

## PROGRAMME 4: NOVEL ANTIMICROBIAL AGENTS

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### Summary of research

Improved treatment for *Burkholderia* infections; The search for an Achilles heel.

*Burkholderia* are not only potentially transmissible between individuals with CF, but due to innate resistance, such infections are also notoriously difficult to treat. Indeed, there are only four cases reported describing the successful treatment of “cepacia syndrome”; all four cases required long-term treatment with multiple antibiotic combinations (Grimwood et al. J Cyst Fibrosis 2009; 8: 291-3). In project 4, we explored the link between the extremely high resistance of *Burkholderia* to cationic antimicrobial peptides (cAMPs) such as human defensins, colistin and polymyxin (PMB). In particular it had been noted by others that *Burkholderia* have a uniquely unusual outer cell wall. This cell wall, called endotoxin (or lipopolysaccharide, LPS) is highly inflammatory to the human immune system and has been linked to the extremely damaging nature of *Burkholderia* infections in the CF lung; the body realises it is infected, raises an immune response to get rid of the infection but *Burkholderia* are resistant to the human response. The chemistry of *Burkholderia* outer wall endotoxin synthesis is unique and is what makes it resistant. However, we discovered that what makes it strong was also a weakness that may be able to target with new antibiotics.

### Major outcomes

Aminoarabinose in *Burkholderia* lipopolysaccharide – an Achilles’ heel? In collaboration with Prof. Miguel Valvano, an international authority on bacterial polysaccharides, we discovered that *B. cenocepacia* strain K56-2, representing the highly virulent and transmissible ET12 lineage, has an essential requirement for genes involved in amino sugar biosynthesis (specifically aminoarabinose, Ara4N). In two publications (Ortega et al, 2007; Loutet et al, 2009) we reported that turning off specific arabinose genes in K56-2 was lethal, suggesting a potential Achilles heel in the search for effective anti-*Burkholderia* therapy. Our 2007 publication was highlighted in Nature Reviews Microbiology “Sweet news for CF sufferers?”. The Ara4N biosynthetic targets we chose in 2005 turned out to be similar to those being studied by Prof. Valvano’s group. DJC presented our research at an International *Burkholderia* Working group meeting in Gent (May 2006) which facilitated a highly fruitful (and on-going) collaboration with Prof. Valvano.

Ara4N biosynthesis and attachment to LPS requires the action of at least 10 enzymes, the first of which is catalysed by UDP-glucuronic acid dehydrogenase (UGD). Genome analysis of *B. cenocepacia* J2315 revealed three UGD genes so Josefin Bartholdson characterised recombinant forms of the three UGD isoforms. Detailed enzymatic studies of both UGDs were carried out. This work complemented the work in Valvano’s group where UGD specific knock-out mutant strains of K56-2 were generated and each had interesting phenotypes (see below). The attachment of Ara4N to LPS is thought to be catalysed by a transferase (ArnT, encoded by *pmrK*). The J2315 *pmrK* gene has been cloned and expressed in *E. coli*. This gene conferred PMB resistance to an *E. coli* (*pmrK*) knock-out strain suggesting the J2315 gene

was active. Similarly, the J2315 *pmrK* gene could complement the lethal phenotype observed on conditional growth media (glucose) suggesting a functional enzyme in this strain. Meanwhile, Valvano and colleagues made a significant breakthrough by generating a K56-2 mutant which is sensitive to the antibiotic PMB. UGD mutant strains of K56-2 produced by Valvano's lab show different PMB sensitivity and only 1 particular (of the 3 possible) UGD gene could complement conditional knock-outs (Loutet et al., 2009). This suggests that Ara4N biosynthesis in *Burkholderia* is unusual and essential to the organism.

*Burkholderia* virulence: Exopolysaccharide production and its impact on mannitol therapy. The factors in *Burkholderia* which make it such a challenging bacterial pathogen remain unclear but undoubtedly involve a complex interplay between the bacterium and host. As evidence, clinical outcome in patients infected by the same strain of *B. cenocepacia* cannot be predicted and can vary asymptomatic survival to rapidly fatal pneumonia. During her PhD studies, Josefin showed that sugar alcohols present in onions, the natural plant host for *Burkholderia*, stimulated the production of bacterial exopolysaccharide (EPS), a key virulence factor in many bacterial pathogens, including mucoid *P. aeruginosa*. Most *Burkholderia* species, which were previously considered to be non-mucoid, produced copious amounts of EPS when onion tissue was provided as the sole nutrient. We narrowed the "onion factor" down to a number of known sugars including mannitol, currently used as an osmolyte to improve mucociliary clearance in individuals with cystic fibrosis. This work was published in Microbiology (Bartholdson et al., 2008) and has been the focus of a commentary article (Reid & Bell. Sugar sweet & deadly. Microbiology 2009) and a press release by the Society for General Microbiology. Surprisingly, we found that the highly transmissible and virulent ET-12 strains J2315 and K56-2 could not produce EPS under any conditions but other *Burkholderia* species did and that EPS production is strain specific. In association with Canadian studies (Zlosnik et al. J Clin Microbiol, 2008), these surprising results suggest that in contrast to the conversion to mucoidy characteristic of *P. aeruginosa* in the CF lung, serious infection with *Burkholderia* follows loss of exopolysaccharide biosynthesis. Loss of EPS production in the most virulent species, *B. cenocepacia* highlights the complexity of *Burkholderia* virulence, but in a positive sense, suggests that the exclusion of ALL Bcc-positive patients from mannitol therapy is unnecessary.

#### **Work carried out as part of the consortium project contributed to the following grant awards :**

To continue this work DJC has received funding from an Edinburgh-based philanthropic charity (The Derek Stewart Charitable Trust) and the School of Chemistry, University of Edinburgh. This award total ~£40k (50% from each contributor) has funded the PhD studies of Karin Bodewits (2007-2010). Here we are trying to understand the molecular basis of the essential requirement that *Burkholderia* have for Ara4N. This has allowed us to continue to collaborate with Prof. Valvano and begin a collaboration with Prof. Christian H. Raetz (Duke University, USA) a world leader in LPS chemistry and biology. Prof. Raetz has supplied an antibiotic targeted specifically at the LPS pathway which we shown to be active against various *Burkholderias* (e.g. *B. multivorans*). This exciting discovery has been submitted for publication to Antimicrobial Agents and Chemotherapy (Nov. 2009) and will be the focus of future studies and proposals.

Furthermore, the work in DJCs lab as part of a larger EPSRC-funded Platform grant (2005-10) on "Mammalian defensins" complements the studies of peptide resistance displayed by *Burkholderia* strains.

### **Publications arising from research**

Loutet SA, Bartholdson SJ, Govan JRW, Campopiano DJ & Valvano MA. (2009) Contributions of two UDP-glucose dehydrogenases to viability and polymyxin B resistance of *Burkholderia cenocepacia*. *Microbiology* 155(6), 2029-2039.

Bartholdson SJ, Brown AR, Mewburn BR, Clarke DJ, Fry SC, Campopiano DJ, & Govan JR (2008) Plant host and sugar alcohol induced exopolysaccharide biosynthesis in the *Burkholderia cepacia* complex. *Microbiology*. **154**, 2513-2521

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