**Department of Respiratory Sciences PhD studentship Project information**

**Funding Source: Self-Funded**

**Proposed project start date:** open

**Closing date for applications:** open

**Eligibility:** EU/International only

**Department/School:** Respiratory Sciences

**Supervisors:** Dr Natalie Garton njg17@le.ac.uk

**Project Title:** Understanding the roles of mycobacterial triacylglycerol in modulating mycobacterial growth and biophysical properties

**Project Description:**

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, *Mycobacterium tuberculosis*, 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 of *M. tuberculosis* H37Rv coincides with induction of TAG synthase, *tgs1*, and accumulation of TAG LBs. When conditions change, accumulated TAG LBs are assimilated; released fatty acids to support regrowth. More recent clinical isolates of *M. tuberculosis* to possess variable *tgs1* expression and TAG LB content during growth. It is not known if such TAG LB accumulation 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.

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.

To measure the relative proportions of cytoplasmic and cell envelope TAG, associated biophysical properties such as cell surface hydrophobicity and host cell interactions.

Experimental approaches used in this study will include:

Culture of *M. tuberculosis* and model mycobacteria in defined conditions

Genetic manipulation of mycobacteria.

Lipid analytical methods to include thin layer chromatography, mass spectrometry.

Fluorimetry and cytological analyses using fluorescence microscopy and transmission electron microscopy.

**References:**

Tarekegn, B. G. *et al*. (2024) ‘Host and pathogen strain interaction in tuberculosis revealed by measurement of exhaled nitric oxide, mycobacterial lipid bodies in sputum and treatment responses: An observational study’. Lancet Microbe. doi: [10.1016/S2666-524(24)00108-3)](https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247%2824%2900108-3/fulltext)

Garton, N. J**.** *et al*. (2008) ‘Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum’, *PLOS Med*., Apr 1;5(4): e75, doi: [10.1371/journal.pmed.0050075](https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0050075)

Baek, S. *et al*. (2011) ‘Metabolic Regulation of Mycobacterial Growth and Antibiotic Sensitivity’, PLoS Biology, 9, e1001065, doi: [10.1371/journal.pbio.1001065](https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001065)

Martinot, A. *et al*. (2016) ‘Mycobacterial Metabolic Syndrome: LprG and Rv1410c Regulate Triacylglyceride Levels, growth rate and Virulence in *Mycobacterium tuberculosi*s’, PLoS Pathogens, 12, e1005351, doi: [10.1371/journal.ppat.1005351](https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005351)

**Tuition Fee details:**

Requires full funding from the prospective applicant, Fee Band 26 (£38,300)

**Project / Funding Enquiries:** Dr Natalie Garton njg17@le.ac.uk

**General enquiries to** **cls-pgr@le.ac.uk**

**To apply please refer to** [**https://le.ac.uk/study/research-degrees/research-subjects/respiratory-sciences**](https://le.ac.uk/study/research-degrees/research-subjects/respiratory-sciences)

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