Research Grant – Successful Submission 2017

Robert Ferguson – Post Doctoral Researcher – Awarded £3000

Using metagenomics for fine-scale resolution of pathogen functional and phylogenetic diversity in farm bioaerosols.

1. Aim of the Project

State of the art metagenomics will be used to give unequalled detail on the pathogens in farm bioaerosols. This data will be used to identify overlooked human health risks (pathogens/antimicrobial resistance) as targets for rapid detection.

Farms (e.g. poultry) have high concentrations of airborne microbes leading to serious health hazards for employees. Here, DNA-based tools will be used to rapidly identify and quantify pathogens at farms so they can be rapidly detected and controlled.

Bioaerosols regulation is based on a very narrow spectrum of pathogens, a broad assessment farm bioaerosols is needed to characterize the health risks. Previous work has focused on amplicon sequencing to characterize bioaerosols. Although providing genus level identification, this falls well short of the standard required for robust pathogen identification and provides no insights into community function.

As part NERC (NE/M010813/1) and EU (HEXACOMM) bioaerosols projects we have developed rapid, sensitive methods for high-throughput bioaerosol sampling. We have also investigated the size fractions of bioaerosols with an Aerosol Society undergraduate bursary.

To build upon our previous work we will reconstruct bacterial genomes with metagenomics; providing the required species/strain pathogen identification. The genomes will be examined for specific genes, e.g. antimicrobial resistance genes (AMR). We will then develop rapid in-field detection methods for the targets identified.

2. Programme of Work

We will use metagenomics to identify genes relevant to health to target with rapid in-field qPCR assays for on-site detection.

See appendix one for scematic of work and how it compliments our current work.

1- Perform metagenomics to measure the occurrence of antibiotic resistance genes, and quantify gene expression changes.
2- Identify and quantify abundance of a broad range of airborne pathogens so we can predict where they are likely to occur and controlled.
3- Develop rapid qPCR assays for rapid monitoring of these targets.

Sampling: Triplicate bioaerosol samples will be collected from 3 farm sites, (targeting livestock, poultry and composting) using fixed samplers. Meteorological conditions and activity onsite will be monitored to determine which time of the day gives the worst impact (with regard to human health risk). DNA and RNA will be extracted from filters and liquid capture using our established protocols.

Metagenomics: Shotgun metagenomic sequencing will be performed to comprehensively sample all genes in the bioaerosol samples. We will use Meta-G-Nome DNA isolation kit (illumina) for the isolation of fosmid cloning ready metagenomic DNA. Library preparation will be performed using Tru-Seq DNA PCR-free sample preparation for whole genome sequencing and sequenced using the illumina HiSeq at TGAC.

Bioinformatics: We will use “Metagenomics Rapid Annotation using Subsystem Technology” (MG-RAST) to assign our reads to metabolic and phylogenetic databases. We will then generate and phylogenetic and Functional profiles of the bioaerosol community.

The functional profile will reveal information on the genetic capacity of the bioaerosol community. The phylogenetic profiles will reveal phylogenetic assignments missed by the gene marker approach and also allow deeper (strain level) assignments. This will identify key pathogens and genes that are present in bioaerosols that have previously been overlooked for monitoring and regulation. Additionally AMR is the most significant health crisis facing humanity. This data will enable us to determine if farms are acting as a reservoir of AMR genes.

Q-PCR assays: Q-PCR will be performed to target a range of bioaerosol pathogens (including Aspergillus spp., Staphylococcus spp, Mycobacterium spp., Legionella spp.) using a suite of published rRNA species-specific primers. qPCR will be applied in field using the Optigene Genie II system which can provide results in under an hour. As part of our NERC bioaerosols project (NE/M010813/1) we are developing rapid qPCR tools for identification of bioaerosols in the field. These are based on identifying pathogens that are already well known to be present in bioaerosols. For each site, we will map pathogen abundance and predict where pathogens are likely to occur and controlled. We can feed the data from the metagenomics into this to provide new overlooked targets for detection. This includes functional genes that may be indicative of pathogenicity.

3. Potential Applications

Metagenomic data will provide a database of bacterial bioaerosol phylogenetic and functional diversity at farms. The qPCR data will provide a spatiotemporally explicit quantification of pathogenic bioaerosol bacterial distributions.

Rapid in-field qPCR methods will be developed, providing a biotoolkit for the farm industry on where, when, and what to sample, for the detection of pathogens. This will provide a much more robust and accurate method for detecting human health risks than the ones currently used.

The project will involve collaborations with Cranfield University, the Environment Agency, and DSTL.

We are collaborating with Cranfield University by combining DNA and chemical marker based bioaerosols analysis. We will be able to combine both data sets to look for links between genes and chemical markers such as VOCs that can be easily monitored (Garcia-Alcega et al. 2017).

We are also working with the EA and DSTL on the development of qPCR assays for specific pathogens and the application of this for rapid in field detection.

There is a need to develop methods to detect bioaerosols rapidly. To do this we need to know what to look for. This work will enable a new level of detail in academic understanding of microbial bioaerosols. In addition to this it will lead to the development of rapid on-site detection of bioaerosols with qPCR enabling more effective real-time monitoring and increase confidence in detection.

The duration of this project will be 12 months.