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Exploring the diversity of conjugative Type IV Secretion Systems

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Akademisk avhandling

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Abstract
The increase of antibiotic resistance is a major threat to human health. The spread of mobile genetic elements (MGEs) via conjugation is a major contributor to this problem, especially in hospital settings. Many MGEs encode Type IV Secretion Systems (T4SSs), which are multiprotein complexes that transfer the MGE from donor to recipient cells. T4SSs are versatile systems that exist in all prokaryotes. While most research has focused on T4SSs from Gram negative (G^-) bacteria, it is important to understand the similarities and differences with T4SSs from Gram positive (G^+) bacteria, given their different cell envelopes. Additionally, there is also variability within G^- T4SSs, which is not yet fully understood.

The aim of this thesis was to explore the diversity of T4SSs, using pKM101 from *E. coli* (G^-) and pCF10 from *E. faecalis* (G^+) as model systems, with a focus on DNA transfer and replication (Dtr) proteins.

We biochemically characterized the relaxase TraI from pKM101, which processes plasmid DNA prior to transfer through the T4SS. We also solved the crystal structure of its transesterase domain with and without its substrate *oriT* DNA, highlighting its conserved mechanism of action. We further explored the relationship between TraI and the accessory protein TraK, using AlphaFold to predict an interaction involving the TraI CTD. This was confirmed experimentally using *in vivo* BPA-crosslinking.

Many conjugative plasmids encode single-stranded DNA-binding proteins (SSBs), which are thought to protect DNA during transfer. pCF10 encodes the protein PrgE, which was proposed to be one such SSB. However, our biochemical studies and X-ray crystallography revealed that PrgE is an OB-fold protein with unexpected DNA-binding behavior. While its benefit for the plasmid remains unclear, our functional studies have shown that it does not play a role in conjugation.

Finally, we analyzed the structural diversity of conjugative T4SSs in G^- and G^+ bacteria, using bioinformatics and structural modelling. This revealed unknown commonalities, which indicate that G^+ T4SS mating channels are likely more similar in structure to G^- T4SSs than expected.

In summary, this thesis provides new insights into the Dtr proteins that play an integral role in T4SS mediated conjugation, knowledge that hopefully can be used in the fight against hospital acquired infections in the future.

Keywords
Antibiotic resistance, Horizontal gene transfer, Conjugation, Type IV Secretion Systems, Relaxases, Single-stranded DNA-binding proteins, Biochemistry, Structural Biology

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