**Master 2 internship project**

**Year 2023-2024**

**Laboratory/Institute:** CIIL, Pasteur Institute of Lille **Director:** Dr Jean Dubuisson

**Team:** Research on *Mycobacteria* and *Bordetella* **Head of the team:** Dr Nathalie Mielcarek

**Name and status of the scientist in charge of the project:** Dr Romain Veyron-Churlet (CRCN, CNRS) **HDR: yes ☑ no ☐**

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**Program of the Master’s degree in Biology:**

**☑** Microbiology, Infectious Diseases and Immunology **☐** Structural Biology of Pathogens

**☐** Physiology, Epigenetics, Differentiation, Cancer **☐** Neurosciences and Neurobiology

**Title of the project: Deciphering protein-protein interaction network among the biosynthesis of arabinogalactan in mycobacteria**

Abstract: ***Mycobacterium tuberculosis*** (*Mtb*) is the etiological agent responsible for **tuberculosis**, which causes 1.6 million deaths per year (WHO, Global TB report 2022). Moreover, there is an alarming increase of tuberculosis cases caused by *Mtb* strains, which are **(multi)resistant to available antibiotics**. In this context, there is an urgent need to **identify** **new targets** and to **develop new antitubercular drugs**.

*Mtb* is capable of infecting, surviving and multiplying within the **alveolar macrophages** of the infected host. Hence, the **mycobacterial cell wall** is the first barrier protecting the bacilli from **host response** and **antibiotic treatment**, making it a key factor of the **mycobacterial pathogenicity**. Therefore, the biosynthesis of several cell wall components (such as mycolic acids or arabinogalactan) is **essential for mycobacterial viability**, constituting a very attractive target for **drug development**.

Objectives: This Master 2 project aims at deciphering **protein-protein interactions** among the **biosynthesis of arabinogalactan**, an **essential compound** of the mycobacterial cell wall. This pathway is of growing interest as being targeted by several **antitubercular drugs** (benzothiazinone, pretomanid, delamanid). For this purpose, we will use the **proximity-dependent biotinylation assay (BioID)**, which was recently developed at the laboratory, by producing fusion proteins in non-pathogenic and fast-growing ***Mycobacterium smegmatis*** (a common model used to study pathogenic mycobacteria). This **innovative approach** may help to identify **new proteins and pathways** that are interconnected with the biosynthesis of arabinogalactan, thus constituting **putative targets** for future **drug development** against mycobacteria.

Methods: This project will use several techniques encompassing **bacteriology** (cultures of *E. coli* and non-pathogenic *M. smegmatis*), **molecular biology** (cloning steps for the production of fusion proteins) and **proteomics** (mass spectrometry analyses), all available at the host laboratory.

Up to 3 relevant publications of the team:

Veyron-Churlet, R., Lecher, S., Lacoste, A. S., Saliou, J. M. and Locht, C. (2023) Proximity-dependent biotin identification links cholesterol catabolism with branched-chain amino acid degradation in *Mycobacterium smegmatis*, FASEB J. 37, e23036.

Veyron-Churlet, R., Saliou, J.M., and Locht, C. (2021) Interconnection of the mycobacterial heparin-binding hemagglutinin with cholesterol degradation and heme/iron pathways identified by proximity-dependent biotin identification in *Mycobacterium smegmatis*. Environ Microbiol 23: 3212-3224.

Veyron-Churlet, R., Saliou, J.M., and Locht, C. (2020) Protein scaffold involving MSMEG\_1285 maintains cell wall organization and mediates penicillin sensitivity in mycobacteria. FEBS J 287: 4415-4426.

Requested domains of expertise (up to 5 keywords): bacteriology, tuberculosis, cell wall biosynthesis, antibiotics, protein-protein interactions.