**2. Publishable Summary (max 2p)**

*Pseudomonas aeruginosa* is a versatile bacterium that can inhabit many environments. In its natural environments, Pseudomonas predominantly grows in organized communities called biofilms. Growth as a biofilm is also an important requirement for the colonization of human tissues. For instance, *P. aeruginosa* grows as a biofilm in the lungs of cystic fibrosis (CF) patients. The pathogenesis of Pseudomonas infections in humans is multi-factorial and complex. *P. aeruginosa* infections are also complicated due to the high intrinsic resistance of this organism, which makes it difficult to control with antibiotics and disinfectants. This intrinsic multidrug resistance is caused by synergy between a low-permeability outer membrane and expression of active efflux systems, including the resistance-nodulation family-division (RND) family of efflux systems. Multidrug resistant clinical isolates of *P. aeruginosa* have been found to hyperexpress efflux systems such as, MexEF-oprN (*nfxC* mutants). These efflux pumps are not only involved in increased antibiotic resistance, they have a more fundamental role in the colonization and persistence of bacteria in the host.
Spontaneous nfxC-type phenotypic mutants of *Pseudomonas aeruginosa* are characterized by chloramphenicol resistance and increased expression of the multidrug efflux pump, MexEF-OprN. These mutants frequently arise during the propagation of bacteria in laboratory conditions. We showed that some of the discrepancies in transcriptomic/phenotypic proﬁles of two mutantsin a small RNA-binding protein RsmA, PAZH13 (*rsmA* mutant in strain PAO1) and PAK delta *rsmA*, are due to the *nfxC*-type nature of PAZH13. This indicates that awareness of the possible occurrence of these spontaneous *nfxC*-type phenotypes is necessary. Screening of *mexE* levels could provide clarity when an *nfxC*-type phenotype is suspected. This work has been published in Environmental Microbiology reports (Mooij et al. 2010).

***Task 1. Determine the role of MexT-regulated genes in biofilm formation.***

The LysR-type transcriptional regulator MexT is a global virulence regulator in *Pseudomonas aeruginosa*. As such it is involved in the regulation of several virulence processes including biofilm formation, type three secretion system and antibiotic resistance through upregulation of the MexEF-oprN efflux pump. To establish the role of MexT in biofilm formation, preliminary flow-cell biofilm experiments had been performed. Analysis of the biofilm structure showed that biofilms of cells overexpressing MexT contain a higher microcolony-density with smaller average size as compared to control cells. These differences were independent of the MexEF-oprN efflux system. Therefore we sought to dissect how MexT modulates biofilm formation. In order to identify if any of the MexT-regulated genes were involved in this process, deletion and overexpression constructs were generated. However, none of these seem to play a role in the MexT-mediated effect on biofilm formation. As we identified that one of the *mexT*-regulated genes, PA4353 was also modulated in a related high-throughput study which aimed to identify genes involved in biofilm formation using artificial sputum media, we continued to characterise this ArsR-type transcriptional regulator.
Flow cell biofilm experiments showed that the architecture of 3-day-old biofilms of the PA4354 (PyeR) mutant differed significantly from that of PAO1 wild-type (Fig. 1). The mushroom-shaped microcolony structures were larger and more abundant in the mutant compared with the wild-type biofilm (Fig. 1). Further characterization of this regulator showed that it has negative autoregulatory properties and binds to a palindromic motif conserved among PyeR orthologues. These characteristics are in line with classical ArsR-family regulators, as is the fact that PyeR is part of an operon structure (pyeR-pyeM-xenB). However, PyeR also exhibits some atypical features in comparison with classical members of the ArsR family, as it does not harbour metal-binding motifs and does not appear to be involved in metal perception or resistance. Hence, PyeR belongs to a subgroup of non-classical ArsR-family regulators and is the second ArsR regulator shown to be involved in biofilm formation. This work has been published in Microbiology (Mac Aogain *et al*. 2012)

**Figure 1:** Modulated 3-day-old biofilm architecture. Visual representation of biofilm stained with SYTO 9 using a confocal scanning microscope. (a, b) Side views of a *z*-stack analysis; (c, d) bright-field overview images. (a, c) PAO1 wild-type pME6032; (b, d) PAO1Δ*PA4354*pME6032. Images shown are representative of three independent biological replicates. Bars: (a, b) 20 µm; (c, d) 200 µm.

decreased expression from the *pyeR* promoter. Data shown are mean values of three biological experiments; error bars,SD.

***Task 2. Study the role of MexT on virulence phenotypes* in vivo *in zebrafish embryos.***

In order to study the role of MexT on virulence phenotypes *in vivo* two different infection models were utilized the wax moth and zebrafish embryo models of infection. Although overexpression of MexT did not alter the virulence phenotype in zebrafish embryos it did alter the lethality of wax moth in an infection assay. The LD50 was approximately 100 fold greater in the PAO1 strain overexpressing MexT (pMEmexT) than the vector control (pME6032), indicating that MexT significantly reduces the *P. aeruginosa*-mediated virulence effects in the wax moth. This effect was partially dependent on the MexEF-oprN multidrug efflux pump as overexpression of MexT in a *mexE* deletion mutant increased the LD50 about 10 fold.

**Socio-economic impact of the research**

This research further established MexT as global virulence regulator and deciphered the role of its target genes in virulence. This is particularly important as in spite of intensive antibiotic treatment and disease management, colonization of the abnormal airway epithelia of cystic fibrosis (CF) patients by *P. aeruginosa* is the predominant cause of morbidity and mortality in these patients. While CF affects ~ 70,000 people worldwide it is most significant in Ireland, which has the world’s highest incidence of CF, (1/19 people). Approximately 80% of the adult CF population is chronically infected with this pathogen. Here we show that by eliminating MexT from the pathogen it significantly reduces its virulence and therefore could provide a target for drugs therapy.

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