ABSTRACT

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Under the Influence: Pf Bacteriophage Modulate *Pseudomonas aeruginosa* Virulence & Pathogenesis

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Pseudomonas aeruginosa is a gram-negative opportunistic bacterial pathogen which is as ubiquitous in the natural environment as it is within hospital settings, where it is a readily acquired nosocomial pathogen. Bacteriophages, viruses which infect bacteria, are a central component of the arsenal *P. aeruginosa* leverages to combat the stresses of infecting mammalian hosts. Over 60% of *P. aeruginosa* clinical isolates are chronically infected with temperate phages within the family of *Inoviridae.* These phages, referred to as Pf, have a filamentous virion structure which accommodates genome sizes between 10-20kbp. Pf is capable of integration into the bacterial chromosome where they can exist in a dormant prophage state, or excise and produce viral progeny which extrude from the bacterial host. Overproduction of Pf resulting from infection of a cell that already harbors a prophage; called superinfection, can lead to membrane stress and ultimately cell lysis. Temperate phages, such as Pf, typically encode superinfection exclusion mechanisms. Twitch motility is mediated by the classical bacterial virulence factor, Type IV Pili (T4P) which mediates bacterial adhesion allowing for biofilm formation. Importantly, T4P serve as the cell surface receptor for Pf, among other phages. We describe inhibition of Pf plaquing and twitch motility by a highly conserved Pf superinfection exclusion gene, *pfsE* (*PA0721)*. Furthermore, we determine that highly conserved aromatic residues facilitate PfsE localization to the inner membrane where PfsE binds to T4P structural subunit PilC, suppressing twitch motility. As most work on Pf phages focuses on Pf4 in the model *P. aeruginosa* strain PAO1, we sought to interrogate how Pf phage modulate virulence-associated phenotypes within diverse clinical isolate hosts. We describe a technique which targets the Pf lysogeny maintenance gene *pflM* (*PA0718*) and demonstrate that *pflM* deletion results in prophage excision but not replication, leading to total prophage loss. We identify conservation of a DUF5447 domain within all examined *pflM* alleles, implicating this domain in Pf lysis/lysogeny decision making. Next, we assess the effects different Pf phages have on virulence associated phenotypes and determine that although impacts on quorum sensing and biofilm formation appear to be strain-specific, nearly all Pf phages suppress pigment production and increase bacterial virulence against bacterivorous nematode *Caenorhabditis elegans.*

Collectively, this research advances our understanding of how filamentous Pf phages influence *P. aeruginosa* pathogenesis.