**University of Leicester**

**BBSRC MIBTP Studentship Project 2024-5 entry.**

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| **Project Reference** |  |

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**Section 2 – *Project Information***

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| **Project Title** | Understanding the roles of triacylglycerol in modulating mycobacterial growth and biophysical properties |
| **Project Summary** | |
| Mycobacteria contain more lipid than any other bacteria. In addition to a characteristically lipid-rich cell envelope, mycobacteria can accumulate triacylglycerol (TAG) as cytoplasmic lipid bodies (LBs). Accumulated prokaryotic TAG represents a reserve of carbon and energy which can be drawn upon to support continued metabolism and growth.  In the host, in response to growth limiting factors such as hypoxia and nitrosative stress, *M. tuberculosis* (Mtb), the agent of tuberculosis (TB), can enter a state of low metabolic activity leading to growth arrest, and sub-populations of differentially culturable bacteria (DCB). DCB cannot be cultured using standard techniques and require addition of culture supernatant. These populations have important impact on TB treatment as they are tolerant to the action of front-line antimicrobials. In TB patient sputum, sub-populations of *M. tuberculosis* containing TAG-LBs revealed during therapy have been associated with treatment failure, or relapse.  *In vitro*, such growth arrest coincides with induction of TAG synthase, *tgs1*, and accumulation of TAG LBs. When conditions change, accumulated TAG is assimilated with release of fatty acids to support regrowth. Studies of the strain Mtb H37Rv, that contains very little TAG during growth, supported the hypothesis that induction of *tgs1* and biosynthesis of TAG reduces carbon flux through the tricarboxylic acid cycle, resulting in growth arrest. However, we have observed more recently isolated strains of *M. tuberculosis* to possess variable *tgs1* expression and TAG LB content during growth. In addition*, Mycobacterium smegmatis*, a rapidly growing non-pathogenic species, contains TAG LBs during growth. It is not known if TAG LB accumulation in growth represents a reduced capacity to respond to growth arresting stimuli *e.g.* hypoxia or nitrosative agents, impacts potential recovery of growth following relief of these stresses, or the detection of DCB.  TAG is also a component of the mycobacterial cell envelope. A TAG transport system has been identified and TAG transport from the cytoplasm has been proposed as a means of modulating growth by regulating TAG LB accumulation. The lipid composition of the cell envelope impacts cell surface hydrophobicity that will influence interactions of mycobacteria with their environment. Such interactions may alter the propensity of mycobacteria to enter aerosols, impacting airborne transmission of disease, or mediate the initial interactions with host cells that would influence pathogenic potential.  The relative proportions of cytoplasmic TAG and TAG in the cell envelope are not understood and may differ between species, strains, culture conditions and growth states. It has been observed that *Mycobacterium abscessus* can increase TAG synthesis without accumulation of TAG LBs, suggesting that there is a significant capacity for cell envelope TAG accumulation.  The aim of this project is to understand the roles of TAG in influencing mycobacterial growth and biophysical properties. Research will be undertaken with different species, strains and mutants in key genes involved in TAG synthesis, assimilation and transport. Specific objectives include:  To determine whether cytoplasmic TAG content impacts response to growth arresting stimuli and regrowth thereafter.  To determine whether during growth, TAG LBs represent  a dynamic metabolic pool continuously being turned over, by measuring flux of fatty acids through various lipid fractions.  To measure the relative proportions of cytoplasmic and cell envelope TAG and associated biophysical properties of cells such as cell surface hydrophobicity and buoyancy.  Techniques that will be undertaken during the project  Actinobacteria culture and growth assays in defined conditions  Genetic modification of Actinobacteria.  Lipid analytical methods to include, chemical extraction, thin layer chromatography, mass spectrometry, radioisotopic labelling, fluorimetry and cytological analyses using fluorescence microscopy and transmission electron microscopy.  Enzyme assays | |
| **References** | |
| Baek, S. *et al*. (2011) ‘Metabolic Regulation of Mycobacterial Growth and Antibiotic Sensitivity’, PLoS Biology, 9, e1001065.  Martinot, A. *et al*. (2016) ‘Mycobacterial Metabolic Syndrome: LprG and Rv1410c Regulate Triacylglcyeride Levels, growth rate and Virulence in *Mycobacterium tuberculosi*s’, PLoS Pathogens, 12, e1005351 | |

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