UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2022-2023

Laboratory/Institute: **Institut de Biologie Structurale** Director: **W. Weissenhorn**
Team: **Bacterial pathogenesis & cellular responses** Head of the team: **I. Attrée**

Name and status of the scientist in charge of the project: **Antoine Maillard, CR** **HDR**: yes ☑
Address: **71, avenue des Martyrs - CS 10090 - 38044 Grenoble cedex 9**
Phone: **04 57 42 86 08** e-mail: **antoine.maillard@ibs.fr**

Program of the Master's degree in Biology:

☑ Microbiology, Infectious Diseases and Immunology ☐ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Project title: ***Secretion & activation of the toxin ExlA from Pseudomonas aeruginosa.***

Objectives (up to 3 lines):

1- Targeted random mutagenesis of ExlA targeting (TPS) domain.
2- Experimental set up of ExlA secretion assays: hemolysis and immunodetection.
3- Sort TPS residues according their relevance to TPS function: targeting / secretion or activation.

Abstract (up to 10 lines):

Pseudomonas aeruginosa (*Pa*) is a major bacterial nosocomial pathogen. The present project focuses on ExlA, a recent addition to *Pa* toxic armament. As a two-partner secretion substrate, ExlA gets translocated across the outer membrane of *Pa* by its dedicated transporter ExlB, to which it is targeted *via* its N-terminal, 250 residue-long TPS domain. ExlB does activate ExlA during transport, which is not yet understood. The trainee will make a TPS domain-targeted variant library of *exlA*. As ExlA is hemolytic and *E. coli* BL21 is not, the library will be expressed in BL21 and screened on blood agar plates. Variants with TPS function preserved or impaired will yield colonies with a halo or no halo, respectively. Variants with C-terminal truncation, hence impaired hemolysis and no halo, will be sorted out by immunodetection of the C-terminal his-tag, thus leading to the selection of full-length proteins that are either (i) secretion-incompetent or (ii) inactive yet secretion-competent. This will pave the way to understand how ExlB activates ExlA.

Methods (up to 3 lines):

Molecular biology, error-prone PCR mutagenesis, recombinant expression in *E. coli*, screening on agar plates, electrophoresis, electro-transfer, Western blots, fluorescence microscopy to check ExlB and ExlA localization.

Up to 3 relevant publications of the team:

Reboud Emeline, Pauline Basso, Antoine P. Maillard, Philippe Huber, Ina Attrée (2017) Exolysin Shapes the Virulence of Pseudomonas aeruginosa Clonal Outliers. Toxins 9(11)
Reboud Emeline, Stéphanie Bouillot, Sabine Patot, et al. (2017) Pseudomonas aeruginosa ExlA and Serratia marcescens ShlA Trigger Cadherin Cleavage by Promoting Calcium Influx and ADAM10 Activation. PLoS Pathogens 13(8)
Job Viviana, Laura Gomez-Valero, Adèle Renier,.. Carmen Buchrieser, Ina Attrée (2022) Genomic erosion and horizontal gene transfer shape functional differences of the ExlA toxin in Pseudomonas spp iScience 25(7)

Requested domains of expertise (up to 5 keywords):

Molecular biology, microbiology, biochemistry.