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Structural Characterization of Novel *Pseudomonas aeruginosa* Type IV Pilins

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Pseudomonas aeruginosa type IV pili, composed of PilA subunits, are used for attachment and twitching motility on surfaces. *P. aeruginosa* strains express one of five phylogenetically distinct PilA proteins, four of which are associated with accessory proteins that are involved either in pilin posttranslational modification or in modulation of pilus retraction dynamics. Full understanding of pilin diversity is crucial for the development of a broadly protective pilus-based vaccine. Here, we report the 1.6-Å X-ray crystal structure of an N-terminally truncated form of the novel PilA from strain Pa110594 (group V), which represents the first non-group II pilin structure solved. Although it maintains the typical T4a pilin fold, with a long N-terminal α-helix and four-stranded antiparallel β-sheet connected to the C-terminus by a disulfide-bonded loop, the presence of an extra helix in the αβ-loop and a disulfide-bonded loop with helical character gives the structure T4b pilin characteristics. Despite the presence of T4b features, the structure of PilA from strain Pa110594 is most similar to the *Neisseria gonorrhoeae* pilin and is also predicted to assemble into a fiber similar to the GC pilus, based on our comparative pilus modeling. Interactions between surface-exposed areas of the pilin are suggested to contribute to pilus fiber stability. The non-synonymous sequence changes between group III and V pilins are clustered in the same surface-exposed areas, possibly having an effect on accessory protein interactions. However, based on our high-confidence model of group III $PilA_{PA14}$, compensatory changes allow for maintenance of a similar shape.

Keywords: Pseudomonas aeruginosa; type IV pili; PilA; X-ray crystallography; fimbriae

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Introduction

A wide range of bacteria express type IV pili (T4P), which are long protein fibers involved in a diverse array of functions ranging from attachment to and twitching motility on living and nonliving surfaces to competence for DNA uptake and electron transfer.[1,2] T4P are required for virulence by a number of pathogenic species including *Pseudomonas aeruginosa*, an opportunistic pathogen of plants, animals, and humans. Each fiber is composed of thousands of subunits of the major pilin protein, whose assembly and disassembly at the inner membrane result in pilus extension and retraction, leading to twitching motility.[3] Two subclasses of T4P have been identified, T4aP and T4bP, which differ in several respects. The major pilins of the two subtypes have limited sequence identity, different lengths of the leader peptide (type IVa pilins have ~6 residue leaders, type IVb pilins have ~15–30) as well as the mature protein (type IVb are larger), and disparate identity of the N-methylated N-terminal residue of the mature subunit (Phe in type IVa, varies in type IVb).[1,2] The differences in the major subunits are mirrored in the architecture of their respective assembly systems, where type IVa pilins are assembled by complex systems encoded across the genome of the host organism while type IVb assembly systems are

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Abbreviations used: T4P, type IV pili; DSL, disulfide-bonded loop; $PilA_{0594}$, PilA from strain Pa110594; SAD, single-wavelength anomalous dispersion; SeMet, selenomethionine; PDB, Protein Data Bank.

composed of fewer components that are typically encoded in single gene clusters, often located on plasmids.[2] The T4aP subclass is found in a broad range of bacterial species including *P. aeruginosa* and *Neisseria* spp., while the T4bP have a more restricted distribution, typically in genera such as *Salmonella* and *Vibrio* and pathogenic *Escherichia coli* species that colonize the mammalian gastrointestinal tract.[1,2,4]

Structures of pilins from both subclasses have been solved[5–12] and reveal a similar overall architecture. Both have a long, hydrophobic N-terminal α-helix, subdivided into α1-N and α1-C. α1-N retains individual subunits in the inner membrane until assembly, when it forms the core of the assembled pilus fiber, while α1-N is embedded in a C-terminal β-sheet and loop domain that forms the exterior surace of the pilus.[4] There is a characteristic disulfide-bonded loop (DSL), often called the D-region, located in the C-terminal regions of both pilin subclasses, which anchors the C-terminus to the β-sheet and is important for function.[13] The hydrophobic α1-C region of the mature pilin is typically truncated for structural work to improve solubility, as it is highly conserved between species and previous studies have shown that full-length and truncated structures of individual pilins are superimposable.[9,11] Despite their general similarities, examination of T4aP and T4bP pilin structures currently available shows that they have distinct folds that arise primarily from differences in the numbers and topology of β-strands in the C-terminus.[4]

A limited number of structures are also available for proteins related to the T4 pilins.[14–19] Minor pilins are pilin-like proteins sharing the conserved N-terminal leader peptide and hydrophobic α1-N helix and are required for expression of surface-exposed pilus fibers[20–23] or in the case of PilX, a minor pilin from *Neisseria meningitidis*, for specific fiber properties.[24] The evolutionarily related type II secretion system's pseudopilins and minor pseudopilins, involved in transport of proteins through the outer membrane, also have a conserved N-terminal leader and α1-N hydrophobic region.[25] The structures of the major pseudopilins PulG[15] and XcpT[19] (from *Klebsiella* and *Pseudomonas*, respectively) and the minor pseudopilins EpsH[17] (from *Vibrio cholerae*), EpsI and EpsJ[16] (from *Vibrio vulnificus*), and GspK, GspI, and GspJ[18] (from *E. coli*) confirmed that they share a common architecture with the type IV pilins, although differences that may relate to specific functions are present.

P. aeruginosa strains express one of five phylogenetically distinct T4aP PilA alleles, three of which were identified only recently.[26] The five PilA proteins differ in their overall sequences and length, the size of the key C-terminal DSL,[13] and the association of the pilin gene with specific downstream accessory genes involved in pilin post-translational modification[27–29] or modulation of pilus assembly[30] (Fig. 1). Structures are available only for *P. aeruginosa* group II pilins,[7–9,11] which are the smallest among the five groups and the only ones lacking associated accessory proteins, making them the exception in the *P. aeruginosa* pilin repertoire.[26]

Phylogenetic analysis suggested that group III, IV, and V pilins are members of a separate family that diverges from the branch containing groups I and II.[26] Group III and V pilins are 43.5% identical with one another over 144 residues in their C-terminal domains but show much lower identity to group I, II, and IV pilins in that region. Pairwise comparisons revealed 17.2% identity between the C-termini of pilins from groups I and III, 26% between groups II and III, and 23.4% between groups III and IV. Similar values are obtained when group V pilins are used as the comparator. Introduction of group III or V alleles of *pilA* into the common group II laboratory strain PAO1 lacking its own *pilA* gene led to poor recovery of motility unless the associated accessory gene was co-introduced.[30] Inactivation of the *tfpY* accessory gene in the group III strain PA14 caused a marked decrease in surface piliation and motility without affecting pilin levels in the cell, suggesting that pilus assembly was impaired in its absence.

Together, the bioinformatic and functional data led us to hypothesize that *P. aeruginosa* group III and V pilins might have an unusual architecture com-

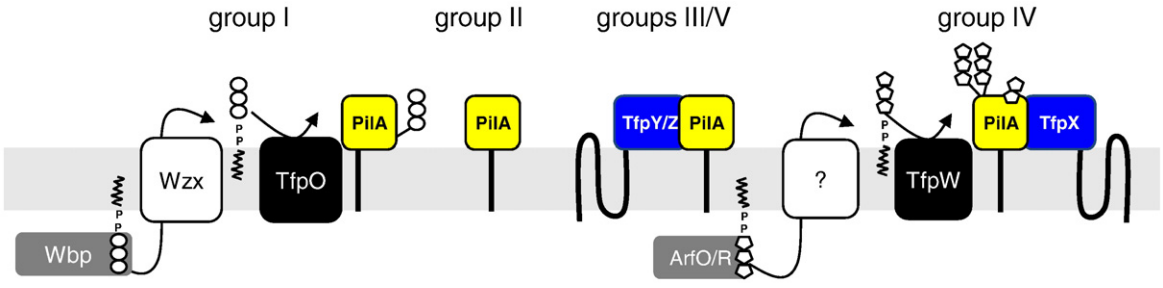

Fig. 1. Pilin modification and assembly systems in *P. aeruginosa*. Group I pilins are glycosylated by TfpO on the C-terminal Ser with an O antigen unit synthesized by the LPS machinery. Group II pilins have no accessory proteins. Group III and V pilins each have a specific accessory protein that promotes their assembly: TfpY for group III and TfpZ for group V. Group IV pilins are glycosylated at several positions by TfpW with mono-, di-, and trisaccharides of D-arabinofuranose synthesized by the ArfO/R proteins (Harvey and Burrows, unpublished data). Group IV pilins have a TfpX accessory protein that is similar to TfpY and TfpZ.