

Pili are essential for conjugation also in many Gram-positive bacteria

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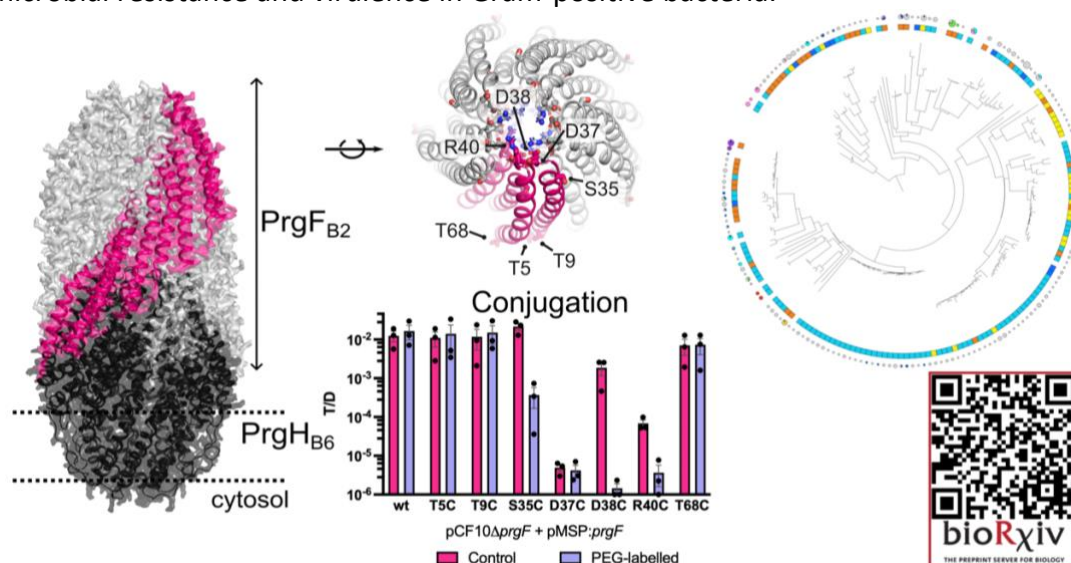
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Type IV secretion systems (T4SS) enable the spread of antibiotic resistance and other virulence factors. The function of T4SSs has been studied for decades, but most studies have focused on a handful of model systems from Gram-negative bacteria. In these systems DNA transfer (conjugation) relies on extracellular conjugative pili, while the prevailing view has been that Gram-positive T4SSs lack pilus-forming VirB2-like proteins and instead depend on adhesins for cell-cell contact. This assumption has fuelled debate on how DNA crosses the thick peptidoglycan layers of the donor and recipient bacteria during conjugation.

We identified a VirB2-like protein, PrgF_{B2}, from the clinically isolated conjugative plasmid pCF10 in *Enterococcus faecalis* and show that it is essential for conjugation. Structural modeling confidently predicts a pilus-like assembly for PrgF_{B2}. We validated this prediction through mutagenesis, conjugation assays, and targeted chemical labeling.

By combining various machine learning bioinformatic techniques, we analysed >1000 Gram-positive conjugative plasmids from diverse species, including major pathogens, and identified pili forming VirB2-like proteins in almost all of them. Our findings overturn the prevailing view that Gram-positive T4SSs lack conjugative pili. This discovery provides a new framework for understanding horizontal gene transfer and highlights critical targets for combating antimicrobial resistance and virulence in Gram-positive bacteria.



<https://doi.org/10.64898/2025.12.18.695078>