Spring 4-14-2020

Clostridium perfringens Adhesion Through Type IV Pili

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Type IV Pili

Bacteria infect host cells through a variety of mechanisms. One mechanism used to adhere to host cells is a surface protein called a type IV pilus. Pili are thin, hair-like appendages on bacteria which play roles in surface motility and adhesion to biotic and abiotic surfaces. Type IV pili (TFP) are noncovalently-assembled fibers composed of protein subunits called pilins, capable of being extended and retracted from bacterial cells, which are utilized for diverse processes in various bacterial species, including host-cell adhesion, bacterial auto-aggregation, biofilm formation, natural competence and twitching motility. Type IV pili were originally characterized in gram-negative bacteria including *Escherichia coli* and *Salmonella,* but advances in high-throughput sequencing have revealed that many Gram-positive species, and even archaea, carry the genes to produce them.

Figure 1. Functions of type IV pili in bacteria

Clostridium (*Clostridioides*) *difficile* is an example of a Gram-positive bacterium possessing a type IV pilus and the first where the structures of the pilin proteins have been elucidated. All members of the genus *Clostridia* contain the genes to produce type IV pili, but those genes differ considerably from those of *C. difficile*, which was recently reclassified because of its genetic divergence from other *Clostridia.*

Clostridium perfringens is a Gram-positive bacteria that is best known as a foodborne pathogen. Preliminary functional data from *C. perfringens* pilin proteins suggest that these pilins may be more similar to the type IV pili of *Neisseria* (type IVa pili) which mediate host cell adherence. Understanding how *C. perfringens* uses type IV pili will help create a model for type IV pili in similar *Clostridia* species and increase knowledge of a poorly understood class of bacterial appendages.

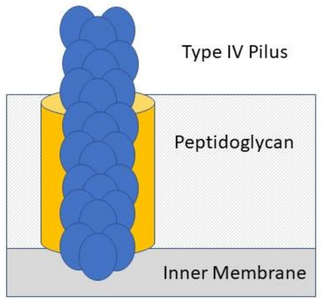

Figure 2. Gram-positive type IV pili

Type IV Pili in *C. perfringens*

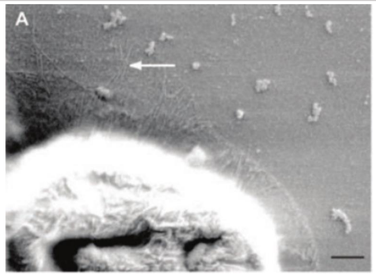

Figure 3. Visualization of TFP in *C. perfringens* strain 13 (3)

Recent findings by Dr. Stephen Melville suggest that the type IV PilA2 pilin in *C. perfringens* is key for cell adhesion, which further supports the conclusion that *C. perfringens* pili are similar to type IVa pili from *Neisseria*.. Understanding how *C. perfringens* uses type IV pili will help create a model for type IV pili in similar *Clostridia* species.

Crystallization

The PilA2 protein was expressed in an *E. coli* host and isolated via a nickel column. The isolated protein was screened in several hundred conditions. Protein crystals formed in a Hampton research Index screening plate condition consisting of 0.1 M citric acid pH 3.5 and 2.0 M ammonium sulfate. Crystal diffraction was achieved though resolution was not high enough to accurately obtain structural data.

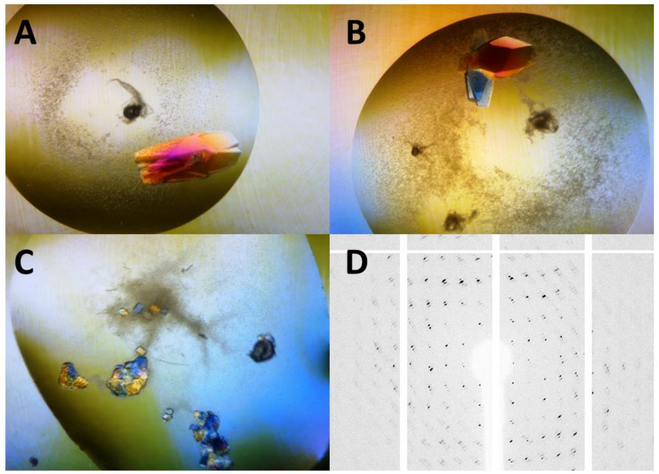

Figure 4. *C. perfringens* PilA2 protein crystals and X-ray diffraction data. Panels A-C display *C. perfringens* PilA2 crystals. Panel D displays the X-ray diffraction data obtained from one of these crystals.

Modelling

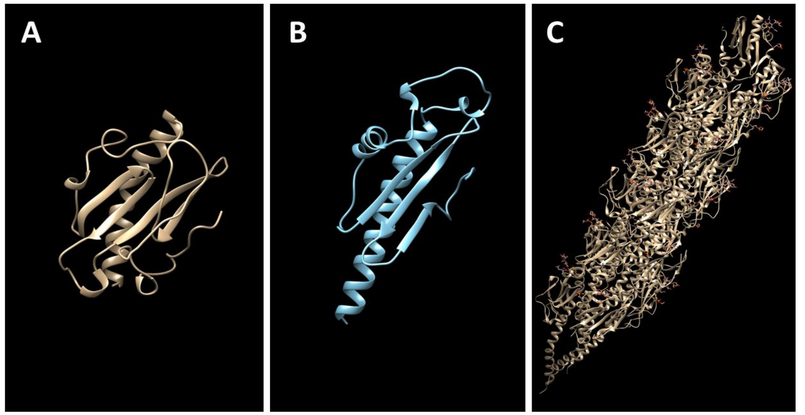

Figure 5. Models of pilin proteins and a complete pilus structure. Panel A shows the solved structure of the type IV pilin protein PilE from *Neisseria meningitidis.* Panel B shows the hypothetical structure of the type IV PilA2 pilin from *Clostridium perfringens* based on ab initio modeling. Panel C demonstrates the complete pilus structure formed from *N. meningitidis* PilE pilins.

Future Directions

We aim to optimize conditions for protein crystallization to produce crystals capable of improved diffraction. Diffraction data will be used to solve the protein structure and produce an informed model of the type IV pilus structure.

References

1. Soncini, S.R., Hartman, A.H., Gallagher, T.M., Camper, G.J., Jensen, R.V., and Melville, S.B. (2020) Changes in the expression of genes encoding type IV pili-associated proteins are seen when *Clostridium perfringens* is grown in liquid or on surfaces. *BMC Genomics.* **21**, 45

2. Rodgers, K., Arvidson, C.G., and Melville, S. (2011) Expression of a *Clostridium perfringens* type IV pilin by *Neisseria gonorrhoeae* mediates adherence to muscle cells. *Infect. Immun.* **79**(8), 3096-3105

3. Varga, J.J., Nguyen, V., O'Brien, D.K., Rodgers, K., Walker, R.A., and Melville, S.B. (2006). Type IV pili-dependent gliding motility in the gram-positive pathogen *Clostridium perfringens* and other *Clostridia*. *Mol. Microbiol.* **62**(3), 680-694