“T6SS-PSEUDO-EFFECTOR”

Publishable summary (wide audience)

Pseudomonas aeruginosa is a human pathogen causing life-threatening infections. It has multiple virulence mechanisms but secretion systems have a key role and function as nanomachines to transporting effectors, toxins and other virulence factors from the bacteria into the environment or target host cells. In recent years, a new secretion system, the Type VI secretion system (T6SS), was shown to be important for *P. aeruginosa* to mediate disease. The T6SS can inject toxins or effector proteins into host cells or other bacteria to either kill them or subvert their function. *P. aeruginosa* has three T6SSs encoded in its DNA. One of these systems, the H1-T6SS, is important for *P. aeruginosa* to compete with other bacteria and kill or block their growth. To do this the H1-T6SS is involved in the secretion of at least seven bacterial effectors, Tse1-7, whereas few effectors have identified for either the H2- and H3-T6SS. We believe that effector proteins (or toxins) secreted by these two systems mediate other important aspects of *P. aeruginosa* virulence and their identification and characterisation are crucial to understanding the H2- and H3-T6SS function in *P. aeruginosa* pathogenesis.

Project objectives

This project will identify these toxins, using three approaches:

1) To ‘switch on the systems’ proteins that control the production of the H2- and H3- will be identified. To achieve this, a gene that encodes an enzyme will be fused to the start site of the H2- and H3-T6SS. Next, when the type six genes are ‘switched on’ the enzyme produced will leads to a coloured product being produced which can be in turn measured.
    a. Random Transposon (small parasitic DNA elements) mutagenesis will be used to disrupt genes in the genome as the transposons insert in the bacteria’s DNA. If an altered level of the coloured product is subsequently detected the disrupted gene is a regulator of the T6SS.
    b. Mapping of the location of these Transposons will identify the regulators.

2) The expression of the T6SSs will be assessed in biofilm conditions. Biofilms are a living community of bacteria attached to a surface. In addition the flowcell assay will be setup at Imperial College. This assay enables bacterial biofilms to be monitored as they develop whilst media is washed over them via fluorescent tagging and confocal microscopy.

3) Aim 1 and 2 will produce conditions or mutants which have increased T6 activity. T6SS toxins will be identified via western blot analysis and mass spectroscopy.

Project Results Achieved

Aim 1: PA14 strains carrying the chromosomally-encoded enzyme fusion were constructed for the H2- and H3-T6SS. Transposon mutagenesis was performed successfully and a panel of putative regulators identified. A subset of selected regulators affected both the H2-T6SS and the H3-T6SS promoters. The regulator mutants were validated via the construction of clean deletion mutants.

Aim 2: The flowcell system was setup at Imperial College and a number of experiments performed. To date we have not observed an increase in T6SS expression under these conditions.

Aim 3: The results of Aim 1 worked exceedingly well and enabled further characterisation of the H2-T6SS. Additional clean mutants were made in *Pseudomonas* strains. We have observed increased secretion of Hcp2 which is the hall mark of an active H2-T6SS in these mutants. This is a key breakthrough as it is allowing us to characterise this system. Furthermore we have identified that several of the known H2-T6SS components are unregulated along with one of the known effector proteins.

Expected final results and their potential impact and use

We now have a handle on several regulators controlling the H2-T6SS and H3-T6SS. This will enable a new avenue of research to be undertaken. We have already identified that known components of the H2-T6SS are ‘switched on’ or ‘switched off’ by altering the levels of these regulators. This control is a powerful tool that will enable the characterisation of the effector proteins (or toxins) secreted or injected into target cells by these systems. Knowing what effectors are delivered will enable specific characterisation of these to discover their potential roles in infection and interbacterial competition.

Two publications have stemmed from this work and additional high impact paper is currently in the final stages of being re-drafted for submission. An additional manuscript is in preparation. This work has been presented at four international meetings to disseminate the results.

Potential Impact

As the three T6SS of the human pathogen *P. aeruginosa* have been implicated in virulence of humans, mice, rats and plant models this is an exciting area of research that will have many fruitful discoveries. In addition these systems appear to be involved in interbacterial competition. This is particularly timely given the threat of antibiotic resistant microbes. If these systems can be correctly harnessed, these bacteria and their T6SS could be deployed as nano-soldiers in microbial warfare enabling the treatment of resistant infections.