The Pam Pilus – a Novel Pilus System within Candidate Phyla Radiation Bacteria

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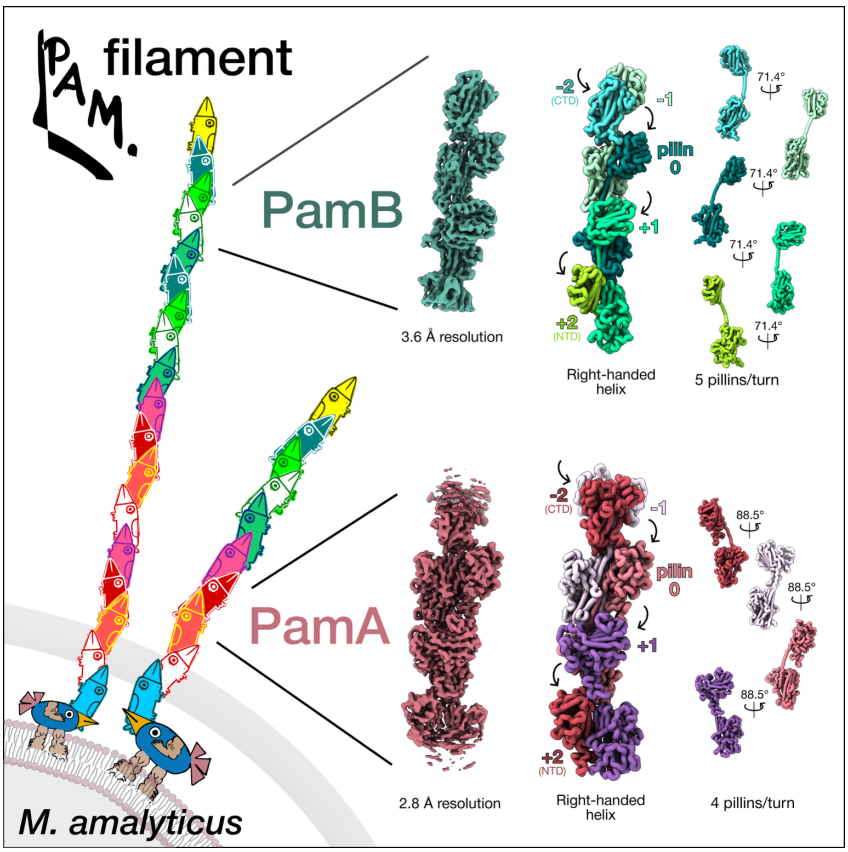
The Candidate Phyla Radiation (CPR) describes a bacterial superphylum often referred to as microbial "dark matter," due to lack of characterisation and primarily identification through metagenomic and single-cell sequencing approaches. Whilst challenging to culture, CPR bacteria are remarkably diverse and widespread, inhabiting environments ranging from mammalian microbiomes to groundwater, deep-sea sediments, and soils. "Candidatus Mycosynbacter amalyticus" is a CPR nanobacterium parasitic to *Gordonia amarae*; a foam-forming actinobacterium problematic in wastewater treatment. Using cryo-electron tomography, we have previously shown that M. amalyticus employs specialized Type IV secretion systems (T4SS) for pathogenic interactions with the host. However, multiple uncharacterized pili were also observed on the cell surface which could have a role in specificity to the hosts mycolic acid–rich cell envelope. Here, we characterised natively isolated filaments from M. amalyticus using single-particle cryo-electron microscopy. We identified two distinct classes of pili and resolved each to sub-4 Å. Using a top-down visual proteomics we identified two different putative pilins, encoded within the same operon, and constructed atomic models for each. The models reveal a donor-strand exchange assembly mechanism reminiscent of the Fim/Pap Type I pili of Gram-negative *E. coli*. Despite these structural parallels, the absence of sequence homology and unique domain architectures distinguish this as a novel pilus system, which we have termed "Pam" (**P**ilus of **am**alyticus - and yes, like the bird!). Gene neighborhood analysis and AlphaFold modeling suggest a dedicated biogenesis pathway analogous to Fim/Pap classical chaperone–usher systems. We propose that Pam pili function cooperatively with the T4SS to mediate host recognition and attachment to the mycolic acid–rich envelope of *G. amarae*. This discovery broadens our understanding of the molecular complexity within CPR bacteria and highlights new mechanisms of interbacterial parasitology.


PAM filament
PamB
3.6 Å resolution
-2
(CTD)
-1
pilin
0
+1
+2
(NTD)
Right-handed
helix
71.4°
71.4°
71.4°
71.4°
5 pillins/turn
PamA
2.8 Å resolution
-2
(CTD)
-1
pilin
0
+1
+2
(NTD)
Right-handed
helix
88.5°
88.5°
88.5°
4 pillins/turn
M. amalyticus