

PROGRAMME 2: Comparison of *Burkholderia cenocepacia* with *Burkholderia multivorans* and *Pseudomonas aeruginosa* in diminishing host defence responses in the cystic fibrosis respiratory tract



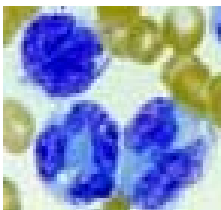
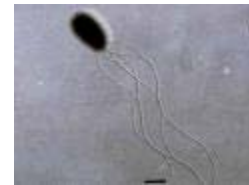
Queen's University, Belfast

Mr Gisli Einarsson (PhD student) & Dr Andrew McDowell (Principal Investigator; School of Medicine & Dentistry)
Prof. J. Stuart Elborn (School of Medicine & Dentistry)
Dr Lorraine Martin and Professor Brian Walker (School of Pharmacy)

Background

Although all nine species of the *B. cepacia* complex (Bcc) can cause CF-related pulmonary infections, epidemiological studies in different geographical regions have revealed that *B. cenocepacia* (formerly *B. cepacia* genomovar III) is the most prevalent organism recovered from CF sputum [Li Puma et al., 2001; McDowell et al., 2004]. In addition, over recent years, clinical data has emerged which shows that patients infected with *B. cenocepacia* have increased morbidity and mortality compared to individuals colonised with other Bcc organisms, such as *B. multivorans*, and patients with *Pseudomonas aeruginosa* [Mahenthalingam et al., 2001]. Currently, our knowledge of the exact mechanisms responsible for the often highly virulent nature of *B. cenocepacia* compared to other Bcc species is poorly understood.

Lipopolysaccharide (LPS) from *B. cenocepacia* induces much higher levels of the major pro-inflammatory cytokine, tumour necrosis factor- α (TNF α) from human monocytic cells compared to other Bcc species [DeSoyza et al., 2004]. This stimulation of TNF α is mediated via the CD14-Toll receptor 4 pathway, which activates the cytoplasmic transcription factor NF- κ B. Previous studies have found that the production of serine, cysteine and metallo-proteinases from human cells, including those from the respiratory tract, is stimulated by NF- κ B as well as TNF α [Marshall et al., 1992; Hozumi et al., 2001; Renesto & Chignard, 1991]. Piliated bacteria, such as the notorious ET-12 strain of *B. cenocepacia* and strains of *P. aeruginosa*, also have the capacity to stimulate NF- κ B production by binding to cellular asialoganglioside-GM1 receptors.



As a consequence, our hypothesis is that pulmonary infection with *B. cenocepacia* is likely to stimulate heightened proteinase production from neutrophils and macrophages recruited to the lung, and possibly local epithelial cells, resulting in greater lung destruction and respiratory failure. In addition, the presence of such proteinases would also lead to enhanced degradation of antimicrobial proteins and peptides, natural proteinase inhibitors and immunoglobulins, thus significantly weakening the host defence response. Other host response mechanisms known to be impaired in the CF lung as a direct result of proteolytic action include opsonisation and phagocytosis of bacteria, and apoptotic cell clearance. Although studies to date have focused primarily on the action of the serine proteinase neutrophil elastase (NE), other classes of proteinase stimulated by NF- κ B and TNF α , such as metalloproteinases (MMPs) and the cysteine proteinases cathepsin B, L and S, have been described in CF sputum and bronchoalveolar lavage. These enzymes all have the capacity to degrade structural proteins within the lung and hence could play pivotal roles in the destruction of lung tissue in CF.

Therefore, as a consequence of these previous studies, the main thrust of our project is:

(1) To measure and characterise the proteinases produced in an *in vitro* model of the host response to *B. cenocepacia*, and compare to similar experiments conducted with *B. multivorans* and *P. aeruginosa*. We are also examining the level of proteinase production by individual strains of *B. cenocepacia*, *B. multivorans* and *P. aeruginosa*.

(2) To examine the effect of proteinases released by the host response on antimicrobial proteins and peptides, as well as immunoglobulins. Such experiments will allow us to assess the potential of small molecule proteinase inhibitors for the treatment of CF patients colonised with *B. cenocepacia*.

Research Platform

The basis of our capacity to rapidly screen and characterise the proteinases produced by both mammalian and bacterial cells is a library of biotinylated, irreversible inhibitors to the serine, cysteine and metallo- classes of proteinases, which act as active site-directed affinity probes [see, for example, Walker & Elmore, 1984; Walker et al., 1993; Hamilton et al., 1993; Lynas & Walker, 1997; Lynas et al., 1998; Walker et al., 2000; Scott et al., 2002]. The combination of a biotin reporter group attached to an irreversible inhibitor enables not only visualisation of active proteinases on Western blots but can also facilitate selective retrieval of the captured enzyme by affinity purification utilising the biotin/streptavidin interaction. The isolated proteinase can subsequently be sequenced using standard proteomic approaches (Figure 1). In this way we hope to identify several novel bacterial proteinases as well as proteinases important in the host response. We also utilise spectrofluorimetric activity assays as well as SDS-PAGE, including gelatin zymography.

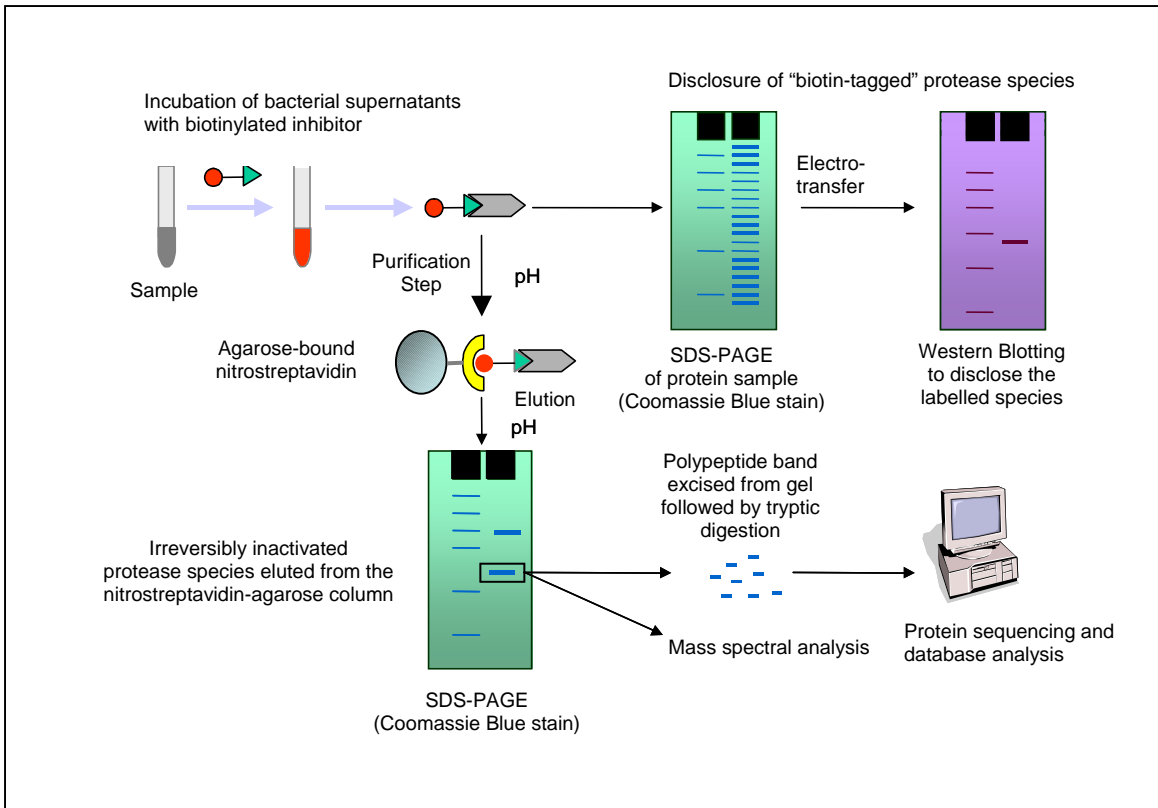


Figure 1: Profiling and Sequencing Strategy for Proteinases

References

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