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Studies on cell wall biosynthesis and remodeling in *Acinetobacter baumannii*

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Abstract
The bacterial cell envelope is a complex and dynamic structure with essential functions in fitness and adaptation. In Gram-negative bacteria, the envelope is composed of an inner (IM) and an outer membrane (OM) that create a space in between called periplasm, where the peptidoglycan (PG) cell wall is located. This PG forms a net-like structure that surrounds the bacteria, determining its shape, counteracting osmotic pressure, and serving as a scaffold for proteins. PG synthesis starts in the cytoplasm, where the membrane-associated PG precursor lipid-II is made through a series of reactions. Lipid-II is then flipped into the periplasm, where it is polymerized to build the mature the sacculus. In rod-shaped bacteria such as *Escherichia coli*, two multiprotein complexes are responsible for PG synthesis: the divisome (septal synthesis) and the elongasome (axial synthesis). Since the discovery of penicillin, PG synthesis has been the focus of research due to its importance as therapeutic target. In this thesis, we explore various mechanisms that contribute to envelope homeostasis in the pathogen *Acinetobacter baumannii*. In the first chapter, we examine the remarkable ability of *A. baumannii* to survive without the elongasome. We phenotypically characterized deletion mutants of the genes encoding the individual components of the elongasome, followed by long-term evolution experiments to identify genetic cues that could explain the non-essentiality of the elongasome in this bacterium. The second chapter of the thesis focuses on the study of ElsL, an uncharacterized protein that allowed *A. baumannii* to keep its rod shape and withstand antibiotics that attack the septum of the cell wall. Although ElsL possesses a YkuD-like domain, which is usually found in periplasmic L,D-transpeptidases, we showed that ElsL is actually a cytoplasmic L,D-carboxypeptidase involved in PG recycling. Absence of ElsL produces a toxic build-up of murein tetrapeptide precursors that negatively affects cell wall integrity. Additionally, inactivation of ElsL perturbs other pathways such as outer membrane lipid homeostasis or L,D-crosslink formation. In the third chapter we focus on the crosstalk between the OM and the PG in *A. baumannii*. This bacterium is an outstanding model to study OM contribution in envelope stability due to its ability to lose its lipooligosacharide (LOS) layer. Using transposon sequencing we found that the elongasome and the PG recycling enzyme ElsL are essential in LOS-deficient *A. baumannii* strains. We further demonstrated that high PBP1A levels impacted negatively on the elongasome function, thus preventing these strains to lose their LOS. In the final chapter of the thesis, we studied how *A. baumannii* employs its type VI secretion system to kill Gram-positive and Gram-negative bacteria. This is dependent on Tse4, a bifunctional enzyme possessing lytic transglycosylase and endopeptidase activities. Additionally, we showed that *A. baumannii* also secretes D-lysine, which gets incorporated into its PG and increased the pH of the environment to enhance Tse4 activity.

Keywords
Peptidoglycan, lipopolysaccharide, bacterial cell wall, antibiotics, *Acinetobacter baumannii*, elongasome

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