



Project Impact - Supervisor: Manuela M. Pereira, BioISI | Co-supervisor: Margarida D. Amaral, BioISI

Title: *The impact of energy metabolism of Staphylococcus aureus on bacterial and host cellular processes*

Objectives: This PhD project aims at elucidating the main players in energy metabolism of *Staphylococcus aureus* and evaluating their impact on fundamental processes of the bacterial and host cells, namely cell division, cell shape and size and host interaction.

Methodology: Energy is at the basis of all life processes. Each organism needs to acquire energy from the environment and convert it to useful forms for its maintenance, growth and reproduction. It is thus surprising that the mechanisms underlying the energy metabolism of many pathogens are still elusive. This PhD project will explore the role of enzymes involved in the energy metabolism of the opportunistic pathogen *Staphylococcus aureus*, aiming at understanding their molecular and cellular roles in both pathogen and host through an integrated perspective.

The molecular approaches will involve biochemical and biophysical characterization of *S. aureus* respiratory enzymes both as wild-type and mutant versions. Some of the target enzymes have already been cloned and expressed in *E. coli* with a His-tag at the host laboratory. Large-scale cultures will be performed, cells will be harvested by centrifugation and disrupted in a French-press and membranes will be obtained by ultracentrifugation. Enzymes will be purified by chromatographic procedures in AKTA systems. All the purifications steps will be monitored by activity assays, UV-Visible spectroscopy and electrophoretic procedures. Functional characterization will be performed and will provide mechanistic insights. The catalytic mechanism will be assessed, both by steady-state and pre-steady-state analyses, determining reaction transfer rates and identifying reaction intermediates. Protein-substrate interaction will be investigated by complementary approaches, which will allow to evaluate interactions at the protein, substrate, and global levels. Binding parameters will be determined and interactions with different substrates and inhibitors will be examined.

The cellular approaches will explore the impact of the enzymes in pathogen cell metabolism and dissect the interplay of the energy metabolism with other fundamental cellular processes, such as cell division and cell shape and size and host interaction. The analyses include knockout *S. aureus* mutants of the genes encoding the different enzymes to assess their impact in bacterial growth and metabolism. We will use the mutants available at the Nebraska Transposon Mutant Library for *S. aureus* and CRISPR-Cas9 technology, which we already implemented in the lab. Cell growth will be studied under several controlled conditions, aeration and temperature in TSB or chemical defined media, supplemented with different carbon sources (e.g. glucose, lactate or acetate). Growth will be monitored by cell density and pH changes. The effect of mutations on the expression of the other enzymes will be assessed by mRNA quantification. Survey of metabolomic profiles of wt and knockout mutant strains will be obtained by mass spectrometry analyses, performed at BioISI MS facility and NMR-based metabolomics. Repercussions of the knockout mutations on cell morphology and cell division will be explored by different methodologies such as fluorescence microscopy and/or atomic force microscopy. The impact of energy metabolism of bacteria on host interaction will be investigated using cells derived from both healthy individuals (controls) or with Cystic Fibrosis (CF) and wt or mutant bacterial strains. The studies will involve immortalized human bronchial epithelial (HBE) cells stably expressing wt- or F508del-CFTR, the most common mutation found in CF individuals. HBE cells will be analysed for viability, proliferation and cytotoxicity.

The project will allow the PhD student to develop critical and analytical, perseverance and problem-solving attitudes. It will provide soft skills on oral and written communications and engagement with science public awareness activities. It will give the opportunity to supervise BSc and MSc students, developing interpersonal, management and leadership competencies.



Supervisor: Manuela M. Pereira

Co-Supervisor: Margarida D. Amaral

Type of fellowship (select the correct option)

National

Mixed (Portugal and abroad:)