MSc Project

Supervisor(s): Margarida Archer Frazão and Mariana Pinho
Supervisor(s) email address: archer@itqb.unl.pt, mgpinho@itqb.unl.pt
Lab/Institution: Membrane Protein Crystallography and Bacterial Cell Biology Labs, ITQB-UNL, Oeiras
TITLE: Structural and functional studies on autolysins from *Staphylococcus aureus*

BACKGROUND

The major component of the Gram-positive bacterial cell wall is peptidoglycan, composed of glycan strands interconnected by short peptides forming a meshwork sacculus that encloses the cell giving it mechanical stability and maintaining its specific shape. Peptidoglycan is a dynamic structure that undergoes constant and simultaneous synthesis and degradation during cell growth. *Staphylococcus aureus* is an important bacterial pathogen and a major cause of hospital-acquired infections. As such, it is a model microorganism for Gram-positive bacteria to study the structure and function of the cell wall. Bacteria produce several peptidoglycan hydrolases (PGHs) that are involved in the degradation of the peptidoglycan. The possible physiological functions include nicking the peptidoglycan for insertion of new monomers involved in remodeling and turnover of the peptidoglycan during cell growth, division and separation. One of these autolysins is Sle1, a 32-kDa soluble protein with N-acetylmuramyl-l-alanine amidase activity. A *sle1* mutant forms clusters, suggesting the involvement of Sle1 in the separation of daughter cells during cell division. This Sle1 mutant revealed a significant decrease in pathogenesis using an acute infection mouse model.

OBJECTIVES

In this project we aim for the structural and functional characterization of *Staphylococcus aureus* Sle1 autolysin “as isolated”, mutant forms and in complex with substrate analogues. Our aim is to purify protein in sufficient amounts to allow the three-dimensional structure determination of Sle1 proteins by X-ray crystallography. The crystal structure will reveal the protein overall fold and allow a detailed atomic characterization of the active site. Because structure is intimately correlated with function, the elucidation of the molecular structure of different forms of Sle 1 will the provide insights into the catalytic mechanism of this enzyme and a meticulous analysis of the peptidoglycan-protein interactions. This structural data is valuable for the design of specific inhibitors of Sle1, which might be of interest to treat infections caused by pathogenic organisms such as *Staphylococcus aureus.* More studies are needed on this topic.

PROJECT DESCRIPTION

The tasks to be performed within this project comprise an experimental component and also involve some computational work. Tasks include gene expression in *Escherichia coli* of Sle1 "as isolated", mutant forms and in complex with peptidoglycan fragments, purification of these proteins; activity assays, crystallization of the purified proteins and subsequent structure determination. The elucidation of the molecular structure of these target proteins will provide insights into the mode of action and specificity of autolysin Sle1.

The work will be done in the Laboratory of Membrane Protein Crystallography at ITQB under supervision of Dr Margarida Archer Frazão, and in a joint collaboration with Dr. Mariana Pinho, Bacterial Cell Biology Lab. In the scope of the project, trips to European synchrotron radiation sources to collect X-ray diffraction data can take place.

Task 1- Gene cloning, expression tests in *Escherichia coli* and purification by affinity chromatography of various Sle1 targets.

Task 2- Crystallization experiments of the purified proteins (apo-, ligand-bound and mutants).

Task 3- X-ray diffraction data measurements, in-house or at synchrotron sources, and data processing

Task 4- Structure determination, model building, crystallographic refinement and structure-function analysis

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										