

## **Full details of the proposed research project**

Understanding the interactions between members of the *Streptococcus anginosus* group and *Prevotella* sp. in the cystic fibrosis lung microbiome.

### **Host**

Dr Fiona Whelan, Anne McLaren Fellow, School of Life Sciences, Faculty of Medicine and Health Sciences.

### **Description of the proposed project**

Background: Cystic fibrosis (CF) affects approximately 12,000 people in the UK and Ireland. Pulmonary exacerbations (PEs) cause most of the morbidity and mortality in this patient population. Because PEs are usually successfully treated with antibiotics, we know that the bacteria that inhabit the lungs play an important role in these events. Previous work from myself and others has determined that the presence of the bacteria in the CF lung do not change before, during, and after PEs, suggesting that interactions between members of the microbiota may be more important in driving PEs than their presence alone.

Previous research suggests important interactions between the *Streptococcus anginosus* group (SAG) and *Prevotella* sp. (Prev) in the CF lung. For example, our group has found an SAG which prevents the growth of an *Prevotella melanogenica*. In this project, we hypothesize that SAG and Prev will interact in a strain-dependent manner. To test this, we will use in vitro competition spot assays to assess the interactions between a variety of SAG and Prev strains isolated from the CF lung microbiota using 2 selective agars – KVLB and McKay.

Specific aims:

1. Use 2 selective agars to culture SAG and Prev present in the microbial community of a fresh sputum sample collected by our collaborators.

The student will begin by culturing a sputum sample obtained from an individual with CF on two selective agars. The first agar, KVLB – Kanamycin, Vancomycin Laked Blood agar – selects for gram-negative anaerobic bacilli. When used to culture samples from the CF lung microbiota, approximately 80% of isolates grown are *Prevotella* sp. The second agar, McKay agar, was specifically designed to select for SAG organisms from the CF lung microbiome. After 3-5 days, the student and I will work together to isolate, record, count, and image each unique phenotype which has grown on these 2 selective agars (weeks 1-2).

2. Use an in vitro competition spot assay to test the interactions between SAG and Prev cultured in Aim 1.

Following isolate collection, the student will use a high-throughput competition spot assay to identify the interactions between SAG-Prev pairs. The competition assay involves setting up a 48-well plate of various SAG isolates and stamping them onto multiple plates of a general media in high-throughput; after 24 hours, each plate will be stamped with 48 colonies of a single Prev strain, pinned directly next to the SAG isolates. The resulting SAG-Prev strain pairs will be categorized as interacting synergistically if one or both colonies have a growth circumference larger than single-strain controls, antagonistically if one or both colonies fail to grow or have a decreased growth circumference, or neutral if there is no effect on growth (compared to controls). (weeks 3-5).

The student and I will collect and summarize the experimental data. Images of each plate will be taken, and spot circumference measured using ImageJ software. We will use R to generate a heatmap of the spot circumference of each SAG-Prev pair. We will categorize isolates as interacting synergistically/antagonistically and decide on the 10 SAG-Prev pairs which best merit further investigation (week 6).

3. Follow up on a subset of interaction pairs using further microbial assays.

A subset of SAG-Prev pairs will be tested with in vitro assays including: (a) determining the importance of physical proximity by spotting on solid agar at various distances; (b) assessing the importance of secreted proteins and/or alterations to the environment via growth of each member of the isolate pair in the sterile spent culture medium of the other; (c) assessing the importance of the metabolic environment by competing on a variety of agar types. These follow up experiments will show whether the observations from the competition assay are generalizable across media types and conditions, and provide the first step in our understanding of the mechanism of interaction (weeks 7-8).

This project would suit a full time student or a student working on a part-time basis over a longer period.

### **What overall scientific training will the student receive during the project?**

This project will benefit the student in several ways. First, the student will learn a variety of microbiological techniques and assays not often included in Undergraduate education. Additionally, because of the high-throughput nature of the project, the student will learn vital skills in data management, organization, and summary which will be vital skills for their future research career goals. The student will also have a chance to use analyses software such as ImageJ and the R programming language; programming and bioinformatics can often seem inapproachable and a hard skill to begin to learn independently. I hope that this introduction will help break down these barriers and allow the student to feel more comfortable exploring other computational data analysis approaches in the future.

### **A personal statement from Fiona**

"I have personally found academic research to be a rewarding and exciting career path, and I am excited to share that passion with a BVS student over the summer. I have been involved in research surrounding the cystic fibrosis lung microbiota, including culture-enrichment and isolation of particular members of the community, for almost a decade. We are still learning new things and gaining improved perspectives of this community with each new project and research direction. This particular research project fits into our larger research programme which includes understanding the bacterial interactions of this community at both the microbe and genetic or gene level, combining classic microbiology with high-throughput sequencing and bioinformatic analyses. The results of this project will help us better understand the microbial community dynamics of the cystic fibrosis lung, and how this contributes to the onset of pulmonary exacerbations in cystic fibrosis."