

Structural and Functional Characterization of Pili P Protein in *Escherichia coli*

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ABSTRACT

Pili P, a type of pili found on the surface of *Escherichia coli*, plays a crucial role in the bacterial adherence to host tissues, contributing to the pathogenesis of various infections. This review aims to explore the structure, function, and role of Pili P in *E. coli*, highlighting its importance in bacterial colonization and virulence. Pili P are composed of protein subunits that assemble into hair-like structures, which facilitate adherence to epithelial cells and the formation of biofilms. This interaction is essential for the survival and persistence of *E. coli* in hostile environments, including the urinary tract, where it can lead to urinary tract infections (UTIs). The structure of Pili P involves an intricate arrangement of pilin subunits, with adhesins at the tip that mediate specific binding to host cell receptors. Additionally, the role of pili in the process of bacterial invasion, immune evasion, and resistance to host defense mechanisms is discussed. The review also covers recent advancements in understanding the molecular interactions between Pili P and host cells, as well as ongoing research aimed at targeting Pili P as a potential therapeutic strategy for treating *E. coli*-related infections. Understanding the structural dynamics and function of Pili P in *E. coli* can

provide valuable insights for developing new antimicrobial agents and treatment strategies.

Keywords: *Adhesion, Biofilm, E. coli, Pili P Protein, Virulence*

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, with *Escherichia coli* being the primary causative agent. The pathogenicity of uropathogenic *E. coli* (UPEC) is largely attributed to its ability to express surface appendages known as P pili, also referred to as pyelonephritis-associated pili. These filamentous structures facilitate bacterial adhesion to host tissues, playing a crucial role in the colonization of the urinary tract and the establishment of infection.

This review provides an in-depth examination of the structure, assembly, and functional roles of P pili, emphasizing their contribution to bacterial adhesion and immune evasion. Additionally, the active site and key amino acid residues involved in host receptor interactions will be discussed in detail, highlighting their significance in *E. coli* pathogenesis (Sheikh et al., 2017).

Structural Features of P Pili

P pili (PDB ID: 6NRV) are hair-like structures extending from the bacterial cell surface, measuring approximately 1–2 micrometers in length. These pili are composed of multiple protein subunits arranged in distinct structural domains: the pilus rod and the pilus tip (Hahn et al., 2001; Zheng et al., 2019).

Pilus Rod

The pilus rod serves as the primary backbone of P pili and is primarily composed of the major structural protein PapA. Around 1,000 PapA subunits polymerize in a right-handed helical configuration, providing the pilus with the mechanical strength necessary to withstand the shear forces present in the urinary tract. This helical arrangement also ensures the stability of the pilus during adhesion to host cells.

Pilus Tip

The distal end of the pilus rod is capped by the pilus tip, a flexible and specialized region essential for host recognition and adhesion. This region consists of several minor pilus proteins, each playing a specific role:

- **PapK:** Functions as an adaptor, linking the pilus rod to the tip fibrillum.

- **PapE and PapF:** Form a fibrillar structure that extends outward, positioning the adhesin for optimal receptor binding.

- **PapG:** The terminal adhesin that mediates bacterial attachment to host epithelial cells.

The spatial organization of these proteins ensures that PapG is optimally positioned for host cell recognition, enabling *E. coli* to establish infections in the urinary tract.

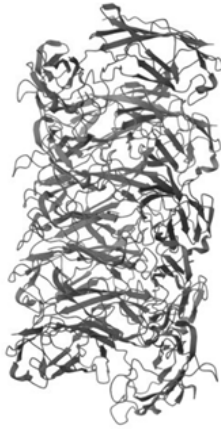


Fig 1: Structure of Pili (Zheng et al., 2019)

Chaperone-Usher Pathway: Assembly Mechanism

The biosynthesis of P pili is directed by the chaperone-usher pathway, a well-conserved mechanism among Gram-negative bacteria. This pathway ensures the proper folding, stabilization, and ordered assembly of pilus subunits

(Werneburg et al., 2018).

Periplasmic Chaperone (PapD)

Once synthesized in the cytoplasm, pilus subunits are translocated into the periplasmic space, where they interact with the chaperone protein PapD. PapD plays a dual role (Sarowar et al., 2016):

- **Stabilization:** It prevents the premature aggregation of pilus subunits by capping their interactive surfaces.
- **Folding Assistance:** It facilitates the proper conformational arrangement of subunits, ensuring they are correctly oriented for subsequent assembly.

PapD-subunit interactions are characterized by a donor strand complementation mechanism, where PapD contributes a beta-strand to complete the immunoglobulin-like fold of the subunit, ensuring structural stability.

Outer Membrane Usher (PapC)

The PapD-subunit complexes are transported to the outer membrane usher protein, **PapC**, which facilitates the stepwise polymerization of pilus components and their translocation to the bacterial surface. Key features of PapC include:

- **Subunit Polymerization:** Ensures the correct assembly of pilus proteins in a sequential manner.
- **Channel Formation:** Forms a transmembrane channel that allows for the extrusion of the growing pilus structure.

Through the concerted action of PapD and PapC, P pili are efficiently assembled, ensuring that the PapG adhesin is positioned at the pilus tip for effective host cell interaction.

Active Site and Key Amino Acids of PapG Adhesin

The PapG adhesin is a crucial determinant of UPEC virulence, as it mediates bacterial attachment to host cells. The active site of PapG is specifically adapted to recognize and bind to globoside receptors, particularly globotriaosylceramide (Gb3) and globotetraosylceramide (Gb4), which are highly

expressed on kidney epithelial cells (Zheng et al., 2019).

Receptor Binding Specificity

The binding specificity of PapG is critical for the tissue tropism of UPEC. Its ability to recognize Gb3 and Gb4 glycolipids enables it to target renal epithelial cells, contributing to the development of pyelonephritis.

Structural Composition of the Binding Pocket

Structural analyses of PapG have revealed a well-defined binding pocket designed to accommodate the carbohydrate moieties of host receptors. This pocket is stabilized by a network of hydrogen bonds and hydrophobic interactions, allowing for a strong and specific attachment.

Essential Amino Acid Residues in PapG

Several amino acids within PapG have been identified as crucial for receptor binding and adhesion:

- **Tyrosine (Tyr):** Forms hydrogen bonds with the galactose residues of host glycolipids, securing the receptor in place.
- **Phenylalanine (Phe) and Leucine (Leu):** Contribute to **hydrophobic interactions**, strengthening the adhesin-receptor complex.
- **Aspartic Acid (Asp) and Glutamic Acid (Glu):** Facilitate **electrostatic interactions**, enhancing binding affinity.

Mutagenesis studies have demonstrated that alterations in these key residues significantly diminish or abolish PapG's binding ability, highlighting their importance in UPEC adhesion.

Functional Role of P Pili in UPEC Pathogenesis

P pili play a crucial role in *E. coli* pathogenesis by promoting bacterial adherence and survival in the urinary tract.

Host Tissue Colonization

The PapG adhesin enables UPEC to attach firmly to kidney

epithelial cells, preventing bacterial clearance by urine flow. This adhesion is a key step in the establishment of UTIs, leading to bacterial invasion and inflammation.

Immune Evasion Strategies

UPEC employs several strategies to evade host immune defenses, including:

- **Mechanical Resilience:** The **helical structure** of the pilus rod allows UPEC to withstand urinary shear forces.
- **Antigenic Variation:** Variability in pilus components, including PapG, enables UPEC to evade immune recognition.

These adaptations contribute to the persistence of *E. coli* infections and increase the likelihood of recurrent UTIs.

Therapeutic Approaches Targeting P Pili

Given their pivotal role in UPEC pathogenesis, P pili have been explored as potential targets for anti-adhesive therapies aimed at preventing bacterial colonization (Duménil, 2019).

Pilus-Targeting Vaccines

Experimental vaccines have been developed to elicit immune responses against pilus subunits, particularly PapG, to block bacterial adhesion.

Small Molecule Inhibitors

Compounds that disrupt the interaction between PapG and host receptors are being investigated as potential therapeutic agents to prevent UPEC colonization.

Monoclonal Antibodies

Monoclonal antibodies directed against PapG could neutralize its adhesin activity, reducing bacterial attachment and enhancing clearance by the immune system.

Conclusion

P pili are essential virulence factors in UPEC, enabling bacterial adhesion and colonization of the urinary tract.

The structural organization of P pili, particularly the PapG adhesin, plays a critical role in host-pathogen interactions. By understanding the active site and key amino acids involved in receptor binding, novel therapeutic approaches targeting bacterial adhesion can be developed to mitigate UPEC infections. Future research should focus on refining vaccine formulations, small-molecule inhibitors, and monoclonal antibody therapies to effectively combat drug-resistant UPEC strains and reduce the burden of UTIs.

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