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Studies on the role of class A penicillin-binding proteins in the bacterial cell envelope

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Abstract
Bacterial cell envelopes are intricate and ever-changing structures that serve numerous defensive and adaptive functions. One of its main structural elements is the peptidoglycan (PG) cell wall, a heteropolymer composed of glycan chains crosslinked by short peptides that forms a net-like structure. The PG layer surrounds the cytoplasmic membrane, providing osmotic stability for the cell and contributing to its shape. PG also holds physiological significance as the enzymes that synthesize and remodel this vital polymer serve as the target of some of our most successful antibiotics, such as penicillin. Penicillin and other β-lactam antibiotics primarily target enzymes known as penicillin-binding proteins (PBPs). This is attributed to the pivotal role of PBPs as the primary enzymes involved in polymerizing and modifying the PG in most bacteria. Due to their importance and therapeutic potential, they have been the focus of research for several decades. In this thesis, we focused on a specific subset of PBPs, the class A PBPs (aPBPs) and their involvement in different cellular processes in two model organisms, *Vibrio cholerae* and *Pseudomonas fluorescens*.

Using an innovative high-throughput analytical pipeline of the chemical structure of the PG of the entire *V. cholerae* transposon mutant library we identified a novel bifunctional PBP, PBP1V. This protein, characterized by a putative domain of 186 amino acids near the transpeptidase active site, is mainly conserved among Gamma- and Betaproteobacteria. Phenotypic analysis of PBP1V revealed that while this protein is not essential in *V. cholerae*, it significantly contributes to its fitness under low osmolarity conditions. The analysis of synthetic lethal interactions involving PBP1V revealed that this protein functionally links the biosynthesis of PG and lipopolysaccharide (LPS). We discovered that unlike the other two aPBPs (PBP1A and PBP1B), PBP1V is needed for LPS homeostasis.

In the final chapter of this thesis, we investigated the role of an aPBP in maintaining bacterial rod shape. Using *P. fluorescens* as a model organism, where the cytoskeletal protein MreB is not essential, we conducted long-term evolution experiments and found that the transpeptidase activity of PBP1A causes lethality when the function of MreB is lost.

Keywords
Bacterial cell wall, peptidoglycan, cell wall synthesis, aPBPs, lipopolysaccharide, *Vibrio cholerae*, *Pseudomonas fluorescens*

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