Elucidating the structure & self-regulation of PrgK-mediated cell wall remodeling in *E. faecalis* during conjugation

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Conjugative type 4 secretion systems (T4SSs) are primary drivers of horizontal gene transfer and antibiotic resistance in Gram-positive pathogens. In the *Enterococcus faecalis* pCF10 system, the essential cell wall hydrolase PrgK facilitates DNA transfer by remodeling the thick peptidoglycan layer. PrgK is a complex modular protein featuring three extracellular domains: a metallo-peptidase (LytM), a muramidase (MUR, formerly SLT), and a cysteine/histidine-dependent amidohydrolase (CHAP). Structural analysis reveals that the LytM domain possesses a degenerate active site, suggesting a regulatory rather than catalytic function. While the MUR domain provides the primary hydrolytic activity, it unexpectedly exhibits muramidase rather than lytic transglycosylase activity. Crucially, the LytM and CHAP domains negatively regulate this muramidase activity. Furthermore, the CHAP domain mediates PrgK dimerization through a conserved Cys766-mediated disulfide bond, a process that further modulates enzymatic efficiency.

To further define the *in vivo* role and regulatory contributions of each domain, we developed a high-fidelity modular assembly approach to systematically replace individual domains and combinations (e.g., ΔLytM-MUR, ΔMUR-CHAP, and ΔLytM-CHAP) with flexible linkers. Using a specialized two-step PCR, we successfully cloned these variants into the pMSP3545 expression vector. Our current work involves complementing these recombinant variants into an *E. faecalis* pCF10 ΔprgK strain to assess their impact on conjugation efficiency and cell viability. Parallel to these functional assays, we are utilizing cryo-electron microscopy (cryo-EM) to solve the high-resolution structure of the PrgK dimer. This integrated approach aims to provide a comprehensive model of how PrgK facilitates DNA transfer through precise, self-regulated cell wall remodeling without compromising the integrity of the donor or the recipient cell.

Keywords

Antibiotic resistance, Conjugation, T4SS, Cell wall remodeling, *E. faecalis*, Cryo-EM