A graduate fellow position (Assegno di Ricerca) is available at the Department of Biology of the University of Padova for the project entitled: “Investigating the role of the integral membrane protein LysX2 in *Mycobacterium tuberculosis* virulence”. **Starting no later than December 1st, 2022**. Duration: 18 months.

State of the Art and Research Program

We have recently identified a new gene of *Mycobacterium tuberculosis* (*Mtb*) encoding an integral membrane protein with a putative aminoacyl-phosphatidylglycerol (aaPG) synthase domain which we named LysX2 (Boldrin F. *et al.* BMC Microbiology 2022, 22(1):85; doi: 10.1186/s12866-022-02493-2). AaPG synthases are widely represented among prokaryotes since several bacterial species use them to produce aminoacyl esters of phosphatidylglycerol (aaPGs) which confer resistance to low pH and antimicrobial cationic peptides (CAMPs) by increasing the net positive charge of the bacterial surface.

Upon heterologous expression of LysX2 in *Mycobacterium smegmatis* (whose genome does not encode this protein), we found that LysX2 conferred resistance to low pH, nitrosative stress and CAMPs. Moreover, the presence of LysX2 delayed biofilm formation, suggesting a modification of surface properties. Indeed, we found that the expression of LysX2 caused a strong decrease of the bacterial net negative charge, suggesting a modification at the level of the mycomembrane.

A remarkable characteristic of LysX2 is the periplasmic localization of its putative aaPG synthase domain. Members of the family of aaPG synthases such as MprF from *Staphylococcus aureus* and LysX from *Mtb*, have their PG synthase catalytic domain localized in the cytosol, consistent with their role in transferring a lysyl residue from a tRNALys to PG. Recently we deleted the LysX2 structural gene in *Mtb* and preliminary data suggest that the mutant is attenuated in a mouse model of infection. Overall, our data indicate that LysX2 is an *Mtb* protein, which could represent an important new virulence factor and a prototype of a new class within the family of aaPG synthases exerting its function through a novel still unknown mechanism, which is important for modulating cell surface and consequently the bacterial fitness during the infection process.

In this project we propose to characterize the role of this protein in its natural host. For this purpose, we will compare the ability of the *Mtb* null l*ysX2* mutant, its complemented strain, and their wild-type progenitor to form biofilms as well as their resistance to low pH, CAMPs and nitrosative stress. Since these stresses are encountered by *Mtb* upon infection of macrophages and pneumocytes, we will also test these strains in resting and activated macrophages and in a cell line of type II pneumocytes. Finally, in collaboration with Dr. Hedia Marrakchi from the Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France, we will also analyze if LysX2 is able to induce any chemical modification of the *Mtb* cell envelope in order to elucidate its mechanism of action.

Eligibility requirements

Applicants must hold a University degree in any of the following Life Sciences: Biology, Molecular Biology, Health Biology, Medical Biotechnologies.

The ideal candidate is an enthusiastic and committed young researcher with experience in molecular biology and cell biology who will be expected to work independently. Team skills and a good knowledge of written and spoken English are requested. As *Mycobacterium tuberculosis* is a risk group 3 microorganism, willingness to work in a Biosafety Level 3 Facility is mandatory.

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