

# ABSTRACT

Charles University in Prague  
Faculty of Pharmacy in Hradec Králové  
Department of Biochemical Sciences

Candidate: Kateřina Musilová

Supervisor: Prof. Ing. Vladimír Wsól, Ph.D.

Tutor: Prof. Paul Nguewa, Ph.D.

Title of diploma thesis: Molecular cloning of *YinP* gene from *Leishmania major* using two red fluorescent pXG-mCherry plasmids. Valuable tools for gene expression location.

In the 21<sup>st</sup> century, leishmaniasis remains a major health problem in numerous developing countries. Around 2 million cases of leishmaniasis are reported every year and estimated mortality is over 20,000 deaths annually. Antileishmanial drugs are often unaffordable for affected people and display severe toxic side effects. Potent human vaccines are not available. This, together with increasing resistance, is a reason why new effective, safe, and affordable medicines are greatly needed.

Leishmaniasis is caused by *Leishmania* species. These parasites are transmitted by phlebotomine sand flies, which also provide to the leishmania environment necessary for their development into infective forms. The process of transformation into a stage infective for vertebrate hosts is called metacyclogenesis. Nowadays, genes, enzymes, and proteins possibly exhibiting a function in the metacyclogenesis are extensively examined. One of the genes suggested to play a role during the development of the *Leishmania* infective stage is *YinP*.

The main objective of this study was to reveal where *YinP* gene is expressed in the leishmanial cell. Two plasmids, pXG-mCherry12-*YinP* and pXG-mCherry34-*YinP*, were constructed to contribute to finding. In these vectors, *YinP* gen was inserted directly next to the gen for fluorescent protein (mCherry), to generate fluorescent fusion proteins expressed in the parasites. The created plasmids were introduced in *Leishmania major* parasites by electroporation.

Fluorescent microscopy disclosed that red fluorescence of mCherry fused with *YinP* was localized only in a part of nucleus. The parasites transformed with pXG-mCherry plasmids without *YinP* inserted displayed red fluorescence in the entire cell. Therefore, our results showed that *YinP* protein is expressed in the nucleus.