**BBSRC MIBTP Studentship Project**

**September 2023**

|  |  |
| --- | --- |
| **First Supervisor** | Prof Chris Bayliss |
| **School/Department** | Genetics & Genome Biology |
| **Email** | [cdb12@leicester.ac.uk](mailto:cdb12@leicester.ac.uk) |

|  |  |
| --- | --- |
| **Second Supervisor** | Prof Russell Wallis |
| **School/Department** | Respiratory Sciences |
| **Email** | [rw73@leicester.ac.uk](mailto:rw73@leicester.ac.uk) |

|  |  |
| --- | --- |
| **Additional Supervisor** | N/A |

|  |  |
| --- | --- |
| **Project Title** | Genetic and structural analysis of pilin-associated adhesins of Neisseria |
| **Project Summary** | |
| Type IV pili are major adhesins of many bacterial organisms. Adhesion by these structures is usually mediated by adhesin proteins located at the tip of a pilus that can extend a significant distance from the bacterial surface. The PilC proteins are proposed to be the major adhesins associated with the Type IV pili of Neisseria species and each strain encodes two of these proteins, PilC1 and PilC2. Surprisingly, these large (~110-kD) proteins are subject to high frequency ON/OFF switching due to polyC tracts present within the reading frames. These proteins exhibit divergence in central portions of the genes both within and between strains indicating that the ligands for these proteins may be divergent between the two proteins and between strains. An alternate view is that amino acid differences are driven by immune escape.  The overall aim of this project is to associate structural differences in these proteins with functional differences. The project will combine comparative genomics, structural techniques and biological assays to test for associations between structure and function. Due to the large sizes of these proteins, it is proposed to utilise CRYO-EM to assess structural features and to look at interactions between the proteins and the Type IV pilus. The critical hypotheses are 1) that the PilC1 and PilC2 proteins have differing ligands due to differences in the ligand-binding pocket; 2) the PilC proteins exhibit antigenic variation in surface-exposed loops between strains; 3) phase variation enables switches in tissue tropism and immune escape.  These hypotheses will be tested through a series of objectives:-  Objective 1. Examination of genetic variation between strains. Comparative genomics will be utilised to compare the sequences of the pilC genes between Neisseria meningitidis isolates. As these genes are often poorly assembled, nanopore sequencing may be utilised on a sub-set of isolates to confirm sequence differences.  Objective 2. Structural studies of the PilC proteins. Full-length genes and sub-domains will be cloned into expression vectors. Proteins will be expressed and purified from E. coli expression strains using His-tag binding technologies. The structures of full-length proteins will be explored through CRYO-EM. Other structural techniques (e.g. NMR) would be utilised for sub-domains.  Objective 3. Examining the biological functions of PilC proteins. Three approaches to examination of biological functions would be pursued. Firstly, purified proteins would be tested for interactions with the purified PilE proteins, the major sub-unit of the pilus, and for ligand binding to glycan arrays or to immortalised eukaryotic cells. Secondly, antibodies would be generated against sub-domains of the proteins or peptides to generate protein-specific antisera. These reagents would complement an existing monoclonal antibody that binds to a common domain on both proteins. These antibodies would be utilised for detecting phase variation, blocking binding to ligands and for serum bactericidal assays. Thirdly, mutations would be generated in the proteins that are predicted to interfere with ligand-binding activities or display of antigenic loops. Mutations may be tested for changes in these activities using assays with purified proteins or following introduction into meningococcal cells.  Techniques that will be undertaken during the project:  A PhD student undertaking this project will develop skills in the areas of structural biology, bioinformatics, genetics/molecular biology and immunological/biological assays. The structural biology aspects will encompass protein expression, protein purification, CRYO-EM and NMR. The bioinformatic techniques will include analysis of whole genome sequences, identification of functional domains in protein database, use of alpha-fold to predict protein structure and nanopore sequencing to analysis poorly-assembled genomic regions. Genetics and molecular biology will include cloning and site-directed mutagenesis of genes and construction of bacterial mutant strains. Immunological and biological assays will cover generation of antisera, ELISA, Western blotting, immunoblots, serum bactericidal assays and adhesion to host cells.  BBSRC Strategic Research Priority: Understanding the Rules of Life – Microbiology, Structural Biology | |
| **References** | |
| Green LR, Al-Rubaiawi AA, Al-Maeni MARM, Harrison OB, Blades M, Oldfield NJ, Turner DPJ, Maiden MCJ, **Bayliss CD** (2020). Localized Hypermutation is the Major Driver of Meningococcal Genetic Variability during Persistent Asymptomatic Carriage. mBio;11(2):e03068-19. PMID: 32209693  Green LR, Dave N, Adewoye AB, Lucidarme J, Clark SA, Oldfield NJ, . . . **Bayliss CD** (2019). Potentiation of Phase Variation in Multiple Outer-Membrane Proteins During Spread of the Hyperinvasive *Neisseria meningitidis* Serogroup W ST-11 Lineage. J. Infect. Dis, 220, 1109-1117. doi:[10.1093/infdis/jiz275](http://doi.org/10.1093/infdis/jiz275)  Bidmos FA, Chan H, Praekelt U, Tauseef I, Ali YM, Kaczmarski EB, Feavers I & **Bayliss CD** (2015). Investigation into the Antigenic Properties and Contributions to Growth in Blood of the Meningococcal Haemoglobin Receptors, HpuAB and HmbR. *PLOS ONE*, *10*(7), 22 pages. doi:[10.1371/journal.pone.0133855](http://doi.org/10.1371/journal.pone.0133855) | |