Rotation Projects: Infection/Immunity Stream

Dr John Morrissey

Dissecting signaliing interactions between bacterial and fungal pathogens

The fungus *Candida albicans* and bacterium *Pseudomonas aeruginosa* are opportunistic pathogens that infect similar vulnerable patients and locations. These include the cystic fibrosis lung, burn wounds, catheters, and medical implants. Both organisms use signalling systems to sense their environment, communicate within their population, and co-ordinate virulence. Recently, it has emerged that interactions via secreted signals between these pathogens can also take place. Our work has shown that there is bidirectional signalling that affects important phenotypes such as production of virulence factors and biofilm formation. This project will continue our work dissecting the molecular mechanism of these interactions. This 6 month project will focus on pursuing a number of very interesting leads and preliminary data that have been generated. The range of techniques that will be brought to bear include molecular biology, RTqPCR, cell culture and confocal microscopy.

There will be a number of specific objectives that will guide the project.

1. We will address the possible effects of bacterially-derived alkyl quinolones on modulating gene expression and impairing biofilm formation in *C. albicans*.
2. We will assess biofilm formation in particular *C. albicans* mutants that have emerged as candidates for involvement in the interaction. These include *efg1* and t*up1*.
3. We will assess the survival of *efg1* and other mutants in a new virulence/survival amoeba model that we have developed.

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David Clarke

Growth dynamics of adherent-invasive Escherichia coli (AIEC) within the macrophage.

Adherent-invasive *Escherichia coli* (AIEC) are a group of bacteria that have been associated with Crohns' disease, a debilitating and chronic inflammatory bowel disease that is on the increase in the Western world. AIEC are distinguished from all other strains of *E. coli* by their ability to persist and replicate within macrophages. Bacterial replication within the macrophage is associated with the production of high levels of pro-inflammatory cytokines such as TNF-a and IL-1b and this would be expected to contribute to the inflammation associated with Crohns' disease. When studying bacterial replication and cytokine production data is normally generated from large populations of macrophages and what is reported is therefore an average of this population. Unfortunately this doesn't take into account the heterogeneity that will occur in this interaction at the single cell level e.g. not all macrophage will be infected by the bacteria and not all infected macrophage will contain replicating bacteria. This heterogeneity is particularly important in chronic infections (like Crohns' disease) where slow or non-growing bacteria may have important clinical consequences. In this study we will be characterising the growth dynamics of AIEC (and certain mutants) in individual macrophages using a reporter plasmid system that was originally developed to monitor *Salmonella* intracellular replication dynamics (Helaine et al., 2010). We will also use this system to characterise the affect of certain drugs that we have already shown to affect the intracellular replication of AIEC e.g. bafilomycin A (inhibits acidification of the phagosome), BAY-11 (inhibits activity of the transcription factor NF-kB) and caspase-1 inhibitors (that prevent IL-1b production and activation of the inflammasome). Finally this system will be used to screen for bacterial mutant that are unable to replicate normally in the macrophage. Such a screen will identify novel virulence factors associated with AIEC and these virulence factors may be useful as potential targets for the treatment of Crohns' disease.

References

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Professor Alan Dobson

Metagenomic approaches to identify novel anti-microbial compounds from unique deep sea marine ecosystems.

Marine invertebrates such as sponges, produce a large range of potent biologically active metabolites, with many currently in clinical trials. Products which are currently in use include the anti-cancer agent ET-743 (Yondelis), which is used to treat ovarian neoplasms and sarcoma. As these marine invertebrates host a large and exteremely diverse microbial community, many of these bioactive metabolites are believed to be microbially derived. The vast majority of these bacteria are however not amenable to culture. To overcome this our group has developed a functional metagenomics based screen to exploit the sponge microbiota. This involves a novel vector system which allows trans-species conjugation and copy number control, with libraries constructed in an *E. coli* fosmid being conjugated into both *Pseudomonas putida* and *Streptomyces lividans* hosts for screening in all three host systems. Using this system we have previously identified anti-bacterial activity from the soil metagenome.

This project involves the construction of a metagenomic library from bacteria isolated from deep sea sponges collected from around 2,700 metres below the ocean surface, in Irish terretorial waters. Following construction this library will be screened for antibiotic actiovities against a range of clinically relevant pathogens such as *Salmonella enterica* serotype Typhimurium, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridium difficile*. Metagenomic clones displaying activities will be further analysed to determine the genetics, biochemistry and regulation of antibiotic biosynthesis. In addition PCR based approaches will also be employed to screen this library for genes encoding polyketide synthase and non riobosomal peptide synthase activites- both of which are known to produce bioactive secondary metabolites.

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Fergal O'Gara

Characterization of proteins that modulate Pseudomonas-infected cystic fibrosis lungs

Pseudomonas aeruginosa is a ubiquitous environmental bacterium that is among the top three opportunistic human pathogens. Furthermore, in spite of intensive antibiotic therapy and disease management, colonization of the abnormal airway epithelial of cystic fibrosis (CF) patients by *P. aeruginosa* is the predominant cause of morbidity and mortality in these patients. Once established, eradication of this pathogen from the lungs is almost impossible due to its natural resistance to antibiotics and its biofilm-like mode of growth. Biofilms are dynamic, three-dimensional communities of bacteria (microcolonies) living in an extracellular polysaccharide matrix that is relatively impenetrable to antibiotics. Therefore, there is a necessity to identify novel mechanisms of antimicrobial action. Proteins involved in biofilm formation could serve as novel treatment targets and provide novel treatment strategies for Pseudomonas infections.

In order to identify novel proteins, a Mariner-Transposon library in *P. aeruginosa* strain PA14 has recently been screened in the BIOMERIT Research Centre for the loss of tight microcolony formation in artificial sputum medium (ASM). This medium was previously developed to mimic the *in vivo* conditions in the chronic infected CF lung (1). Microcolony formation was inhibited in 30 out of the 5200 mutants tested. Three mutants contained a transposon insertion in a putative transcriptional regulator of which two of those regulators, belonged to the family of LysR-type transcriptional regulators. PA2432, is a bistable expression regulator (BexR) involved in the expression virulence factors including downregulation of the multidrug efflux pump MexEF-oprN (2). Previously, we and others have shown that the MexEF-oprN efflux pump is associated with the inhibition of biofilm formation, so it is likely that PA2432 regulates biofilm formation through MexEF-oprN (3,4). The second LysR-type transcriptional regulator is PA2877 and this regulator is currently being characterised within the BIOMERIT Research centre. The third transcriptional regulator which is the focus of this project is PA3771, a putative transcriptional regulator with a luxR family signature. This signature is very interesting since it implicates that this putative transcriptional regulator is involved in one of the key pathogenic mechanisms, the quorum sensing circuitry.

Objectives: The primarily objectives of this project are (1) the characterisation of the regulatory pathway of PA3771, and (2) to study its role in biofilm formation.

Methods of Research: The main technologies / techniques to be used in this project include; Molecular biology techniques, such as PCR, cloning, site-directed mutagenisis, reporter fusions, Southern/Northern-blot analysis Proteomic techniques, such as SDS-PAGE, 2-D gel electrophoresis, Western-blot analysis, MALDI-TOF Mass Spectrometry, and phenotypic assays will involve, attachment, biofilm, eukaryotic cell culture, cytotoxicity and as a virulence model, zebrafish / mice will be used.

Michael B Prentice

Bioengineering of bacterial microcompartments

Traditional descriptions of the interior of prokaryotic cells imply bacteria are small bags containing freely diffusing enzymes and substrates. In fact, genome sequences show at least 20% of bacteria can compartmentalize their cytoplasm for specific enzyme-catalysed metabolic activities within selectively-permeable thin-shelled protein microcompartments. The genes encoding this machinery show frequent horizontal transfer suggesting evolutionary advantages of enzyme compartmentalisation which have not been quantified. Many commensal gut bacteria and enteric pathogens express microcompartments, suggesting the mammalian gut is an environment where microcompartment-mediated metabolism is important.

The supervisor has shown that expression of this ultrastructure, and the metabolic function it enables, can be transferred between bacteria by standard genetic techniques [1]. As a self-contained system with defined components, inputs and outputs, the native bacterial microcompartment is an interesting systems biology paradigm. However, in a major advance, we have assembled empty compartments devoid of enzymes [2], and now targeted novel enzymes to the interior. We can thereby reassemble bacterial internal structure to metabolise novel substrates, or native substrates in novel ways (synthetic biology).

Possible projects include compartmentalising a enzyme to create a novel therapeutically or biotechnologically useful microcompartment by gene cloning in *E.coli* or *Lactococcus*, or phenotypic characterisation of a pre-existing construct by electron microscopy, phenotypic microarray, HPLC and mass spectrometry.

References

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Colin Hill

A role for bacteriocins in microbial infection

The commensal bacteria of the intestinal tract and food environments play an important role in host health, including playing a significant part in determining the susceptibility of the host to infection with intestinal pathogens. However, with few exceptions, the mechanisms by which commensal bacteria prevent or alleviate infection are not clear. One mechanism which has recently been identified is the production of bacteriocins by commensal bacteria. We will use well characterised model systems, for example infection of cell lines and/or murine models with *Listeria monocyctogenes* and other pathogens, to explore the ability of bacteriocins to act as therapeutic agents in limiting infection. We will use molecular microbiology, immunology and genetics tools to investigate bacteriocins as alternate therapies.