Laboratory/Institute: CIIL, Pasteur Institute of Lille **Director:** Dr Jean Dubuisson
Team: Research on *Mycobacteria* and *Bordetella* **Team leader:** Dr Nathalie Mielcarek

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Master 2 Project: Deciphering the protein-protein interaction network during mycobacterial cell wall biosynthesis

Summary: ***Mycobacterium tuberculosis*** (*Mtb*) is the causative agent responsible of **tuberculosis** (TB), which causes 1.3 million deaths each year (WHO, Global TB report 2023). TB cases caused by *Mtb* strains that are **multidrug resistant to available antibiotics** are increasing at an alarming rate. There is an urgent need to **identify new targets** and to **develop new anti-TB drugs**. *Mtb* is able to infect, survive and replicate within the **alveolar macrophages** of the infected host. The **mycobacterial cell wall** is therefore the first barrier that protects the bacilli from the **host response** and **antibiotic treatment**, making it a key factor in **mycobacterial pathogenicity**. In addition, as mycobacterial cell wall biosynthesis is **essential for bacterial viability**, making it a very attractive target for **drug development**.

Aims: This Master 2 project aims to decipher **protein-protein interactions** during **the mycobacterial cell wall biosynthesis**, which involves several metabolic pathways that are crucial for bacterial viability. Indeed, several anti-TB drugs (isoniazid, ethambutol, etc.) specifically target the biosynthesis of essential compounds (mycolic acids, arabinogalactan, etc.). To this end, we will use the **proximity-dependent biotinylation identification (BioID)** recently developed in 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 new pathways** that are interconnected and thus represent **putative targets** for future **drug development** against mycobacteria.

Methods: This project will make use of several techniques, including **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 in 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.