Breaking Barriers: pCF10 Type 4 Secretion System relies on a self-regulating muramidase to modulate the cell wall

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The combination of hospital acquired infections and antimicrobial resistance is a large threat to public health. It is therefore of high interest to understand how antibiotic resistance genes spread between bacteria. A major route of this transfer is via Type 4 Secretion Systems (T4SS). T4SSs are membrane spanning megadalton-sized protein complexes that facilitate delivery of mobile genetic element, including virulence factors or antibiotic resistant genes, from donor to recipient cells. The assembly of the T4SS channel requires a regional lesion in the cell wall, which is introduced by peptidoglycan remodelling enzymes that are usually part of the T4SS. In contrast to their Gram-negative counterparts, Gram-positive T4SSs are less well-characterized, with very limited structural and functional data available.

PrgK, the cell wall remodelling enzyme of the enterococcal pCF10 T4SS, is essential for efficient conjugation and plasmid transfer. PrgK is predicted to have three extracellular enzymatic domains: LytM, SLT and CHAP. In this study, we present the structure of these three domains of PrgK by a combination on crystallography and AlphaFold modelling. Our crystal structure show that the LytM domain has a degenerate active site. The other two domains, SLT and CHAP, on the other hand, have conserved active sites. Furthermore, AlphaFold modelling predicted that PrgK interacts with another pCF10 T4SS protein, namely PrgL that is thought to be involved in T4SS channel assembly. This PrgK-PrgL interaction was proven by size-exclusion chromatography coupled to multi angle light scattering (SEC-MALS). *In vitro,* only the SLT domain was active against cell wall extracts of *E. faecalis*. Furthermore, we show that this domain does not have its predicted lytic transglycosylase activity, but rather muramidase activity. The CHAP domain, as the LytM domain, shows no significant catalytic activity *in vitro*. Instead, these two domains regulate the activity of the SLT domain. Surprisingly, this regulatory effect of LytM and CHAP is lost when SLT acts on peptidoglycan from Vibrio cholerae, a Gram-negative bacterium. Our finding here provides new pieces to the puzzle of how the structure, function and assembly of Gram-positive T4SSs.