**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** | **The impact of bacterial metabolism on successful bacteriophage infection**  |
| **Project Summary**  |
| Bacteriophages, or phages are bacterial viruses and the most abundant and diverse biological entities on Earth.  Throughout the biosphere they outnumber their bacterial hosts by at least 10 to 1 and strikingly, most of the functions of most genes encoded by phages remains unknown.  What is known, is that their impact on life ranges from the maintenance of healthy microbiota to the promotion of biodiversity in soil and oceans. Phages also have many potential practical uses, from the treatment of infections caused by antibiotic resistant bacteria to preventing antibiotic resistance build up within food animals and increasing the shelf life of food products to reduce global food waste.    To optimise and extend the applications of phages, it is important to understand how specific phages impact their host bacteria, particularly to determine how phages manipulate bacterial metabolism.  Bacteria-phage interactions differ extensively according to physiological conditions, for example interactions between urinary tract causing bacteria and their phages alter when they interact in urine compared to in standard media.  This is because the bacteria are undergoing different metabolic pathways in these scenarios and phages can interact with some pathways but not others.  Furthermore, our lab and work of others have shown using metabolomic profiling experiments that phages can profoundly alter the metabolites that bacteria express [1-2] and reviewed and contextualised in our review [3].    However, despite knowing this occurs, our understanding of the underlying molecular and genetic mechanisms by which this happens is limited. To unravel the process of phage interactions with bacterial physiology, the student will work in an interdisciplinary way; in a phage-focused laboratory, with analytical chemists, and within a group focused on determining how metabolism impacts bacterial phenotype.   To determine the impact of metabolic status on phage interactions we will study *Klebsiella* and its associated phages as a model system.  We will study how our diverse, well characterised *Klebsiella* phage collection interact with *Klebsiella* under different metabolic conditions.  *Klebsiella* are Gram-negative bacteria, some of which cause diseases and others are found in the environment as free-living microbes.  They have diverse metabolic adaptive capabilities including the ability to fix nitrogen. *K. pneumoniae* causes many diseases including pneumonia, urinary tract infections, bacteremia, and septicaemia in humans and animals, showing that the microbe is environmentally adept very likely due to its inherent metabolic flexibility.  Worryingly, many strains are antibiotic resistant thus pose a serious health threat, hence it is one of the few pathogens for which there is an urgent need for effective antibiotics as declared by the WHO.  Phages could be one such approach so determining how they interact with metabolic status is paramount to progress.  **Aims and Objectives** In this project, we aim to test the hypothesis that particular phage types target specific bacterial pathways and determine the mechanistic basis for such targeting, which will help us better develop phages for therapy.     **Specific Objectives are:** 1. Determine how phages interact with bacterial metabolism by using RNASeq and and analytical chemistry in order to identify which metabolic pathways allow successful infection and how they are altered during this process.

 1. Examine our well characterised phages to determine if phage specific infection strategies for the phages tested exist.  For example, establish if phages that are particularly effective at treating strains impact the same metabolic pathways.

 1. Confirm our observations by making bacterial knockout mutants of whole or partial metabolic pathways targeted by phages and determine how phages interact with these bacterial mutants.

 1. Determine if phages with different metabolic strategies are more effective at treating bacteria when combined with each other or within one ‘metabolic type’.

 **Importance** This project aims to provide a fundamental understanding of the relationship between specific phages and their bacterial hosts according to bacterial physiology and metabolism.  In doing so, it aims to compare how bacterial ‘takeover strategies’ differ between phages, and how this impacts their success as phages to kill bacteria in disease relevant settings. This dataset and outputs from the thesis will provide novel insights to assess phages for therapeutic and industrial applications.  **Environment** The project combines molecular biology and microbiology facilities available within the University of Leicester's ecosystem (Centre for Phage Research and Respiratory). It also involves multidisciplinary collaborations from LISCB (Leicester Institute of Chemical and Structural Biology) to conduct analytical chemistry.   techniques that will be undertaken during the project1. Maintenance, handling and manipulation of bacterial cultures and bacteriophage stocks
2. Bacteriophage enumeration and phenotypic characterisation
3. Transcriptional profiling by RNAseq and quantitative reverse transcriptase real time PCR assays
4. Analytical Chemistry using Gas Chromatography Mass Spectrometry
5. Targeted mutation and associated molecular techniques
6. In vivo assays using mouse and/or *Galleria mellonell*a
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| **References** |
| Howard-Varona, C., Lindback, M.M., Bastien, G.E. et al. Phage-specific metabolic reprogramming of virocells. ISME J 14, 881–895 (2020). [https://doi.org/10.1038/s41396-019-0580-z](https://www.google.com/url?q=https://doi.org/10.1038/s41396-019-0580-z&sa=D&source=editors&ust=1629818009447000&usg=AOvVaw0HvQfOGBZcPdQNYDTqyJnE)  De Smet, J., Zimmermann, M., Kogadeeva, M. et al. High coverage metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa* physiology during infection. ISME J 10, 1823–1835 (2016). <https://doi.org/10.1038/ismej.2016.3>  Francesca E. Hodges, Thomas Sicheritz-Pontén, and Martha R.J. Clokie. PHAGE.Mar 2021.16-25.[http://doi.org/10.1089/phage.2020.0041](https://doi.org/10.1089/phage.2020.0041)  |

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