

PROGRAMME 5: BREAKTHROUGH PROJECTS AND ISSUES OF DIRECT CLINICAL RELEVANCE

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Summary of Research

Organisms of the *Burkholderia cepacia* complex (Bcc) cause serious infections within the CF lung. As outlined below, we undertook a diverse set of studies which aimed to address numerous aspects of how these organisms adapt to the CF lung and establish infection, how those infections can be diagnosed, and how *Burkholderia* infections might be treated in the future with novel antimicrobial agents.

- **Rapid diagnostics for Bcc**

Diagnosis of Bcc infection can be complex and time-consuming. We optimized rapid diagnostics for Bcc using a technology called fluorescent *in situ* hybridization (FISH). This technique can be applied directly to sputum, resulting in the identification of Bcc within sputum within 2-3 hours (as opposed to 3-4 days by conventional methods).

- **Do Bcc have an Achilles' heel?**

Bcc infections are extremely difficult to treat as the bacteria are highly resistant to antibiotics. Consequently, there is considerable interest in identifying any weaknesses in the Bcc that might be exploited by novel antimicrobial agents. Working in collaboration with Professor Miguel Valvano of the University of Western Ontario, we identified one potential weakness – the Bcc require their outer surface to be modified with a particular sugar (called aminoarabinose). Without that sugar modification, the bacteria die. Thus, a novel antimicrobial agent that could prevent this sugar modification in Bcc would be expected to be an effective antibiotic.

Additionally, research focused on 'two-component systems' (TCSs) within Bcc. TCSs allow bacteria to respond to their local environment and switch on genes that allow them to adapt to that environment. In many bacteria, TCSs play an essential role in their ability to cause infection. Bcc contain a large number of TCSs but their significance is largely unknown. Building on preliminary studies conducted in the course of the Microbiology Consortium, a thorough investigation of Bcc TCSs is now being led by Dr Alan Brown (now based at the University of Exeter). Funded by the Medical Research Council, the project aims to investigate the role that these TCSs play in the ability of Bcc to establish infection, and to assess if inhibition of these TCSs weakens the Bcc.

- **The anti-oxidant response of Bcc**

During infection, bacteria are engulfed by cells of our immune system which try and kill the bacteria by producing toxic 'reactive oxygen species'. In order to survive, bacteria have numerous 'anti-oxidant' responses which neutralize the reactive oxygen species. We have characterized a novel anti-oxidant of Bcc, and investigated the role it plays in macrophage survival. These studies provide further insight into how Bcc survives within the CF lung environment.

- **Virulence factors and strain markers in *B. multivorans***

The dominant Bcc species within the UK CF population is now *B. multivorans*. However, compared to *B. cenocepacia*, relatively little is known about factors possessed by *B.*

multivorans that contribute to the infection process (virulence factors). Similarly, little is known about *B. multivorans* strain markers that can be used to identify and follow particular strains within the UK CF population. We undertook analysis of the genome of one particular strain of *B. multivorans* that appears to cause more serious infection than other strains. This has provided insight into the genes possessed by this strain that may contribute to infection, and may subsequently allow a diagnostic assay for the identification of this strain.

Publications arising from research:

Clarke DJ, Mackay CL, Campopiano DJ, Langridge-Smith P & Brown AR (2009). Interrogating the molecular details of the peroxiredoxin activity of *Escherichia coli* bacterioferritin comigratory protein using high resolution mass spectrometry. *Biochemistry* 48(12), 3904-3914.

Brown AR & Govan JR (2007) Assessment of fluorescent *in situ* hybridization and PCR-based methods for the rapid identification of *Burkholderia cepacia* complex organisms directly from sputum samples. *J. Clin. Microbiol.* **45**, 1920-1926.

Ortega XP, Cardona ST, Brown AR, Loutet SA, Flannagan RS, Campopiano DJ, Govan JR & Valvano MA. (2007) A putative gene cluster for aminoarabinose biosynthesis is essential for *Burkholderia cenocepacia* viability. *J. Bacteriol.* **189**, 3639-3644.

Govan JR, Brown AR & Jones AM. (2007) Evolving epidemiology of *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex in cystic fibrosis lung infection. *Future Microbiology* **2**, 153-164.

To access summaries of any of these articles, go to the 'Publications' page of the Microbiology Consortium website and click on 'Abstract' for the relevant article.