BBSRC Strategic Research Priority:

Food Security

- Microbiology

Principal Supervisor: Dr. Chris Bayliss  cdb12@le.ac.uk

Co-supervisor: Dr Andrey Morozov

PhD project title: **Understanding how mutability facilitates survival of alternating selection and bottlenecks by the major food-borne pathogen *Campylobacter jejuni***

University of Registration: University of Leicester

---

Project outline

1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns)

Bacterial pathogens are subject to two strong pressures during spread within and transmission between hosts. The first is fluctuating selection pressures with a bacterial population in a benign host environment being suddenly exposed to an inflammatory response induced by a co-infecting virus or the appearance and rapid spread of a bacteriophage through the bacterial population. The other major pressures is a non-selective bottleneck as occurs during transmission where the bacterial population is ejected into the environment and is randomly reduced in numbers by desiccation before entering a new host.

How do bacterial pathogens survive these rapid fluctuations in the environment? One mechanism is through hypermutable sequences that generate large numbers of bacterial variants in bacterial populations prior to an environment change or bottleneck. These variants permit survival of the altered environment and transmission of genetically variable populations to new hosts.

*Campylobacter jejuni* contains multiple hypermutable sequences. A combination of experimental and theoretical modelling will be utilised to determine how the mutation rates of these hypermutable sequences enable *Campylobacter* to survive the combined effects of fluctuating selection and non-selective bottlenecks. These experiments will be important for understanding how hypermutable sequences contribute to the pathogenesis and transmission of this important pathogen.

*C. jejuni* is a major agent of food-borne gastroenteritis. *C. jejuni* is a commensal of the gastrointestinal tract of birds and most importantly poultry, the major source of human infection. Transmission between birds is via the fecal-oral route whereas transmission to humans involves survival on external and internal surfaces of meat products. Within hosts, *C. jejuni* colonises the mucosa and elicits inflammatory responses. Additionally multiple bacteriophages have been isolated for this species. There is therefore stringent selection for variation in surface structures.
The hypermutable sequences of C. jejuni are mainly polyG tracts whose mutations mediate ON/OFF switches in gene expression. These ‘phase-variable’ genes modify surface structures impacting on adhesion to host tissues, bacterial aggregation and phage resistance. Switching of individual and multiple genes may have critical effects on the expression of particular phenotypes. Using a rapid, high through-put analysis method (as developed in Dr. Bayliss laboratory; Bayliss et al. 2011 and Lango-Scholey et al. 2016), the extent of variation in 27 phase-variable genes of C. jejuni strain 11168 has been explored during in vitro passage, infections of chickens and after imposition of non-selective bottlenecks.

A recent development in Dr. Bayliss laboratory is the setting up of cyclical assay for exploring alternating selection on a phase-variable locus. Gene, cj1421, is a phase-variable gene involved in modifying the capsule. A phage F336 is used to select for OFF variants whilst incubation in human serum selects for ON variants. This assay can be used to show the importance of hypermutable sequences in survival of fluctuating environments.

Another recent development is the generation of a theoretical model of the impact of bottlenecks on the genetic diversity produced by multiple phase-variable loci. This model predicts that small bottlenecks will reduce survival of certain selective pressures.

The aim of this project is to combine cyclical selection with non-selective bottlenecks to explore how these twin pressures will impact on the adaptability and survival of C. jejuni populations. A combination of theoretical modelling and experimental investigation will be utilised to explore how the mutability of the polyG tracts facilitates survival of both stringent selection and non-selective bottlenecks.

**Techniques that will be undertaken during the project.**

**Molecular Microbiological Techniques**

Experimental skills will include multiplex PCR and GeneScan analyses, cloning and site-directed modification of DNA sequences, and growth of bacterial pathogens. Performance of selection assays using phage and antisera. This part is the Molecular Microbiology to be supervised by Dr. Bayliss.

**Mathematical Techniques**

Statistical analysis of data sets using the program, R. Use of MatLab and Python to develop models of bacterial behaviour using experimental data sets. Retiterative utilisation of in silico models to explore the parameter space and to inform biological model development. This part is the mathematical modelling to be supervised by Dr. Morozov.