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The effect of corticosteroids on quorum sensing in pseudomonas.
Fundació Institut d'Investigació Sanitària Illes Balears, Palma de Mallorca, Spain

Rationale for the travel:

I am a clinical doctor enrolled in the national specialised foundation programme (research stream): a programme that allows doctors in post medical school training dedicated time for research. With an interest in infectious disease and microbiology I was excited to have found scientists willing to provide me with the opportunity to undertake a project in this discipline. My limited prior laboratory training necessitated first hand intense training to realise the potential of this opportunity. The identified project builds on prior research from Dr Jordana-Lluch at the Fundació Institut d'Investigació Sanitària Illes Balears, Spain and the plans benefit from the expertise in molecular microbiology within the laboratories within the Biodiscovery Institute at the University of Nottingham. The ideal training was identified by myself and the supervisory team to incorporate training in the required experimental techniques and analysis under the guidance of Dr Jordana-Lluch followed by undertaking the data acquisition independently back in Nottingham.

Scientific background to the project:

Chronic Obstructive Pulmonary Disease is a chronic, progressive respiratory disease characterised by progressive airflow limitation and tissue damage due to chronic inflammation. Inhaled corticosteroids are widely used to prevent abnormal inflammatory responses (exacerbations) associated with higher morbidity in chronic obstructive pulmonary disease (COPD). The conditions in the damaged lungs promote chronic bacterial colonisation, including by *Pseudomonas aeruginosa.* Colonisation by potentially pathogenic bacteria in turn accelerates lung function decline, most probably due to more frequent exacerbations.

Dr Jordana-Lluch previously demonstrated that corticosteroids used to treat these very exacerbations promote biofilm formation in *Pseudomonas aeruginosa*. Co-ordinated biofilm communities are associated with chronic infection, potentially explaining the high incidence of *Pseudomonas aeruginosa* in COPD patients. This raises a dichotomy whereby *Pseudomonas aeruginosa* colonisation both drives more frequent exacerbations and is promoted by the very treatment for these exacerbations. Biofilm formation in *Pseudomonas aeruginosa* is regulated by three quorum sensing (QS) pathways (*las*, *rhl*, *pqs*). Contrary to what was observed for the parent strain, corticosteroid treatment of mutants unable to synthesize QS signaling molecules do not increase biofilm biomass, leading to the hypothesis that quorum sensing signaling molecules are implicated in mediating this phenotype.

Project aim: to investigate the effects of corticosteroids on quorum sensing in *Pseudomonas aeruginosa*.

Outcome of research:

Receiving a travel grant from the Royal Society of Biology enabled me to travel to Palma de Mallorca to learn and optimise a protocol to investigate the effects of corticosteroids on quorum sensing in *Pseudomonas aeruginosa* under the guidance of Dr Jordana-Lluch at the Fundació Institut d'Investigació Sanitària Illes Balears. This would prepare for subsequent research using the expertise and bacterial strain collection at the University of Nottingham where the aim is to chemically complement single quorum sensing signaling molecule deficient mutants with exogenous quorum sensing molecules to determine which quorum sensing molecules are required for the responsiveness to corticosteroids and at what concentrations.

The first day was spent optimising the protocol, and we began going through a first run through of the experiment by setting up the cultures of bacteria which were incubated overnight to grow. In this case we were using the non-mutated parent strain and a *pqs* knockout strain, each with solvent control, budesonide and fluticasone. Each strain had been modified to produce a bioluminescent reporter

such that we could quantify the amount of quorum sensing gene expression over time by way of luminescence. I quickly learnt many key techniques I'm sure laboratory scientists take for granted from how to use a pipette to how to determine the volume of solvent needed to dissolve my steroids in for a given concentration, and how to perform a serial dilution of the quorum sensing molecules.

The next morning it was time to set up the final experimental conditions. Each strain was treated with either solvent alone, budesonide and fluticasone and then the knockout strain with each steroid or solvent would then also be grown with 0, 0.5, 1 or 10 micromolar of exogenous PQS quorum sensing molecules. Dr Jordana-Lluch talked me through how to calculate the concentrations of steroids and PQS molecules I would need to add for each condition such that not only were we reaching the final concentration we wanted, but that the total solvent added in each condition from the PQS and steroid was identical for each. I soon had my calculations completed and my incubation plate mapped out. I set up all the necessary combinations of bacteria, steroids and quorum sensing molecules before setting up my experimental plate. What seems so simple when condensed into a paragraph really was a steep learning curve but by evening the plate was ready to go into the specialized equipment (TECAN) for overnight growth measurement alongside monitoring of the bioluminescent reporter.

An anxious overnight wait was rewarded with a huge spreadsheet of data, which I was trained how to analyse. Much to my delight when we created the graphs, we showed that the initial results fit what we expected: no quorum sensing occurring in the mutant with no exogenous molecules with quorum sensing being restored with exogenous molecules proportional to the amount reintroduced. We spent the next few days fine tuning the experiment and running a few repeats before it was time for my return to the UK.

I am continuing the work back in the Biodiscovery Institute at the University of Nottingham, carrying out repeats with different concentrations of quorum sensing molecules to determine the exact concentration at which levels are restored to wild type levels. I will also carry out the experiment chemically complementing the knockouts of the other two quorum sensing pathways. This trip to Mallorca to work with Dr Jordana-Lluch was invaluable to teach me the skills I need to perform this work, and I am very grateful to the Royal Society of Biology, and everyone at the University of Nottingham and Fundació Institut d'Investigació Sanitària Illes Balears who made it possible.