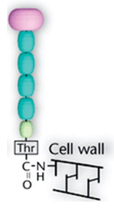


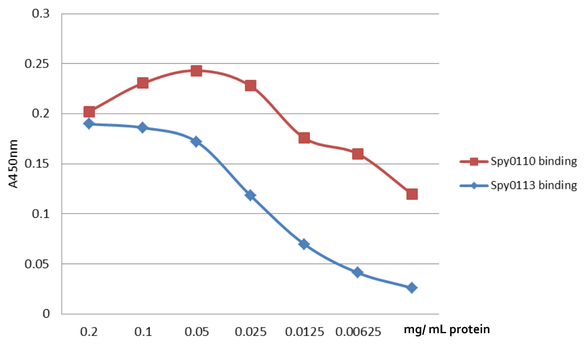
Western blot for pili expression



CELL-BASED ELISA BINDING ASSAY



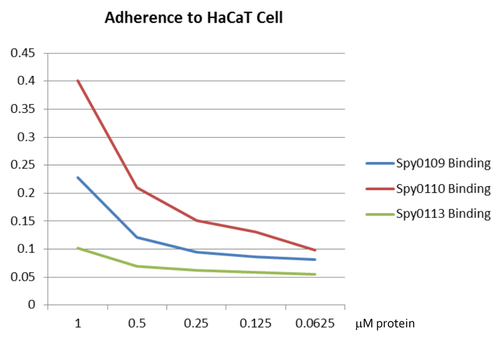


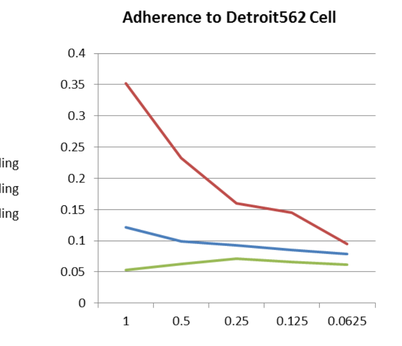


In vitro binding assay was done using purified recombinant minor pilin proteins and poly-clonal antibodies, based on ELISA method. Both M2 pilus ancillary proteins bind to the keratinocyte-derived HaCaT cell line.

Spy0113 is the predicted M2 pilus adhesin that directly interacts with the host cells; however, Spy0110 is assumed to be the cell wall linker that sits at the bottom of pili structure and not involved in attachment. This suggested the binding properties need to be confirmed by other assays.

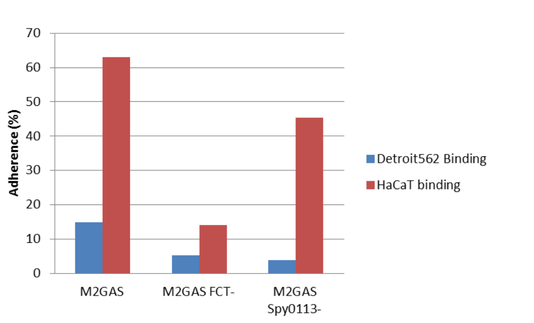
BACTERIA BINDING AND INHIBITION ASSAY

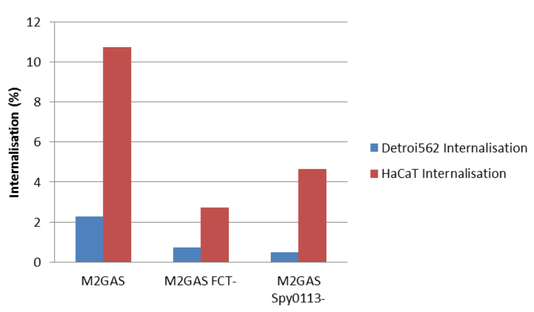




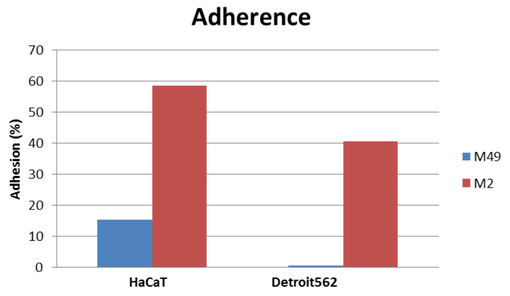
M2 pilus subunits showed different levels of adherence in HaCat or Detroit562 cell-based ELISA binding assays (upper panel). To verify the binding property of each subunit, recombinant proteins were incubated with M2 GAS before infection, which resulted in various inhibition effects (lower panel).

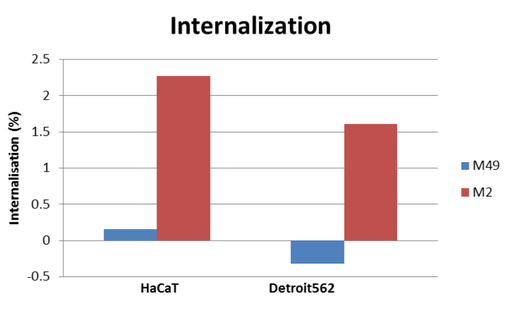
BIOLUMINESCENT ADHERENCE AND INTERNALIZATION ASSAY





The adherence or internalisation of wild-type or mutant strains lacking either the whole pilus operon (*FCT*-) or assumed adhesin (*Spy0113*-) was expressed as percentage relative to initial inoculum (100%). All strains showed stronger binding to keratinocyte-derived HaCaT cells than pharyngeal Detroit562 cells. Adherence and internalisation of pili-defective mutants (M2GAS *FCT*-) to HaCaT cells were significantly reduced, but this could only partially be explained by the loss of predicted adhesin Spy0113 as shown by the M2GAS *Spy0113*-.





CONCLUSION AND FUTURE PERSPECTIVE

The preliminary results showed each pilus subunit plays different roles in host cell adherence and invasion; furthermore, the specific binding may vary according to the streptococcal strain and the host tissue type. The similarity of the FCT regions of serotype M2 strain GAS and GBS suggests that the ability of M2 GAS strains to cause puerperal sepsis infections might be due to the pili. The bioluminescent bacteria generated in this project can serve as a useful tool for studying the function and tropism of this unique pilus. The assays demonstrated here can be applied to different human cell lines, including cervical and endometrial cells, to test the virulence of M2 pilus on infecting these potential host targets.

REFERENCES

1. Beres, S.B. and J.M. Musser, Contribution of exogenous genetic elements to the group A Streptococcus metagenome. PLoS One, 2007. 2(8): p. e800.
2. Abbot, E.L., et al., Pili mediate specific adhesion of Streptococcus pyogenes to human tonsil and skin. Cell Microbiol, 2007. 9(7): p. 1822-33.
3. Bessen, D.E. and S. Lizano, Tissue tropisms in group A streptococcal infections. Future Microbiology, 2010. 5(4): p. 623-638.
4. Darmstadt, G.L., et al., Role of group A streptococcal virulence factors in adherence to keratinocytes. Infect Immun, 2000. 68(3): p. 1215-21.

ACKNOWLEDGEMENT

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

hrc Health Research Council of New Zealand