

Student's Name:

Student email address:

No.

Supervisor(s): Prof. Miguel Teixeira, Dr. Filipe Folgosa

Supervisor(s) email address: miguel@itqb.unl.pt, f.folgosa@itqb.unl.pt

Lab/Institution: Instituto de Tecnologia Química e Biológica António Xavier, UNL

Scientific area: Biochemistry

TITLE: Unraveling a key survival mechanism in anaerobic Clostridia pathogens

BACKGROUND

Nosocomial infections are a major health problem worldwide, maintaining a pool of affected people of over 1.4 million; in Portugal for example, an average of 12 deaths per day is due to hospital acquired infections. These are caused by many pathogens, from which bacteria are the most common ones, namely gram-positive bacteria as *Clostridium* sp., or gram-negative bacteria such as *Escherichia coli*.

The ability of human immune system to deal with the infections caused by some of these organisms relies on several defence mechanisms, including the generation of nitric oxide (NO). Besides NO, anaerobic pathogens, such as *Clostridium difficile*, are also obligated to tackle the presence of O₂, and respective reactive oxygen species (ROS). In this sense, evolution has provided defence mechanisms that enable these organisms to survive to these molecules.

One of these mechanisms is based on flavodiiron proteins, FDPs. Widespread among all Kingdoms, FDPs may have a dual role regarding O₂ and NO detoxification, contributing to the microbial survival to the stress imposed by host immune system. All FDPs have a central core composed by a metallo-β-lactamase-like diiron containing domain, followed by a flavodoxin one. In *Clostridium difficile*, there are two FDPs encoded in its genome: one with the two canonical domains, and another with two extra domains, predicted as a small-rubredoxin-like and a NAD(P)H:rubredoxin oxidoreductase.

OBJECTIVES

The main objectives of this Project are to analyse complex FDPs from Clostridiales, namely *C. difficile*, a very important human pathogen, and to establish their function as O₂ and/or NO reductases, thus contributing to the determination of the role of these enzymes in these members of the human microbiome, as detoxifiers of those gaseous compounds.

Also, these studies will lead to the identification of the nature of the extra domains and of their function, leading to an extended view on the diversity of the important family of flavodiiron enzymes.

PROJECT DESCRIPTION

The project will be divided in several tasks, towards achieving the main goals of this project.

Task 1 – Enzyme production and purification: The clones of all enzyme targets, and truncated forms (separated domains) are already available at the host laboratory, and preliminary tests revealed the overexpression of the targets. Therefore, this task will involve their production, by overexpression in *E.coli*, and purification, by standard chromatographic processes.

Task 2- General biochemical characterization: Determination of molecular masses and quaternary structure, metal and flavin contents, using several analytical tools (SDS PAGE, gel filtration, ICP, reverse phase HPLC).

Task 3 – Kinetic, thermodynamic and spectroscopic analysis: This task will start by an overall analysis of the thermodynamic (redox) and spectroscopic studies (UV-Visible, Electron Paramagnetic Resonance), aiming at determining the the properties of the novel enzymes analysing the newly studied enzymes/domains. The first target will be the enzymes from *C. difficile* strains. Thorough kinetic study, by steady state (using specific NO and O₂ electrodes) and single turnover measurements, by fast kinetics (stopped-flow) will be undertaken, to assess the reactivities of these enzymes, to establish their function.

Task 4 – Complementation assays: To further analyse the wild-type enzymes, complementation assays will be performed in an *E.coli* strain deleted in the FDP encoding gene, also available at the host Laboratory.

TIMELINE

	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										