**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Self control: regulating expression of essential protein kinases in mycobacteria  |
| **Project Summary**  |
| *Background* Mycobacteria are a group of versatile microorganisms which include medically important pathogens and environmental bacteria. *Mycobacterium tuberculosis* and *Mycobacterium bovis* cause tuberculosis (TB) in humans and cattle. Bovine TB remains a challenge for production industry in England and Wales, since both herd incidence and herd prevalence remain relatively stable and high1. Thus, a better understanding of mycobacterial biology is required for implementation of control measures. Mycobacteria differ from other bacteria by a complex lipid rich cell wall, unique way of growing at cell poles and very slow replication. Mycobacteria can also survive and persist in harsh conditions such as extreme pH, the presence of reactive oxygen species, nitrosative stress, hypoxia and starvation. These abilities allow mycobacterial pathogens to grow within eukaryotic cells and persist host immune-mediated pressure. One of the key mechanisms that controls mycobacterial growth and survival is serine threonine protein kinase (STPK) signalling. *M. bovis* genome encodes 11 STPK and two of them, PknA and PknB, are essential for growth. *PknA* and *pknB* are transcribed from the same promoter and their expression is tightly regulated. Our recent findings suggest that dysregulation of PknB has a detrimental effect on mycobacterial survival under nitrosative stress; however, very little information on molecular mechanisms controlling PknA and PknB expression is available. An essential global transcriptional regulator Rv0023 linked to NAD+/NADH homeostasis is annotated to govern transcription of *pknB* and *pknA.* Additionally, an antisense transcript has been identified within the coding region of *pknA* and *pknB* which is differentially transcribed under various conditions. *Aims and objectives* The proposed project will be focused on investigation of molecular mechanisms underpinning regulation of *pknA* and *pknB* expression during growth, transition to dormancy and resuscitation, persistence in macrophages. The proposed objectives will include: 1. Investigating the effect of Rv0023 over-expression and silencing on expression of *pknA* and *pknB* and bacterial survival; 2. Establishing the role of antisense transcript within the *pknA/pknB* coding region in bacterial growth, dormancy and persistence in macrophages. 3. Exploring proteomics and phosphoproteomics profiles of mycobacteria with altered *pknB* and *pknA* expression under nitrosative stress and during persistence in activated macrophages. *Experimental approaches* Previously established models of dormancy, including hypoxia driven non-replicating persistence and nitric oxide induced growth arrest will be used. Additionally, we will use murine and human macrophage cell lines activated with interferon gamma for investigation of mycobacterial persistence. We will mainly focus on *M. bovis* BCG, a vaccine strain currently used for laboratory research. *pknA* and *pknB* silencing will be achieved by application of CRISPR genetics; over-expression systems will be used for dysregulation of kinase expression. Within the project we will also develop methods for proteomics/phosphoproteomics of mycobacteria in infected macrophages. Techniques that will be undertaken during the projectCultivation of mycobacteria Genetic manipulation of mycobacteria, gene silencing and over-expression  Proteomics and phosphoproteomics SDS-PAGE and immunoblotting Macrophage infection studies  |
| **References** |
| 1. Quarterly TB in cattle in Great Britain statistics notice: June 2024. <https://www.gov.uk/government/statistics/incidence-of-tuberculosis-tb-in-cattle-in-great-britain>
2. Turapov O et al. (2018). Two faces of CwlM, an essential PknB substrate, in *Mycobacterium tuberculosis*. *Cell Rep* 25(1): 57-67. e 5.
3. Gap-Gaupool B, Glenn SM, Milburn E et al. Nitric oxide induces the distinct invisibility phenotype of *Mycobacterium tuberculosis*. Commun Biol. 2024 Sep 28;7(1):1206. doi: 10.1038/s42003-024-06912-0.
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