

**International  
*Burkholderia cepacia*  
Working Group**

13<sup>th</sup> Annual Meeting  
April 23-26, 2009



Munk Centre for International Studies  
University of Toronto  
Toronto, Ontario, Canada

This Meeting has been made possible by the generous contributions of



# IBCWG 2009 Conference Program

**THURSDAY, APRIL 23, 2009**

**6:00-9:00** Welcome Reception –Toronto Marriott Bloor Yorkville Hotel  
Yorkville Room

**FRIDAY, APRIL 24, 2009**

**7:30-8:30** Registration and Continental Breakfast- Munk Centre Lobby

**8:30-8:45** Welcome to University of Toronto  
**Dr. W. Conrad Liles**  
Professor and Vice-Chair of Medicine  
Director, Division of Infectious Diseases  
University of Toronto/University Health Network  
Toronto General Hospital

**8:45-9:00** Introductory Remarks- Liz Tullis and Pam Sokol

**Keynote Presentations- Chair- Pam Sokol**

**9:00-9:45** *Burkholderia* epidemiology and outcomes of infection in CF  
John Lipuma, University of Michigan, Ann Arbor, MI

**9:45-10:30** Molecular pathogenesis and ecology of the *Burkholderia cepacia* complex:  
from genes to genomes  
Esh Mahenthiralingam, Cardiff University, Cardiff, Wales

**10:30-11:00** Break

**11:00- 12:15** Session I. Chair - Tom Coenye

**ADAPTATIONS TO SURVIVAL IN THE ENVIRONMENT, BIOFILMS AND CF INFECTIONS**

**I-a** Wastewater treatment lagoons are reservoirs of *Burkholderia cepacia* complex species  
Manuelle Neto, Arnault Graindorge, Evelyne Borges, Françoise Maurin, Laurence Villard, and **Benoit Cournoyer**

**I-b** Regulatory components and pathogenic features of the phenylacetic acid catabolic pathway of *Burkholderia cenocepacia* K56-2  
Robyn J. Law, Jason N. R. Hamlin, Kali Teichroeb, Tom Ward and **Silvia T. Cardona**

**I-c** Transcriptional responses in *Burkholderia cenocepacia* J2315 biofilms following exposure to chlorhexidine, H<sub>2</sub>O<sub>2</sub> and NaOCl  
**Elke Peeters**, Andrea Sass, Eshwar Mahenthiralingam, Hans J. Nelis & Tom Coenye

**I-d Aggregation properties of cepacian. A model for biofilm formation.**  
Bruno Cuzzi, Martin Frank, Paola Cescutti and **Roberto Rizzo**

**I-e Flagella-mediated motility is essential for *Burkholderia cenocepacia* biofilm formation.**  
**Katarzyna M. Stevens**, Dolina D. Kerr, Jen Amon, Susan Koval, and Douglas G. Storey

**12:15 Plans for IBCWG 2010 and beyond**

**12:30- 1:30 Lunch – Munk Centre Lobby**

**1:30-3:00 Session II. Chair - Vittorio Venturi**

#### **VIRULENCE MECHANISMS- QUORUM SENSING AND ADHERENCE**

**II-a A comparative transcriptomic, proteomic and phenotypical analysis of the *Burkholderia cenocepacia* H111 quorum sensing network**  
**Silja Inhülsen**, Kathrin Riedel and Leo Eberl

**II-b Transcriptome analysis of quorum sensing regulation by CepR and CciR in *Burkholderia cenocepacia*.**  
**Eoin P. O’Grady**, Duber F. Viteri, Rebecca J. Malott, Audrey A. Plourde and Pamela A. Sokol

**II-c Production of the antifungal compound pyrrolnitrin is quorum sensing-regulated in members of the *Burkholderia cepacia* complex**  
Silvia Schmidt , Judith F. Blom, Jakob Pernthaler, Gabriele Berg, Adam Baldwin, Eshwar Mahenthiralingam and **Leo Eberl**

**II-d Adhesion of *Burkholderia multivorans* and *Burkholderia cenocepacia* to lung epithelial cells and its inhibition.**  
Ciara Field-Corbett, Gillian Herbert, Minu M. Thomas, Trinidad Velasco-Torrijos, Stephen Carberry, Sean Doyle, Máire Callaghan and **Siobhán McClean**

**II-e Novel adherence factors of pathogenic *Burkholderia cenocepacia*: mechanisms of action and their role in virulence.**  
**Dalila Mil-Homens** and Arsénio M Fialho

**II-f *Burkholderia cenocepacia* *cblA* and *adhA* are required for inflammation *in vitro* and *in vivo***  
**J. B. Goldberg** and U. Sajjan

**3:00-3:30 Break**

**3:30-5:00      Session III. Chair - Jane Burns**

**ANTIBIOTIC RESISTANCE AND NOVEL THERAPEUTICS**

- III-a    Antibiotic resistance and Lipid A biosynthesis of the *Burkholderia cepacia* complex (Bcc)**  
K. Bodewits, H. S. Chung, C. R. H. Raetz, J. R. W. Govan, D.J. Campopiano
- III-b    An investigation of the basis of intrinsic aminoglycoside resistance in *Burkholderia vietnamiensis***  
Agatha N. Jassem, James E. A. Zlosnik, Robert K. Ernst, Robert E. W. Hancock, and David P. Speert
- III-c    Changes in gene expression of *B. cenocepacia* J2315 in response to different classes of antibiotics**  
Andrea M Sass, Angela Marchbank, Pavel Drevinek and Eshwar Mahenthiralingam
- III-d    Inactivation of the Essential Gene Acyl Carrier Protein in *Burkholderia cepacia* complex by Phosphorodiamidate Morpholino Oligomers**  
David E. Greenberg, Kimberly Marshall-Batty, Patrick L. Iversen, Steven M Holland, and Bruce L. Geller
- III-e    Efficacy of bacteriophage therapy in a model of *Burkholderia* pulmonary infection**  
Lisa A. Carmody, Jason J. Gill, Uma S. Sajjan, Carlos F. Gonzalez, Ryland F. Young, and John J. LiPuma
- III-f    Development of Phage Therapy for the *Burkholderia cepacia* complex**  
Karlene H. Lynch, Kimberley D. Seed, Amanda D. Goudie, and Jonathan J. Dennis

**7:00 - CONFERENCE DINNER- location TBA**

**SATURDAY, APRIL 25, 2009**

**8:00-9:00      Breakfast- Munk Centre Lobby**

**9:00-10:30     Session IV. Chair - Roberto Rizzo**

**PHASE VARIATION, COLONY MORPHOLOGY AND EXOPOLYSACCHARIDES**

- IV-a    Phase variation in *Burkholderia multivorans* clinical isolates**  
Inês N. Silva, Ana X. Ferreira, Jörg D. Becker, Christian G. Ramos, Dalila Mil-Homens, Jorge H. Leitão, Arsénio M. Fialho, David P. Speert, and Leonilde M. Moreira
- IV-b    Influence of ShvR on colony morphology and gene expression in *Burkholderia cenocepacia***  
David T. Nguyen, Paola Cescutti, Eoin P. O'Grady, Cora Kooi and Pamela A. Sokol

- IV-c Exopolysaccharide production in BCC and links to clinical outcome**  
**James E. A. Zlosnik**, Paulo C. Costa, Trevor J. Hird, Paul Mori, Deborah A Henry, David P. Speert
- IV-d Cepacian biosynthesis in the *Burkholderia* genus: mechanisms and role in virulence**  
**Ana S. Ferreira**, Inês N. Silva, Jörg D. Becker, Sílvia A. Sousa, Dalila Mil-Homens, Jorge H. Leitão, Arsénio M. Fialho, and Leonilde M. Moreira
- IV-e Determination of the biological repeating unit of cepacian translocated to the periplasmic space by a flippase coded by the *bceQ* gene**  
**Paola Cescutti**, Linda Furlanis, Cristina Lagatolla, Michela Foschiatti, Roberto Rizzo
- IV-f Towards identification of a phage depolymerase enzyme for the cepacian**  
**Sarah Routier** and Jonathan J. Dennis

**10:30-11:00 Break**

**11:00-12:30 Session V. Chair - Liz Tullis**

#### **EPIDEMIOLOGY AND CLINICAL OUTCOMES OF BCC INFECTIONS**

- V-a Molecular characterization and species distribution of *Burkholderia cepacia* complex (BCC) isolates in cystic fibrosis (CF) and non-CF patients in New Zealand.**  
**Christopher E. Pope**, Patricia Short and Philip E. Carter
- V-b Strain markers and virulence determinants in an outbreak strain of *Burkholderia multivorans***  
**Alan R Brown**
- V-c Epidemic of *Burkholderia cenocepacia* ET12 in Toronto adult cystic fibrosis clinic: lessons learned**  
Kieran McIntyre, Matthew Muller, Sylvia Ota, Anne Stephenson and **Elizabeth Tullis**
- V-d Expanding multilocus sequence typing for *Burkholderia* species**  
**Theodore Spilker**, Adam Baldwin, Amy Bumford, Chris Dowson, Eshwar Mahenthiralingam, and John J. LiPuma
- V-e Hypermutability of *Burkholderia cenocepacia* in cystic fibrosis**  
**Jane L. Burns** and Adam Griffith
- V-f Species-associated outcomes of *Burkholderia cepacia* complex infection in CF**  
**Mary Corey**, Jeffery Charbeneau, Susan Murray and John J. LiPuma

**FREE TIME TO NETWORK AND EXPLORE TORONTO**

**SUNDAY, APRIL 26, 2009**

**8:00-9:00 Breakfast- Munk Centre Lobby**

**9:00-9:45 Session VI. Chair - Esh Mahenthiralingam**

**NEW RESOURCES AND APPROACHES**

**VI-a The Burkholderia genome database: Facilitating the comparison and visualization of updated genome data and analyses.**

Geoffrey L. Winsor, Raymond C. Lo, Bhav Khaira, Thea Van Rossum, Matthew D. Whiteside and **Fiona S.L. Brinkman**

**VI-b Multilocus sequence typing of *Burkholderia cepacia* complex without a need of bacterial culture**

**Pavel Drevinek**, Sarka Vosahlikova and Ondrej Cinek

**VI-c Mapping the *Burkholderia cenocepacia* niche response via high-throughput sequencing**

**D.R. Yoder-Himes**, P.S.G. Chain, Y. Zhu, O. Wurtzel, E.M. Rubin, J.M. Tiedje and R. Sorek

**9:45-12:00 Session VII. Chair - David Speert**

**BCC – HOST OR CELLULAR INTERACTIONS**

**VII-a Visualization of the intracellular life style of *Burkholderia cepacia* complex strains in zebrafish embryos**

**Annette Vergunst** and David O'Callaghan

**VII-b Differential modulation of human dendritic cell functions by *Burkholderia cenocepacia* and *Burkholderia multivorans***

**Kelly L. MacDonald** and David P. Speert

**10:15-10:45 Break**

**10:45- 12:00 Session VII- continued**

**VII-c Elucidation of intracellular gene expression by *Burkholderia cenocepacia* within murine macrophages**

**Jennifer S. Tolman** and Miguel A. Valvano

**VII-d The activation of the phosphatidyl-inositol-3 kinase (pi3k) pathway in response to the *Burkholderia cepacia* complex**

**Billie Velapatiño**, and David P. Speert

**VII-e *Burkholderia cenocepacia* induced loss of cortical actin in RAW264.7 membranes and accumulation of actin and Rho at the bacterial-containing vacuolar membrane.**  
**Jonathan Plumb, Kassidy Huynh, Iskra Peltekova, Miguel Valvano and Sergio Grinstein**

**VII-f *BcsK*, a putative transglycosylase critical for the Type 6 secretion system activity of *Burkholderia cenocepacia*.**  
**Daniel Aubert, Douglas K. McDonald and Miguel A. Valvano**

**VII-g *Pseudomonas aeruginosa* alginate promotes *Burkholderia cenocepacia* infection by interfering with host innate defense mechanism**  
**Umadevi Sajjan, Rachana Murthy, Joanna B. Goldberg, Marc B. Hersenson**

**12:00 Closing Remarks**

**IBCWG 2009 GRATEFULLY ACKNOWLEDGES THE GENEROUS SPONSORSHIP OF**

**CYSTIC FIBROSIS FOUNDATION**

**GILEAD SCIENCES, INC.**

**CANADIAN CYSTIC FIBROSIS FOUNDATION**

**CANADIAN INSTITUTES OF HEALTH RESEARCH**

**INNOVOTECH, INC.**

## I-a

### WASTEWATER TREATMENT LAGOONS ARE1 RESERVOIRS OF *BURKHOLDERIA CEPACIA* COMPLEX SPECIES

Manuelle Neto, Arnault Graindorge, Evelyne Borges, Françoise Maurin, Laurence Villard, and Benoit Cournoyer  
Research group on “bacterial opportunistic pathogens and environment”, UMR5557 Microbial Ecology, CNRS, ENVL, and University Lyon 1, Villeurbanne, France.

Contact : Benoit Cournoyer, cournoye@biomserv.univ-lyon1

In several countries, little towns (less than 2000 inhabitants) are moving towards the use of the lagooning approach to clean up domestic wastewaters. Lagooning implies a gravitational transfer of wastewaters into a series of three ponds of sizes defined according to the number of inhabitants connected to the system. The lagooning purifying process then simply aims at reducing by about 60% the values of total nitrogen and phosphorus by the metabolic activities of bacteria and algae entering the lagoons through natural routes or the wastewater themselves. The efficacy of these systems is rarely estimated in terms of reduction of microbial contaminants despite knowing that wastewaters are vectors of several infectious agents. Every year bathing zones are contaminated by wastewaters, resulting in the infection of millions of bathers worldwide, with the main clinical outcomes being gastroenteritis and pneumonia (World Health Association, 2006).

*Burkholderia cepacia* complex (Bcc) bacteria are well-known for their abilities at surviving and thriving in various water environments including rivers (e. g. Olapade et al. 2005. Microb Ecol. 49, 461–467). These abilities make wastewater treatment lagoons (WWTL) potential reservoirs and sources of contamination of natural streams by the Bcc. In this presentation, we will show our first results concerning Bcc species richness and genetic diversity among WWTL. These investigations required an improvement of Bcc isolation procedures and molecular identification schemes. An *ecf* gene Bcc-specific DNA probe was developed based on previous data regarding the distribution biases of sigma 70 gene sequences (Menard et al. 2007. BMC Genomics. 8:308). The sensitivity of this probe was tested on 15 species of Bcc, and its specificity against closely related Bcc-species. This probe was used to detect Bcc colonies growing on TB-T (trypan blue tetracycline) medium. Bcc-like colonies were validated using the *ecfB* (Graindorge et al., submitted) and *recA* PCR screenings, and VITEK2 phenotypic analyses. The *recA* PCR RFLP approach, and *recA* and *ecfB* phylogenetic analyses were performed to relate the WWTL isolates to previously described Bcc species. These analyses showed a significant genetic proximity between DNA sequences of certain WWTL isolates and sequences from *B. stabilis*, and, in other cases, showed close phylogenetic relationships between WWTL isolates DNA sequences and sequences from respectively *B. cenocepacia*, *B. pyrrocinia* and *B. cepacia*. Data concerning the distribution of Bcc species among the influents, ponds, and effluents of WWTL will be presented.

## I-b

### REGULATORY COMPONENTS AND PATHOGENIC FEATURES OF THE PHENYLACETIC ACID CATABOLIC PATHWAY OF *BURKHOLDERIA CENOCEPACIA* K56-2

Robyn J. Law<sup>1</sup>, Jason N. R. Hamlin<sup>1</sup>, Kali Teichroeb<sup>1</sup>, Tom Ward<sup>2</sup> and Silvia T. Cardona<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

<sup>2</sup>Manitoba Chemical Analysis Laboratory, Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada

Contact: Silvia T. Cardona, cardona@cc.umanitoba.ca

The phenylacetic acid (PA) catabolic pathway is the central route where degradation pathways of many aromatic compounds converge and are directed to the TCA cycle. In *Escherichia coli*, the PA catabolic pathway is comprised of four steps, the activation-ligation of PA-CoA, performed by PaaK, the ring-hydroxylation step, for which the PaaABCDE enzymatic complex is responsible, enoyl-CoA isomerization/hydration and ring opening performed by PaaG and PaaZ, and the  $\beta$ -oxidation step carried out by PaaF and PaaH. Recently, we initiated the functional characterization of the PA degradation pathway in *Burkholderia cenocepacia* K56-2 and found that while the putative PA-CoA ring hydroxylation system was required for full pathogenicity in *Caenorhabditis elegans*, interruption of the lower steps by insertional mutagenesis of *paaZ* and *paaF*, slightly increased virulence (Law et al., 2008). In order to gain insight into the molecular basis of these pathogenic phenotypes, we aimed to identify regulatory elements and PA-catabolic intermediates. An eGFP translational reporter system was used to assess gene expression in minimal media containing PA and different carbon sources. To investigate whether the observed difference in pathogenicity of the mutants was due to over production of toxic metabolites, the supernatants of insertional-mutant cultures, grown with glycerol and phenylalanine, were analyzed by HPLC and toxin filter diffusion assays.

Our results show that *B. cenocepacia* K56-2 PA catabolic genes are induced by PA and phenylalanine, are negatively regulated by PaaR, a TetR-type regulator, and are subject to catabolic repression by succinate and glucose. In accordance with distinct HPLC profiles, worm survival was adversely affected by the addition of wild type and *paaF* mutant supernatant in comparison to that of the *paaA* mutant, which showed no significant effect on survival. Current research is focused on the identification of PA-catabolic intermediates that may be responsible for different pathogenic phenotypes and regulatory mechanisms that are relevant during infection.

### I-c TRANSCRIPTIONAL RESPONSES IN *BURKHOLDERIA CENOCEPACIA* J2315 BIOFILMS FOLLOWING EXPOSURE TO CHLORHEXIDINE, H<sub>2</sub>O<sub>2</sub> AND NaOCl

Elke Peeters<sup>1</sup>, Andrea Sass<sup>2</sup>, Eshwar Mahenthiralingam<sup>2</sup>, Hans J. Nelis<sup>1</sup> & Tom Coenye<sup>1</sup>

<sup>1</sup>Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Gent, Belgium

<sup>2</sup>School of Biosciences, Cardiff University, Cardiff, UK

Contact : Tom Coenye, Tom.Coenye@UGent.be

*Burkholderia cepacia* complex bacteria are opportunistic pathogens that can cause severe respiratory tract infections in patients with cystic fibrosis. Treatment is particularly difficult because these microorganisms are highly resistant against various antibiotics. Consequently, infection control guidelines focus on the prevention of acquisition of *B. cepacia* complex organisms by advising patients to clean and disinfect their respiratory equipment on a regular basis. Unfortunately, problems regarding the efficacy of several disinfectants for the removal and/or killing of these micro-organisms have been reported. The biofilm-forming capability of *B. cepacia* complex bacteria may contribute significantly to their resistance against multiple disinfectants. The goal of the present study was to compare the transcriptional responses of sessile *Burkholderia cenocepacia* J2315 cells following exposure to chlorhexidine (CHX; 0.015%, 15 min), H<sub>2</sub>O<sub>2</sub> (0.3%, 30 min) or NaOCl (0.02%, 5 min) with those in untreated biofilms using custom made Agilent microarrays.

The exposure of sessile *B. cenocepacia* cells to CHX resulted in an up-regulation of the transcription of 582 genes and a down-regulation of the transcription of 285 genes (2-fold difference; p<0.05). Probably as a result of the membrane damage caused by this disinfectant, many of the up-regulated genes encode membrane-related proteins. In addition, the transcription of six genes belonging to two RND-operons was also clearly up-regulated. One of these tripartite efflux pumps shows a strong similarity with the MexCD-OprJ multidrug efflux pump, which is involved in the CHX resistance in *Pseudomonas aeruginosa*.

The exposure of the biofilms to H<sub>2</sub>O<sub>2</sub> and NaOCl resulted in an up-regulation of the transcription of 380 and 452 genes, respectively, whereas for 238 and 402 genes the transcription was down-regulated. Many of the up-regulated genes are involved in (oxidative) stress responses, including genes coding for AhpC, AhpF, Ohr proteins and RNA polymerase sigma factors. In addition, the H<sub>2</sub>O<sub>2</sub> treatment also resulted in the up-regulation of the transcription of multiple genes encoding (putative) catalases, a non-heme chloroperoxidase, an OsmC-like protein and an OsmB-like lipoprotein. Unexpectedly, the latter treatment also resulted in an increased transcription of multiple phage-related genes (BCAS 0539-BCAS 0554; BcepMu16-BcepMu1).

### I-d AGGREGATION PROPERTIES OF CEPACIAN. A MODEL FOR BIOFILM FORMATION

Bruno Cuzzi<sup>1</sup>, Martin Frank<sup>2</sup>, Paola Cescutti<sup>1</sup> and Roberto Rizzo<sup>1</sup>

<sup>1</sup>Department of Life Sciences, University of Trieste, 34127 Trieste, Italy.

<sup>2</sup>German Cancer Research Center, 69120 Heidelberg, Germany.

Contact : Roberto Rizzo, rizzor@units.it

Cepacian is the most abundant exopolysaccharide produced by the majority of the clinical and environmental strains of the *Burkholderia cepacia* Complex (BCC). It is produced either alone or as a mixture of different saccharidic polymers, depending on the culture medium. The cepacian repeating units is rather complex including seven monosaccharides, four of them are involved in lateral chains. The presence of a glucuronic acid residue makes the polymer negatively charged. Its biological role is not fully clarified, but certainly it takes part of the formation of a microenvironment surrounding bacteria and protecting them against external threats. Under certain conditions, the polymeric network can be used by micro-organisms, in synergy with different (macro)molecules, to develop a biofilm. Therefore, the definition of the conformational and aggregation properties of cepacian are useful to better understand its biological activity. The aggregation ability of cepacian has been recognised by means of capillary viscosity and molecular mass determination experiments using different solvent systems<sup>1</sup>. The use of dimethylsulfoxide, compared with water, revealed a net decrease in viscosity associated with the halving in molecular mass, thus pointing at the disruption of the interpolymeric complexes present in aqueous media. The evaluation of the intramolecular Overhauser effects in NMR spectroscopy revealed a number of intramolecular non-bonded interactions which explained the intrinsic rigidity of the cepacian backbone, a relevant property for the formation of an efficient polymeric network.

This contribution is focused on the molecular modelling of the interaction of cepacian molecules as obtained from simulation of the conformation and dynamics of single and multiple polymer chains in water solutions. The computer simulation of the interaction of two stretches of cepacian backbone could nicely explain the formation of polymer aggregates which can possibly result in the formation of an extended polymeric network. The evaluation of the intermolecular NMR Overhauser effects were used to correlate the results of the molecular modelling with experimental evidences.

1) Herasimenka Y., Cescutti P., Sampaio Noguera C.E., Ruggiero J.R., Urbani R., Impallomeni G., Zanetti F., Campidelli S., Prato M., Rizzo R. Macromolecular properties of cepacian in water and in dimethylsulfoxide. *Carbohydr. Res.* 343 (2008) 81–89.

I-e

## FLAGELLA-MEDIATED MOTILITY IS ESSENTIAL FOR *BURKHOLDERIA CENOCEPACIA* BIOFILM FORMATION

Katarzyna M. Stevens<sup>1</sup>, Dolina D. Kerr<sup>1</sup>, Jen Amon<sup>1</sup>, Susan Koval<sup>2</sup>, and Douglas G. Storey<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Calgary, Calgary, Canada

<sup>2</sup>Department of Microbiology and Immunology, University of Western Ontario, London, Canada

Contact : Katarzyna M. Stevens, kstevens@ucalgary.ca

*Burkholderia cenocepacia* is an opportunistic pathogen of Cystic Fibrosis (CF) patients, establishing infections with unpredictable outcomes. These infections range from asymptomatic carriage to the invasive, often lethal, cepacia syndrome. Although this bacterium affects a small percentage of patients, it is of particular concern due to its intrinsic antibiotic resistance. It has been well established that biofilm formation plays a significant role in antibiotic resistance. Not surprisingly, *B. cenocepacia* forms biofilms alone and in cooperation with the predominant CF pathogen, *Pseudomonas aeruginosa*. Moreover, biofilm formation is believed to play a significant role in the persistence of CF airway infections. Hence, this study aims to further the understanding of *B. cenocepacia* biofilm formation. To address this aim, biofilm defective mutants of *B. cenocepacia* K56-2 were isolated and characterized at the genetic level. It was determined that almost half of these mutants had transposon insertions in motility-associated genes. Of particular interest were FlhC and FlhD, subunits of a flagella master regulator protein, FlhA, a flagella export pore protein, FlhB, a regulator of flagella export, flagellin type II, and a hook associated protein. Preliminary assays of this mutant group suggested that motility is required for normal biofilm formation for K56-2. To determine their role in flagella formation, we examined these mutants, and their complemented counterparts, by Transmission Electron Microscopy (TEM). TEM results determined that all the flagella mutants lacked intact flagella. Interestingly, when the interrupted genes were complemented *in trans* in all cases there was some restoration of swim motility, but not all of these mutants regained an intact flagella. Furthermore, all flagella mutants were unable to swarm and, in contrast to swim motility, did not complement for swarm phenotype. Confocal laser scanning microscopy images of the mutant biofilms were analyzed for structural differences using the DAIME 3D visualization program. DAIME analysis of the flagella mutants revealed significant structural defects, reduced number of adhering cells, and a lack of microcolonies, which were almost fully restored to wild type levels when complemented in all cases. In conclusion, flagella-mediated motility is necessary for normal biofilm formation in *B. cenocepacia*.

## II-a

### A COMPARATIVE TRANSCRIPTOMIC, PROTEOMIC AND PHENOTYPICAL ANALYSIS OF THE *BURKHOLDERIA CENOCEPACIA* H111 QUORUM SENSING NETWORK

Silja Inhülsen, Kathrin Riedel and Leo Eberl  
Department of Microbiology, Institute of Plant Biology,  
University of Zurich, Zurich, Switzerland  
Contact: Silja Inhülsen, [sinhuelsen@botinst.uzh.ch](mailto:sinhuelsen@botinst.uzh.ch)

Many *Burkholderia* species are problematic opportunistic pathogens in both cystic fibrosis (CF) patients, chronic granulomatous disease (CGD), and nosocomial outbreaks. Polyphasic-taxonomic studies revealed that most of these organisms comprise a group of 17 distinct but closely related species, referred to as the *Burkholderia cepacia* complex (Bcc). *Burkholderia cenocepacia* is with *Burkholderia multivorans* the most common species in CF lung infections.

Like many Gram-negative bacteria, *B. cenocepacia* employs a cell-to-cell communication (“quorum sensing”; QS) system to express various functions in a population density-dependent manner. The predominant QS system of *B. cenocepacia* consists of the AHL synthase CepI, which directs the synthesis of *N*-octanoylhomoserine lactone (C8-HSL), and the transcriptional regulator CepR. Previous work has demonstrated that the production of extracellular proteases, chitinases, and siderophores, swarming motility, biofilm formation and expression of pathogenic traits is QS-regulated in the large majority of Bcc strains.

In order to identify QS-regulated genes we compared the transcriptome of *B. cenocepacia* H111 with that of an isogenic *cepR* mutant. As a control we included the complemented *cepR* mutant. Furthermore, we also mapped the QS proteome employing an alternative liquid chromatography (LC)-MS/MS-based technology (iTRAQ). In this case we compared the proteome of the wild type with that of the *cepR* mutant and with that of the *cepI* mutant grown in the presence or absence of 200nM C8-HSL. Finally, we also employed phenotypic microarrays to analyse the importance of QS for the utilization of various substrates.

Here we report on the identified genes/proteins. Many of these factors provide novel links to previously identified QS-regulated phenotypes, including the production of extracellular hydrolytic enzymes, biofilm formation, and pathogenicity. Our data moreover suggest that expression of flagellar genes is negatively affected when CepR is over-expressed. In addition, QS signalling appears to only slightly affect carbohydrate, nitrogen and phosphorous utilization of *B. cenocepacia* H111.

## II-b

### TRANSCRIPTOME ANALYSIS OF QUORUM SENSING REGULATION BY CepR AND CciR IN *BURKHOLDERIA CENOCEPACIA*

Eoin P. O’Grady, Duber F. Viteri, Rebecca J. Malott, Audrey A. Plourde and Pamela A. Sokol  
Department of Microbiology and Infectious Diseases,  
University of Calgary, Calgary, Alberta, Canada  
Contact: Eoin O’Grady, [epograd@ucalgary.ca](mailto:epograd@ucalgary.ca)

*Burkholderia cenocepacia* is an opportunistic pathogen which causes chronic and life-threatening respiratory infections in people with cystic fibrosis and chronic granulomatous disease. Quorum Sensing (QS) is a mechanism employed by diverse bacteria to coordinately regulate gene expression. *B. cenocepacia* has two QS systems, the CepIR system which is present in all species of the *Burkholderia cepacia* complex, and the CciIR system, which is only present in *B. cenocepacia* containing the *cenocepacia* genomic island found in ET12 strains. CepR and CciR are transcriptional regulators that modulate gene expression including that of specific virulence determinants when activated by their respective signal molecules. Several approaches have been used to identify the CepIR regulon, but considerably less is known about genes regulated by the CciIR system. Transcriptional profiling using *B. cenocepacia* microarrays was used to determine genes differentially expressed in strain K56-2, K56-2*cepR*, K56-2*cciR* and K56-2*cepRcciR*. Both positive and negative regulation was exerted by CepR and CciR on all three chromosomes as well as the plasmid. Using a 2-fold difference in expression as a cut-off, 646 open reading frames (ORFs) were identified that were positively regulated by CepR and 214 ORFs were identified that were negatively regulated by CepR. CciR positively influenced the expression of 100 ORFs and negatively regulated the expression of 495 ORFs. Although many genes on the microarrays were independently regulated by either CepR or CciR, 196 ORFs were regulated by both CepR and CciR. The majority of these genes were positively regulated by CepR and negatively regulated by CciR. K56-2*cepRcciR* had increased expression of 176 ORFs and decreased expression of 313 ORFs in comparison to K56-2. The majority of CepR and CciR co-regulated genes had a similar expression pattern in the *cepR* and *cepRcciR* mutants. Many genes shown to be CepR-regulated by other approaches were validated in this study by transcriptome data. QS regulation was observed for genes on the *cenocepacia* genomic island, as well as genes involved in flagellar motility, biofilm formation, virulence and secretion. Additionally, both CepR and CciR influenced expression of genes negatively regulated by the recently-identified orphan LuxR homolog CepR2. QS regulation of selected genes was confirmed using promoter fusions and quantitative RT-PCR. The CepIR and CciIR systems usually inversely influence the expression of co-regulated genes, likely enabling fine tune control of gene expression in *B. cenocepacia* strains with the *cenocepacia* genomic island.

## II-c

### PRODUCTION OF THE ANTIFUNGAL COMPOUND PYRROLNITRIN IS QUORUM SENSING-REGULATED IN MEMBERS OF THE *BURKHOLDERIA CEPACIA* COMPLEX

Silvia Schmidt<sup>1</sup>, Judith F. Blom<sup>2</sup>, Jakob Pernthaler<sup>2</sup>, Gabriele Berg<sup>3</sup>, Adam Baldwin<sup>4</sup>, Eshwar Mahenthiralingam<sup>5</sup> and Leo Eberl<sup>1\*</sup>

<sup>1</sup> Department of Microbiology, Institute of Plant Biology, University of Zurich, Zurich, Switzerland

<sup>2</sup> Department of Limnology, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland

<sup>3</sup> Department of Environmental Biotechnology, Graz University of Technology, Graz, Austria

<sup>4</sup> Department of Biological Sciences, Warwick University, Coventry, UK

<sup>5</sup> Cardiff School of Biosciences, Cardiff University, Main Building, Cardiff, UK

Contact : Leo Eberl, leberl@botinst.uzh.ch

Members of the genus *Burkholderia* are known for their ability to suppress soil-borne fungal pathogens by the production of various antibiotic compounds. In this study we investigated the role of *N*-acylhomoserine lactone (AHL)-dependent quorum sensing (QS) in the expression of antifungal traits. Using a quorum quenching approach, i.e. by heterologous expression of the *Bacillus* sp. AiiA lactonase, we show that expression of antifungal activities is AHL-dependent in the large majority of the investigated strains belonging to various *Burkholderia* species. We demonstrate that in certain strains of *B. ambifaria*, *B. pyrrocinia*, and *B. lata*, one of the QS-regulated antifungal agents is pyrrolnitrin (prn), a common broad-spectrum antibiotic that is also produced by some *Pseudomonas* and *Serratia* species. To investigate the underlying molecular mechanisms of AHL-dependent prn production in better detail, we inactivated the AHL synthase *cepI* as well as *cepR*, which encodes the cognate AHL receptor protein, in *Burkholderia lata* 383. Both QS mutants no longer produced prn as assessed by gas chromatography-mass spectrometry analysis and as a consequence were unable to inhibit growth of *Rhizoctonia solani*. Using fusions of the *lacZ* gene to the promoter of the *prnABCD* operon, which directs the synthesis of prn, we demonstrate that expression of prn is positively regulated by CepR at the level of transcription.

## II-d

### ADHESION OF *BURKHOLDERIA MULTIVORANS* AND *BURKHOLDERIA CENOCEPACIA* TO LUNG EPITHELIAL CELLS AND ITS INHIBITION

Ciara Field-Corbett<sup>1</sup>, Gillian Herbert<sup>1</sup>, Minu M. Thomas<sup>1</sup>, Trinidad Velasco-Torrijos<sup>3</sup>, Stephen Carberry<sup>2</sup>, Sean Doyle<sup>2</sup>, Máire Callaghan<sup>1</sup> and Siobhán McClean<sup>1</sup>

<sup>1</sup>Centre of Microbial Host Interactions, Institute of Technology Tallaght Dublin, Belgard Road, Tallaght, Dublin 24, Ireland. <sup>2</sup>Dept of Biology and <sup>3</sup>Dept of Chemistry National University of Ireland, Maynooth, Co. Kildare, Ireland.

Contact : Siobhán McClean, siobhan.mcclean@ittdublin.ie

The mechanisms of attachment and pathogenesis of *Burkholderia cepacia* complex are poorly understood. In spite of strict segregation measures, Bcc continues to be acquired from the environment. Elucidation of the mechanisms of colonization and lung epithelial adhesion should allow the development of therapies which can minimize the risk of colonization, on exposure to Bcc in the environment. Previously, it has been shown that Bcc strains bound to many glycolipid receptors, including asialoGM1, asialoGM2 and globosides, on lung epithelia. We have demonstrated that these glycolipid receptors are essential for invasion. We have developed a method to determine the levels of binding of Bcc to the lung epithelial cells, using real-time PCR. We have used this method to evaluate inhibition of this adhesion by a series of glycoconjugate derivatives. In addition, we are examining the bacterial outer membrane proteins that are involved in the direct interaction with lung epithelial cells, by MALDI-ToF analysis. Determination of the proteins involved in attachment to the epithelia will allow the design of specific therapies which could also reduce lung colonization.

This project has been funded under the Programme for Research in Third Level Institutions (PRTLII) administered by HEA & Technology Sector Research Strand III.

## II-e

### NOVEL ADHERENCE FACTORS OF PATHOGENIC *BURKHOLDERIA CENOCEPACIA*: MECHANISMS OF ACTION AND THEIR ROLE IN VIRULENCE

Dalila Mil-Homens and Arsénio M Fialho  
IBB-Institute for Biotechnology and Bioengineering, Center for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal  
Contact : afialho@ist.utl.pt

Members of the *Burkholderia cepacia* complex (Bcc) are capable of causing lung infections in cystic fibrosis patients. The Bcc consists of at least 17 species; the virulence is variable and *B. cenocepacia* is the dominant species. To initiate infection, *B. cenocepacia* must be able to colonize the respiratory epithelium by binding to extracellular-matrix components. This step, although not fully characterized, is mediated by several proteins collectively termed adhesins, which are surface-exposed proteins encoded by phase-variable genes that are in general associated with close repetitive DNA sequences. The genome of the epidemic strain *B. cenocepacia* J2315 was searched for close repetitive DNA sequences, aiming to identify novel adhesin genes. We identified two adhesin genes that are members of an adhesion gene cluster located downstream to the *cci* island that is a marker for virulent *B. cenocepacia* strains. This cluster represents a prototype of a two-component phosphorelay system and is organized with four adhesins, one lipoprotein, one sensor histidine kinase and two response regulators. We analysed by PCR 3 genes among 35 representatives Bcc clinical isolates, to investigate if the adhesin cluster was conserved. Notably, these three genes were present in the epidemic isolates of the ET-12 lineage of *B. cenocepacia* but absent in all the other members. Furthermore, the *BCAM 0224* adhesin was exclusively detected in this epidemic lineage. By RT-PCR analysis it was found that these adhesin genes were expressed in *B. cenocepacia* K56-2. Environmental factors such as osmolarity, oxygen limited conditions and oxidative stress significantly enhance the expression levels of the adhesin cluster at the transcriptional level. These changes in expression occurred preferentially for cells grown under static conditions and required the presence of extracellular matrix (ECM) proteins, such as fibronectin, collagen type II and laminin. Knock-out mutants of *B. cenocepacia* K-56 BCAM 0219 and BCAM 0224 adhesins were constructed and assayed for adherence to ECM proteins and biofilm formation. We also used the killing of *Galleria mellonella* (the greater wax moth) caterpillar as a model host to study the role of the adhesin genes in virulence. A summary of these observations and their relevance for *Burkholderia* pathogenicity will be presented.

Supported by FCT PhD fellowship to D. Mil-Homens (SFRH / BD / 41760 / 2007)

## II-f

### *BURKHOLDERIA CENOCEPACIA* CblA AND AdhA ARE REQUIRED FOR INFLAMMATION *IN VITRO* AND *IN VIVO*

J. B. Goldberg<sup>1</sup> and U. Sajjan<sup>2</sup>  
<sup>1</sup>Department of Microbiology, University of Virginia, Charlottesville, VA, USA  
<sup>2</sup>Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI, USA  
Contact: Joanna B. Goldberg, jbg2b@virginia.edu

Previously, we have demonstrated that both CblA and the 22kDa adhesin, AdhA, contribute to adherence of *Burkholderia cenocepacia* strain BC7 to well-differentiated airway epithelial cells. To assess whether the defective adherence of these mutants to epithelial cells affected the inflammatory potential, we examined IL-8 production by CF airway epithelial cells in response to infection with wild-type BC7, the CblA-, or the AdhA- mutant. Wild-type BC7 stimulated 2.7 and 3.2 fold higher levels of IL-8 compared to the CblA- and the AdhA- mutant, respectively. Next by using a new mouse model of infection, we examined the requirement of CblA and AdhA in bacterial persistence and inflammation *in vivo*. Eight to ten weeks old C57BL/6 mice were infected by the intratracheal route with wild-type BC7, the CblA-, or the AdhA- mutant ( $1.5 - 2 \times 10^6$  CFU) suspended in purified alginate isolated from mucoid *Pseudomonas aeruginosa*. Five days later, mice were sacrificed and assessed for pulmonary bacterial load and inflammatory markers such as myeloperoxidase, KC, and MIP-2. Mice infected with wild-type BC7 showed higher bacterial loads than mice infected with the CblA or the AdhA mutants. Mice infected with wild-type BC7 also showed increased levels of inflammatory markers compared to mice infected with either the CblA- or the AdhA- mutant, consistent with the increased bacterial pulmonary load. Examination of bacterial distribution in the lungs by immunolocalization one day after infection, revealed that while majority of the wild-type bacteria were localized to peribronchiolar and perivascular areas, the CblA- and AdhA- mutants were found in airway lumen, indicating that wild-type BC7 efficiently invade and cause inflammation *in vivo*. Taken together, these results suggest that CblA and AdhA are required not only for initial adherence and related pro-inflammatory response *in vitro*, but also for dissemination of infection and to cause maximal inflammation in mice.

This work was supported by grants GOLDBE07G0 and SAJJAN08G0 from the Cystic Fibrosis Foundation.

### III-a 3 ANTIBIOTIC RESISTANCE AND LIPID A BIOSYNTHESIS OF THE *BURKHOLDERIA CEPACIA* COMPLEX (BCC)

K. Bodewits<sup>\*1,2</sup>, H. S. Chung<sup>3</sup>, C. R. H. Raetz<sup>3</sup>, J. R. W. Govan<sup>2</sup>, D.J. Campopiano<sup>1</sup>

<sup>1</sup>School of Chemistry; <sup>2</sup>Centre for Infectious Diseases, Medical School, University of Edinburgh, Edinburgh, UK; <sup>3</sup>Department of Biochemistry, Duke University Medical Center, North Carolina, USA.

Contact : Karin Bodewits, k.bodewits@sms.ed.ac.uk

Lipid A is the hydrophobic anchor moiety of LPS that builds the surface monolayer of the asymmetrical outer membrane that shields Gram-negative bacteria. The first six enzymes in the lipid A biosynthesis are vital in *Escherichia coli* and inhibition of one of these six enzymes is lethal.

Since the lipid A structure and composition of *Burkholderia cepacia* complex (Bcc) members is distinct from the lipid A of other bacteria we are investigating the biosynthesis of lipid A and potential new drug targets in Bcc. A novel antibiotic CHIR-090 (*N*-aroyl-L-threonine hydroxamic acid) showed good antimicrobial activity against several Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*. The primary mechanism of activity of CHIR-090 is inhibition of the zinc-dependent UDP-(3-O-(*R*-3-hydroxymyristoyl))-*N*-acetylglucosamine deacetylase, called LpxC. This enzyme catalyses the second, irreversible step in the lipid A biosynthetic pathway and therefore is essential for growth in the majority of Gram-negative bacteria. Here we present the inhibitory activity of CHIR-090 against different members of the Bcc and investigate the molecular basis for species-specific susceptibility and resistance.

### III-b AN INVESTIGATION OF THE BASIS OF INTRINSIC AMINOGLYCOSIDE RESISTANCE IN *BURKHOLDERIA VIETNAMIENSIS*

Agatha N. Jassem<sup>1</sup>, James E. A. Zlosnik<sup>2</sup>, Robert K. Ernst<sup>4</sup>, Robert E. W. Hancock<sup>2,3</sup>, and David P. Speert<sup>1,2,3</sup>

Departments of Pathology and Laboratory Medicine<sup>1</sup>, Pediatrics<sup>2</sup>, Microbiology and Immunology<sup>3</sup>, University of British Columbia, Vancouver, Canada

<sup>4</sup>Department of Microbial Pathogenesis, University of Maryland, Baltimore, USA

Contact : Agatha Jassem, agatha@interchange.ubc.ca

The *Burkholderia cepacia* complex (Bcc) is a group of closely-related Gram-negative bacterial species that are opportunistic, highly virulent pathogens in immunocompromised individuals, particularly those with cystic fibrosis or chronic granulomatous disease. Treatment of Bcc infections is hampered by their intrinsic resistance to many antimicrobials, including polycationic aminoglycosides. Mechanisms of aminoglycoside resistance in the Bcc are not fully understood, however it is thought that characteristics of the outer membrane (OM), particularly lipopolysaccharide (LPS), are involved, since cationic antimicrobials enter other Gram-negative bacteria through LPS-mediated self-promoted uptake, relying especially on lipid A binding sites. In a survey of Bcc bacteria we observed that, unlike the other species, most environmental and clinical isolates of *B. vietnamiensis* were intrinsically susceptible to aminoglycosides, yet remained highly resistant to other cationic agents (natural and synthetic cationic antimicrobial peptides, and polymyxin B). Our overall goal is to elucidate the basis of the observed differential aminoglycoside intrinsic resistance in *B. vietnamiensis*, in order to better understand the mechanisms governing aminoglycoside resistance in the Bcc. We hypothesize that unique properties of the *B. vietnamiensis* OM confer aminoglycoside susceptibility. MALDI-TOF mass spectroscopy analysis of lipid A revealed no structural differences between aminoglycoside susceptible and resistant *B. vietnamiensis* isolates. In addition, no permeability differences to the fluorescent hydrophobic probe 1-*N*-phenyl-naphthylamine (NPN) following incubation with gentamicin (an aminoglycoside antibiotic) were observed between aminoglycoside susceptible and resistant *B. vietnamiensis* isolates. These data suggest that in *B. vietnamiensis* different mechanisms of resistance exist for different classes of cationic antimicrobial agents and that aminoglycoside entry does not occur via self-promoted uptake.

### III-c CHANGES IN GENE EXPRESSION OF *B. CENOCEPACIA* J2315 IN RESPONSE TO DIFFERENT CLASSES OF ANTIBIOTICS

Andrea M Sass, Angela Marchbank, Pavel Drevinek and Eshwar Mahenthiralingam  
Cardiff School of Biosciences, Cardiff University, Cardiff Wales, UK.  
Contact : SassAM@cardiff.ac.uk

*B. cepacia* complex (BCC) bacteria can cause severe lung infections in CF patients which are difficult to treat due to the high intrinsic antibiotic resistance of the bacteria involved. Recently several members of the BCC have been fully sequenced. The availability of the genome sequences made it possible to develop microarrays for the study of gene expression on a genomic scale. To investigate BCC antibiotic resistance mechanisms, microarray experiments were performed using *B. cenocepacia* J2315 as a model organism. Gene expression during growth of J2315 in the presence of subinhibitory concentrations of three antibiotics of different mechanism of action was analysed. The antibiotics included: (1) Meropenem as a clinically relevant example of a beta-lactam; (2) trimethoprim/sulfamethoxazole (1:5), a folate-synthesis blocker, and (3) amikacin as a model aminoglycoside. Additionally we studied the gene expression in spontaneous J2315 mutants with elevated resistance to each of the latter antibiotics. The results of gene expression analysis showed that the wild type J2315 up- or downregulates only a minimal number of genes in the presence of sub-inhibitory concentrations of antibiotics. Among the up-regulated genes were: two beta-lactamases, encoded on the medium and small chromosome respectively, in the presence of meropenem; an acetyltransferase in the presence of amikacin and *aidA* in the presence of trimethoprim. The microarray observed expression changes were also confirmed by qPCR. The functional involvement of the acetyltransferase (in amikacin resistance) and small chromosome beta-lactamase (in meropenem resistance) was also confirmed by transfer of the respective genes to *B. vietnamiensis* G4 which lacked the homologous genes. The study of gene expression in spontaneous mutants with elevated resistance revealed constitutive change in expression of a much larger number of genes that observed in the exposure of the wild type to subinhibitory antibiotics. The meropenem-resistant mutant displayed constitutive up-regulation of genes implicated in phenylacetic acid degradation. Further MIC tests and qPCR experiments revealed that the expression of these genes was suppressed by glucose in J2315, and that resistance to meropenem decreased in the presence of glucose. These data suggests that the capacity of *B. cenocepacia* to degrade a multitude of xenobiotic compounds may also plays a role in its intrinsic antibiotic resistance. The trimethoprim-resistant mutant displayed strong (>100 fold) constitutive up-regulation of the *ceo* efflux pump confirming its major role in antibiotic resistance. The *ceo* efflux pump was however not up-regulated in the wild type in the presence of subinhibitory amounts of trimethoprim. In addition to the microarray experiments relating to antibiotic resistance we also performed microarray

experiments in a range of standard conditions; like the comparison of exponential growth phase with stationary phase cultures. Many genes found to be up-regulated in the presence of antibiotics were also up-regulated in stationary phase cultures. Overall these data demonstrate that *B. cenocepacia* has multiple innate pathways of antibiotic resistance and can develop further adaptive resistance by eliciting an active stress response characteristic stationary phase growth.

### III-d

4

#### INACTIVATION OF THE ESSENTIAL GENE ACYL CARRIER PROTEIN IN *BURKHOLDERIA CEPACIA* COMPLEX BY PHOSPHORODIAMIDATE MORPHOLINO OLIGOMERS

David E. Greenberg<sup>1</sup>, Kimberly Marshall-Batty<sup>1</sup>, Patrick L. Iversen<sup>2</sup>, Steven M Holland<sup>1</sup>, and Bruce L. Geller<sup>3</sup>

<sup>1</sup> Immunopathogenesis Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, US Department of Health and Human Services, Bethesda, MD 20892, USA

<sup>2</sup> AVI BioPharma Inc, Corvallis, OR

<sup>3</sup> Oregon State University, Corvallis, OR

Contact : David Greenberg, degreenberg@niaid.nih.gov

The *Burkholderia cepacia* complex (Bcc) comprises seventeen phenotypically similar but genetically distinct species. Although these environmental bacteria rarely cause disease in healthy individuals, they can cause significant morbidity and mortality in chronic granulomatous disease (CGD) and cystic fibrosis (CF). Many of the strains of Bcc found in patients with CF are antibiotic resistant making therapy challenging in these patients. Novel approaches to develop antimicrobial therapy include the use of antisense technologies such as phosphorodiamidate morpholino oligomers (PMOs). We tested various peptide-conjugated PMOs (PPMOs) in different strains of Bcc. In overnight culture, PPMOs targeting the gene acyl carrier protein (Acp) were bacteriocidal in various clinical isolates of *Burkholderia cepacia*, *B. multivorans* and *B. cenocepacia* with a > 5 log reduction in colony forming units/ml (CFU/ml) ( $p > 0.001$ ). A PPMO with a scrambled oligo sequence had no effect on growth in any *Burkholderia* species. MIC values of the Acp PPMO across numerous species of Bcc ranged from 2.5-10  $\mu$ M. A clinical CGD isolate of *B. multivorans* was used for further PMN killing assays and mouse studies. PMNs infected with *B. multivorans* showed enhanced killing with the addition of the Acp PPMO compared with scrambled sequence PPMO or no PPMO present. The enhanced killing effect persisted over time with > 1 log difference in CFU/ml between Acp PPMO and PMN +/- scrambled PPMO at 2 and 4 hours. The difference increased to > 3 log difference by 24 hours. A series of challenge studies were performed in gp91<sup>-/-</sup> CGD mice. Mice were infected i.p. with 5x10<sup>6</sup> CFU followed by immediate injection of Acp PPMO (200 $\mu$ g), scrambled PPMO (200  $\mu$ g) or water. Mice received a second dose of active PPMO or placebo at 3 and 6 hours. Compared to the water treatment group, the Acp PPMO treatment group showed a 79% reduction in the risk of dying at day 30 ( $p < 0.0001$ ). Compared to the scrambled PPMO treatment group, the Acp PPMO group showed a 53% reduction in the risk of death by day 30 ( $p = 0.037$ ). Mice that survived showed mild to no pathology in the spleen, liver or lymph nodes. In conclusion, the Acp PPMO shows treatment effects against Bcc in vitro, ex vivo and in vivo. The use of antisense technologies may be a viable treatment approach against this group of opportunistic pathogens.

### III-e

#### EFFICACY OF BACTERIOPHAGE THERAPY IN A MODEL OF *BURKHOLDERIA* PULMONARY INFECTION

Lisa A. Carmody<sup>1</sup>, Jason J. Gill<sup>2</sup>, Uma S. Sajjan<sup>1</sup>, Carlos F. Gonzalez<sup>3</sup>, Ryland F. Young<sup>2</sup>, and John J. LiPuma<sup>1</sup>

<sup>1</sup>Department of Pediatrics and Communicable Disease, University of Michigan Medical School, Ann Arbor, MI, USA

<sup>2</sup>Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA

<sup>3</sup>Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX, USA

Contact: John LiPuma; jlipuma@umich.edu

The therapeutic potential of bacteriophage in a mouse model of acute *B. cenocepacia* pulmonary infection was assessed. Phage were administered by either intranasal (i.n.) inhalation or intraperitoneal (i.p.) injection. Bacterial density, macrophage inflammatory protein-2 (MIP-2), and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) levels were significantly reduced in lungs of mice treated with i.p. phage. No significant differences in lung bacterial density or MIP-2 levels were found between untreated mice and mice treated with i.n. phage, i.p. UV-inactivated phage, or i.p.  $\lambda$  phage controls. Mock-infected mice treated with phage showed no significant increase in lung MIP-2 or TNF $\alpha$  levels compared to mock-infected / mock-treated mice. Immunofluorescent localization of phage and bacteria in lung revealed i.n. delivered phage were found sequestered in alveolar macrophages, while i.p. delivered phage were found in perivascular areas and alveolar septa where they co-localized with degraded bacteria. In the absence of treatment, bacteria were found primarily in perivascular and peribronchiolar areas, eventually forming microcolonies in regions of consolidated lung parenchyma. In vitro, phage were unable to transmigrate across polarized bronchial epithelial cells. We have demonstrated the efficacy of phage therapy in an acute *B. cenocepacia* lung infection model. Systemic administration of phage was more effective than inhalational administration, and immunofluorescent localization of phage indicated that circulating phage have better access to bacteria in lung compared to topical phage.

**III-f**  
**DEVELOPMENT OF PHAGE THERAPY FOR THE**  
***BURKHOLDERIA CEPACIA* COMPLEX**

Karlene H. Lynch, Kimberley D. Seed, Amanda D. Goudie,  
and Jonathan J. Dennis  
Department of Biological Sciences, University of Alberta,  
Edmonton, Alberta, Canada T6G 2E9  
Contact : Jon Dennis, jon.dennis@ualberta.ca

The *Burkholderia cepacia* complex (BCC) are pan-resistant to all major classes of antibiotics and therefore new therapeutic strategies are needed. One such strategy that holds significant promise is phage therapy. Unfortunately, the majority of phages identified that specifically infect the BCC have a number of undesirable characteristics, a major one being that they integrate into the bacterial genome (lysogenize) to become prophages rather than lysing the bacterial cell. In a proof-of-principle experiment, we have modified the genome of lysogenic phage KS9 using molecular techniques in order to convert it into a lytic phage. Successful experiments using lytic phages in the alternative animal infection model (*Galleria mellonella* larvae) demonstrate that BCC-specific phages are effective at reducing or eliminating BCC infections. These studies indicate that although in vitro analysis can predict the host range of particular phages against the BCC, in vivo experimentation is important for determining phage persistence and activity. Because the BCC infections to be treated are located in the lungs of patients, we envision that phages against the BCC will be instilled in aerosol form using nebulizers. Our recent studies have shown that phages specific to the BCC are effectively aerosolized without inactivation during in vitro application. We are now in the process of performing in vivo experiments using aerosolized BCC and phage instillations in pulmonary animal models.

#### IV-a PHASE VARIATION IN *BURKHOLDERIA* *MULTIVORANS* CLINICAL ISOLATES

Inês N. Silva<sup>1</sup>, Ana S. Ferreira<sup>1</sup>, Jörg D. Becker<sup>2</sup>, Christian G. Ramos<sup>1</sup>, Dalila Mil-Homens<sup>1</sup>, Jorge H. Leitão<sup>1</sup>, Arsénio M. Fialho<sup>1</sup>, David P. Speert<sup>3</sup>, and Leonilde M. Moreira<sup>1</sup>

<sup>1</sup>Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal

<sup>2</sup>Instituto Gulbenkian de Ciência, Oeiras, Portugal

<sup>3</sup>Division of Infectious and Immunological Diseases, Department of Pediatrics, University of British Columbia, Canada

Contact : Inês Silva; ines.silva@ist.utl.pt

Bacteria belonging to *Burkholderia cepacia* complex (Bcc) emerged as important opportunistic pathogens in cystic fibrosis patients. One of the hypothesized virulence traits in Bcc bacteria is the exopolysaccharide (EPS) produced by strains from all Bcc species (1,2). The mucoid phenotype seems to be affected by phase variation. Recent studies of clinical Bcc isolates demonstrated that the transition of mucoid to nonmucoid phenotype occurs frequently during the course of infection (2). In order to understand the mechanisms underlying phenotype switch in Bcc bacteria, a transcriptional profiling was carried out. To analyze *Burkholderia* global transcripts, an Affymetrix custom microarray was designed based on *B. cenocepacia* J2315 and *B. multivorans* ATCC 17616 genomes. This array has a total of 14824 probe pair sets encompassing 12489 genes from both genomes. A global genome expression analysis of two isogenic mucoid and nonmucoid sequential clinical isolates – *B. multivorans* D2095 and *B. multivorans* D2214, respectively – grown in EPS producing media at 30 °C and 37 °C was then performed. Genes belonging to the *bce-I* and *bce-II* clusters, directing the biosynthesis of cepacian, were down-regulated in nonmucoid *B. multivorans* D2214, as well as genes involved in flagellar assembly, chemotaxis and type VI secretion system. Motility assays confirmed the repression of the flagella biosynthetic regulon since the nonmucoid *B. multivorans* D2214 showed lower swimming and swarming motility than the mucoid strain at 30 °C and 37 °C. Both isogenic strains were capable of producing biofilms at 30° C and 37 °C, however, the mucoid *B. multivorans* D2095 produces biofilm with a lower size when compared to the nonmucoid *B. multivorans* D2214. Virulence assays in the *C. elegans* and *G. mellonella* infection models pointed out the mucoid *B. multivorans* D2095 as more virulent than the isogenic nonmucoid clinical isolate. We are currently characterizing other phenotypic traits that can account for differences in virulence of both strains.

1. Richau *et al.*, *J Clin Microbiol*, 38:1651-1655, 2000
2. Zlosnik *et al.*, *J Clin Microbiol*, 46 (4): 1470-3, 2008

#### IV-b INFLUENCE OF ShvR ON COLONY MORPHOLOGY AND GENE EXPRESSION IN *BURKHOLDERIA* *CENOCEPACIA*

David T. Nguyen<sup>1</sup>, Paola Cescutti<sup>2</sup>, Eoin P. O'Grady<sup>1</sup>, Cora Kooi<sup>1</sup> and Pamela A. Sokol<sup>1</sup>

<sup>1</sup>Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada and <sup>2</sup>Department of Life Sciences, University of Trieste, Trieste, Italy

Contact: Pamela Sokol, psokol@ucalgary.ca

*Burkholderia cenocepacia* typically exhibits rough colony morphology on solid medium but has been shown on occasion to spontaneously undergo a stable transformation producing shiny colony variants (shv). These shv lack the extracellular matrix present in the rough morphotypes. Shv are generally reduced in biofilm production and less virulent in plant and animal infection models than the wildtype. Previously, we have determined that BCAS0225, a LysR-type transcriptional regulator we have designated *shvR*, influences the colony morphology of strain K56-2. A *shvR* mutant exhibits all of the phenotypes associated with the spontaneous shv including shiny colony morphology, reduced biofilm production, avirulence in the alfalfa infection model, and scant extracellular matrix production. Exopolysaccharide (EPS) analysis, protein profiles and transcriptome analysis were used to investigate the mechanisms by which ShvR influences colony morphology and virulence. The quantity of EPS production was not affected in the *shvR* mutant. Nor were there any differences in carbohydrate composition between the *shvR* mutant and wildtype as determined by <sup>1</sup>H-NMR. Transcriptome analysis using the *B. cenocepacia* J2315 microarray identified a number of genes that are differentially expressed in *shvR* mutant. The expression of genes involved in the biosynthesis of four exopolysaccharides characterized in *B. cenocepacia* (cepacian, cellulose, capsule and poly-N-acetylglucosamine) was not altered in the microarray under the conditions examined adding further evidence that alterations in EPS production are not involved in the conversion from the rough to the shv morphotype. Genes with at least two-fold or greater differences in expression were identified on all three chromosomes plus the plasmid, with 102 genes showing increased expression and 88 genes showing decreased expression. Quantitative RT-PCR was used to validate the differential expression of selected genes. Comparisons of extracellular and cell-associated protein profiles revealed at least two proteins that were differentially expressed between the wildtype and *shvR* mutant. Studies are in progress to investigate the influence of the differentially expressed genes and proteins on colony morphology and virulence of *B. cenocepacia*.

#### IV-c EXOPOLYSACCHARIDE PRODUCTION IN BCC AND LINKS TO CLINICAL OUTCOME

James E. A. Zlosnik, Paulo C. Costa, Trevor J. Hird, Paul Mori, Deborah A Henry, David P. Speert  
Division of Infectious and Inflammatory Diseases, Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada.  
Contact: David P. Speert, dspeert@cw.bc.ca

Lung infections caused by bacteria of the *Burkholderia cepacia* complex (BCC) have an unpredictable outcome in cystic fibrosis (CF). We recently described the prevalence of the mucoid phenotype in *Burkholderia cepacia* complex bacteria, showing that the more pathogenic species, *B. cenocepacia*, was more usually nonmucoid while *B. multivorans* isolates were predominantly mucoid. Additionally, we showed that phenotypic switches, from mucoid to nonmucoid, can take place in longitudinal isogenic BCC isolates from CF patients in both *B. cenocepacia* and *B. multivorans* infections. This is counter to the paradigm established for the more common *Pseudomonas aeruginosa* infections. These observations raise two important questions: 1) what is the clinical impact of the different morphologies on the outcome of CF infections and 2) what factors could cause phenotypic switches? We have addressed the first question by conducting a large chart review of 100 BCC infected patients in Vancouver between 1981 and 2007. We plotted FEV1 vs. time and determined the best fit slope to determine the rate of pulmonary deterioration. These data, suggest a more rapid pulmonary deterioration in patients infected with nonmucoid as compared to those infected with mucoid bacteria; patients infected with nonmucoid bacteria had a steeper decline in FEV1 than those infected with mucoid. We will present these data and discuss them, including the challenges of conducting such a study as well as other outstanding questions. We will also describe data that show that EPS phenotype variants can be isolated *in-vitro* by exposure to certain clinically relevant antibiotics.

#### IV-d CEPACIAN BIOSYNTHESIS IN THE *BURKHOLDERIA* GENUS: MECHANISMS AND ROLE IN VIRULENCE

Ana S. Ferreira<sup>1</sup>, Inês N. Silva<sup>1</sup>, Jörg D. Becker<sup>2</sup>, Sílvia A. Sousa<sup>1</sup>, Dalila Mil-Homens<sup>1</sup>, Jorge H. Leitão<sup>1</sup>, Arsénio M. Fialho<sup>1</sup>, and Leonilde M. Moreira<sup>1</sup>  
<sup>1</sup>Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal  
<sup>2</sup>Instituto Gulbenkian de Ciência, Oeiras, Portugal  
Contact : Ana Sofia Ferreira, sofiaferreira@ist.utl.pt

Many strains of the *Burkholderia* genus produce the exopolysaccharide cepacian, composed of a branched acetylated heptasaccharide repeat-unit with D-glucose, D-rhamnose, D-mannose, D-galactose and D-glucuronic acid in the ratio 1:1:1:3:1, respectively (1). The genes directing cepacian biosynthesis are located in two genomic regions of the chromosome 2, named *bce-I* and *bce-II* (2). Three genes, encoding the putative acyltransferases *bceO*, *bceS* and *bceT* are located within cluster *bce-II*. A *B. multivorans* ATCC 17616 *bceS* insertion mutant was obtained. This strain showed a three-day delay in cepacian production in S medium, and a decrease of about 25% of the EPS acetylation content when compared to the wild-type strain. A reduction in the biofilm size and an attenuated virulence phenotype in the *Galleria mellonella* model of infection were also observed for the *bceS* mutant, implicating a role for *bceS* on *B. multivorans* virulence. Together, these results point out for the importance of the acetyl content of the polysaccharide in several properties of the producing bacteria. Two previously characterized genes involved in cepacian biosynthesis were the ones encoding the tyrosine kinase BceF (3,4) and the repeat-unit polymerase BceI (4). Studies using gp91<sup>phox-/-</sup> mice to assess virulence of *bceF* and *bceI* mutants, unable to produce cepacian, showed that the *bceF* mutant strain has significant virulence attenuation on the mice, when compared to the behaviour of the *bceI* mutant. These results suggested a role of the tyrosine kinase in other cellular functions, besides cepacian biosynthesis. In order to identify these other roles, a transcription profiling study was performed using an Affymetrix *Burkholderia* custom DNA microarray. Pools of total RNA were extracted from *B. cepacia* IST408 wild-type and *bceF* mutant strains grown at 37°C. Data analysis is being conducted and the results obtained will be presented and discussed.

- (1) Cescutti *et al.*, *Biochem Biophys Res Commun*, 273: 1088-1094, 2000
- (2) Moreira *et al.* *Biochem Biophys Res Commun*, 312: 323-333, 2003
- (3) Ferreira *et al.* (2007) *Appl Environ Microbiol* 73(2): 524-534
- (4) Sousa *et al.* (2007) *Cell Microbiol* 9(12): 2817-2825

**DETERMINATION OF THE BIOLOGICAL REPEATING UNIT OF CEPACIAN TRANSLOCATED TO THE PERIPLASMIC SPACE BY A FLIPPASE CODED BY THE *bceQ* GENE**

Paola Cescutti, Linda Furlanis, Cristina Lagatolla, Michela Foschiatti, Roberto Rizzo

Department of Life Sciences, University of Trieste, Trieste, Italy

Contact : Paola Cescutti, pcescutti@units.it

Most of the species belonging to the *Burkholderia cepacia* complex (Bcc) produce an exopolysaccharide named cepacian. Its primary structure is rather complicated and its importance as virulence factor is still under investigation. Besides characterising its macromolecular, solution and antioxidant properties, efforts have been made to determine the genes involved in its biosynthesis. One 16 kb gene cluster, including 12 genes (*bceA-bceL*) and located on chromosome 2 of the Bcc species, was initially described by Moreira et al. [1]. Then, a second region, located on the same chromosome 150 kb apart, was proposed [2]. By transposon mutagenesis of an Italian clinical Bcc isolate (BTS7), a mutant derivative (BTS7c) unable to produce cepacian was obtained. The inactivated gene showed high homology (94%) with that coding for a “polysaccharide biosynthesis protein” of two reference Bcc strains, J2315 and *B. sp.* 383, located in the second cluster. *In silico* analysis revealed that this region is approximately 15 kb long and contains 9 genes, that were named *bce* from M to U. Most of them corresponded to those previously described [2], except for three which code for three different acyltransferase, with no homology among each other. The “polysaccharide biosynthesis protein” is encoded by the *bceQ* gene and it showed high homology (26% identities / 48% positives) with the Wzx protein of *E. coli* [3], an integral membrane protein which mediates the transfer of isoprenoid lipid-linked glycans from the cytoplasmic side of the inner membrane to the periplasmic space, known as flippase. Therefore, inactivation of the *bceQ* gene would result in accumulation of the repeating unit linked to the lipid moiety on the inner membrane. In the present work we investigated the function of the protein encoded by the *bceQ* gene in the BTS7 isolate looking for the repeating unit of cepacian linked to the cytoplasmic membrane in the wild type strain and in its mutant derivative. The repeating unit was isolated by size exclusion chromatography and characterised by electrospray mass spectrometry. Fragmentation spectra of the permethylated derivatives were useful to establish the biological structure of the repeating unit.

[1] Moreira, L. M.; Videira, P. A.; Sousa, S. A.; Leitão, J. H.; Cunha, M. V.; Sá-Correia, I. *Biochem. Biophys. Res. Comm.* 2003, 312, 323-333.

[2] Ferreira, A. S.; Leitão, J. H.; Sousa, S. A.; Moreira, L. M. International *Burkholderia cepacia* working group (IBCWG), 2008, April 14-17, Italy.

[3] Rick, P. D.; Barr, K.; Sankaran, K.; Kajimura, J.; Rush, J. S.; Waechter, C. J. *J. Biol. Chem.* 2003, 278, 16534-16542.

**TOWARDS IDENTIFICATION OF A PHAGE DEPOLYMERASE ENZYME FOR THE CEPACIAN**

Sarah Routier<sup>1</sup> and Jonathan J. Dennis<sup>2</sup>

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

Contact : Sarah Routier; sjt2@ualberta.ca

Bacterial exopolysaccharides (EPS) are known to be involved in evasion from host immune systems, adhesion to surfaces, resistance to antimicrobials and formation of biofilms. Therefore, the EPS of bacterial pathogens must be considered when investigating virulence and potential therapy methods. This includes investigations into the *Burkholderia cepacia* complex (BCC) and the alternative therapy method, phage therapy. Preliminary experiments suggest that a recently isolated phage, DC1, carries a depolymerase enzyme that hydrolyzes the EPS of *B. cepacia* LMG18821 and *B. cenocepacia* PC184. EPS degradation can be observed when the bacterial strains are infected with DC1 phage on media that stimulates EPS production as large zones of clearing surrounding the plaques. Although the EPS composition of PC184 is not known, the EPS of LMG18821 contains galactose, ketodeoxyoctulosonic acid (Kdo) and cepacian, the most common EPS component among members of the BCC. In order to determine if the DC1 activity is involved in the degradation of cepacian specifically, we have attempted to mutate each BCC strain so that it is unable to produce this EPS using targeted mutagenesis of *bceB*. The undecaprenyl-phosphate glycosyl-1-phosphate transferase gene encoded by *bceB* involved in the synthesis of cepacian possesses an 11 bp deletion frameshift mutation in ET12 BCC isolates (e.g. *B. cenocepacia* K56-2). This mutation is correlated with the inability of these BCC strains to synthesize the cepacian. A plasmid carrying a copy of *bceB* interrupted by an antibiotic resistance cassette will be introduced into LMG18821 and PC184 in order to create *bceB* knockout mutants. Loss of DC1 halo activity on these BCC mutants will confirm that its enzymatic activity is directed towards the cepacian. Alternatively, BCC K56-2 cepacian production will be complemented by a plasmid carrying a functional copy of *bceB* from LMG18821 and DC1 halo activity on this BCC strain will confirm cepacian as the enzymatic target. The creation of BCC modified strains and their subsequent phenotypic changes along with EPS zones of clearing by DC1 will be discussed.

**MOLECULAR CHARACTERIZATION AND SPECIES DISTRIBUTION OF *BURKHOLDERIA CEPACIA* COMPLEX (BCC) ISOLATES IN CYSTIC FIBROSIS (CF) AND NON-CF PATIENTS IN NEW ZEALAND.**

Christopher E. Pope<sup>1</sup>, Patricia Short<sup>1</sup> and Philip E. Carter<sup>1</sup>  
<sup>1</sup>Institute of Environmental Science & Research Ltd (ESR),  
Kenepuru Science Centre, Porirua, Wellington, New Zealand.  
Contact : Chris Pope, Chris.Pope@esr.cri.nz

Little is known about the distribution of BCC species among individuals within New Zealand. In total 64 BCC positive clinical isolates submitted between 2003 and 2007 to ESR were analyzed, 39 isolates from CF patients and 25 from non-CF patients. Species were identified for the 64 isolates through biochemical tests and molecular techniques. The strains were further characterized using the multilocus sequence typing (MLST) scheme for BCC. All isolates were successfully speciated by *recA* sequence analysis. The most common BCC species identified among the 39 CF patients were *B. multivorans* (79.5%), followed by *B. cenocepacia* (12.8%), *B. stabilis* (5.1%) and *B. vietnamiensis* (2.6%). Within the non-CF group *B. multivorans* predominated (28%), followed by *B. lata*, and *B. stabilis* with a prevalence of 24% each, then *B. cenocepacia* (16%), *B. cepacia* (4%) and *B. seminalis* (4%). MLST analysis among the 39 CF isolates identified 29 sequence types (STs) and included 15 STs not previously described. As expected CF siblings shared the same STs. Overall, five CF isolates and four non-CF isolates were identified as *B. cenocepacia*. In the CF group four were identified as *B. cenocepacia* IIIB strains and one as *B. cenocepacia* IIIA. MLST data showed each isolate to be a distinct ST. In the non-CF group three isolates were identified as *B. cenocepacia* IIIA and one as *B. cenocepacia* IIIB. The diversity of the MLST types observed suggests CF patients in New Zealand are most likely to acquire the BCC from environmental sources although sibling MLST results show person-to-person spread can occur. This diversity also suggests successful infection control measures in New Zealand may prevent person-to-person spread of BCC in CF patients. The low number of CF patients in New Zealand (n~400) may also decrease the likelihood of person-to-person spread in social and clinical settings. This study represents the first in-depth investigation into the epidemiology of BCC in New Zealand showing that person-to-person spread or acquisition of common strains from the clinical setting are unlikely.

**STRAIN MARKERS AND VIRULENCE DETERMINANTS IN AN OUTBREAK STRAIN OF *BURKHOLDERIA MULTIVORANS***

Alan R Brown  
School of Biosciences, University of Exeter, Exeter. UK  
Contact : a.r.brown@exeter.ac.uk

*Burkholderia multivorans* is now the most frequently isolated member of the *Burkholderia cepacia* complex (Bcc) from cystic fibrosis (CF) patients in the UK. In contrast to *B. cenocepacia*, little is known about strain markers and virulence determinants in *B. multivorans*. This study focuses on the 'Glasgow strain' of *B. multivorans*, represented by C1576 (LMG16660) within the published Bcc strain panel. This strain was responsible for an outbreak within a paediatric CF unit in Glasgow in the early 1990s, from which none of the infected children survived to adulthood. The aim of this study was to investigate strain markers and putative virulence determinants within the Glasgow strain. Suppression subtractive hybridization (SSH) was used to compare the genomes of *B. multivorans* ATCC 17616 and C1576, resulting in the identification of approximately 70 sequences which are unique to the Glasgow strain. Based on surveillance by pulsed-field gel electrophoresis (PFGE), it was previously assumed that the Glasgow strain was restricted to that one outbreak in the early 1990s, as the characteristic PFGE pattern had not been observed elsewhere. However, using the strain markers identified by the SSH study, it is now clear that the Glasgow strain remains within the UK's CF population, albeit having undergone significant genomic rearrangements resulting in unique PFGE profiles. The SSH study also identified putative virulence determinants within the Glasgow strain including lipase/esterase-like products and a filamentous heamagglutinin (FHA) locus. Investigations are ongoing to map these putative virulence determinants to genomic islands and characterize their associated phenotypes.

with CF through internet communication may lead to increased physical contact between patients. A rapid and coordinated response by CF and Infection control teams hopefully limited the epidemic.

**V-c**  
**EPIDEMIC OF *BURKHOLDERIA CENOCEPACIA* ET12 IN TORONTO ADULT CYSTIC FIBROSIS CLINIC: LESSONS LEARNED**

Kieran McIntyre<sup>1</sup>, Matthew Muller<sup>2</sup>, Sylvia Ota<sup>2</sup>, Anne Stephenson<sup>1</sup> and Elizabeth Tullis<sup>1</sup>

<sup>1</sup>Toronto Adult Cystic Fibrosis Clinic, <sup>2</sup>Division of Infectious Disease and Microbiology

<sup>2</sup>Department of Medicine, St Michael's Hospital and University of Toronto, Toronto, Ontario, Canada

Contact : Elizabeth Tullis, tullise@smh.toronto.on.ca

Toronto has faced the challenges of caring for patients with cystic fibrosis (CF) infected with *B. cepacia* complex (Bcc) for 3 decades. The dominant genomovar of Bcc has been *B. cenocepacia* ET 12. Since the institution of infection control measures in 1992, the prevalence of infection with Bcc has decreased and currently ~ 20% of our 380 patients are infected. Since 1998, there have been 19 new acquisitions of *Burkholderia*, 9 were *B. multivorans*, 8 *B. cenocepacia*, and 2 *B. gladioli*. The last patient to acquire *B. cenocepacia* was in 2003. Between September 16 and October 7, 2008, 5 CF patients were newly infected with *B. cepacia* complex. Box PCR genotyping (LiPuma lab) revealed that all patients had identical *B. cenocepacia* ET 12. Microbiological testing, including MCBT testing, revealed all isolates were highly multi-drug resistant. All 5 patients had overlapping hospital stays on the CF ward in August 2008 and positive cultures for Bcc occurred 2-6 weeks following discharge from hospital. A patient with known multi resistant *B. cenocepacia* ET12 was also admitted to the CF unit in August and expired due to cepacia syndrome.

A chart review and interviews with patient and staff indicated that there was no common or shared respiratory equipment and that none had consecutive procedures such as bronchoscopy or pulmonary function. No direct linkages were identified between the patient with known *B. cenocepacia* and these 5 patients. Discussions with patients indicated a widespread change in behavior with complex and tight-knit social groups and a variety of 'high risk' activities in outpatient and inpatient contexts. Contact tracing was conducted on all inpatients in hospital during the original admission of the 5 case patients, as well as any outpatients known to have contact with these patients. No new cases have been identified from the 51/54 (94%) contacts screened. Environmental swabs from the unit, clinic, and pulmonary function lab were all negative for Bcc. The clinical outcome of this outbreak has been sobering. 4 of 5 patients have died between 37 and 104 days post acquisition. One patient is still alive and well 155 days post acquisition.

*B. cenocepacia* has been a part of our CF population for decades but a new "vulnerable" patient population had emerged. The epidemic curve suggests a point source transmission of *B.cenocepacia* on the CF ward. The etiology of the spread is uncertain – contact between patients, indirect contact spread through health care workers hands, or environmental contamination are all possible. There is no room for complacency in CF clinics with patients with Bcc. Ongoing education of infection control measures is necessary for staff and patients. The increased ability to "meet" others

## V-d

### EXPANDING MULTILOCUS SEQUENCE TYPING FOR *BURKHOLDERIA* SPECIES

Theodore Spilker<sup>1</sup>, Adam Baldwin<sup>2</sup>, Amy Bumford<sup>1</sup>, Chris Dowson<sup>2</sup>, Eshwar Mahenthiralingam<sup>3</sup>, and John J. LiPuma<sup>1</sup>

<sup>1</sup> Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI, USA

<sup>2</sup> Department of Biological Sciences, Warwick University, Coventry UK

<sup>3</sup> Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Contact: John J. LiPuma; jlipuma@umich.edu

The multilocus sequence typing (MLST) scheme for *Burkholderia cepacia complex* (Bcc) has provided important insights into population dynamics, diversity and recombination events. It has also been used to classify novel species and can be effective for the identification of previously misclassified *Burkholderia* species in the complex. The expansion of the Bcc with the addition of seven new species, and our appreciation that other *Burkholderia* species, particularly *B. gladioli*, are also involved in human infection, provides an opportunity to expand the capacity of the current MLST scheme. We thus sought to redesign the primers targeting the loci in the current MLST scheme to (i) more reliably amplify these seven loci from all Bcc species; (ii) amplify these loci from all *Burkholderia* species, including *B. gladioli* and as yet unclassified *Burkholderia* species; and (iii) enable the use of a single primer set per locus for both amplification and DNA sequencing. We aligned all seven loci in the current MLST scheme (*atpD*, *gltB*, *gyrB*, *recA*, *lepA*, *phaC* and *trpB*) from all available sequenced *Burkholderia* strains to design new primers. The new genus-level primers were specifically designed to amplify sequences that include the regions amplified by the original MLST primers; therefore, the new primers provide results that are entirely compatible with the current MLST scheme. To confirm that the new primers more reliably amplify loci from Bcc strains, we successfully amplified 41 loci (7 *atpD*, 10 *gltB*, 17 *gyrB*, 2 *recA*, 3 *phaC*, and 2 *trpB*) from 25 Bcc strains that failed to amplify with the original primers. To assess the utility of the new primers at the genus level, we amplified all seven loci from 74 strains representing 38 named *Burkholderia* species (including all 17 Bcc species) as well as another 14 unclassified *Burkholderia* strains. In all cases, the new genus-level primer sets provided PCR products of the expected size that were confirmed as the intended target by DNA sequence analyses. The re-designed MLST primers expand the utility of the current *Burkholderia* MLST scheme.

## V-e

### HYPERMUTABILITY OF *BURKHOLDERIA CENOCEPACIA* IN CYSTIC FIBROSIS

Jane L. Burns and Adam Griffith

Division of Infectious Diseases, Seattle Children's Research Institute, Seattle, Washington, USA

Contact : Jane Burns, jane.burns@seattlechildrens.org

*Burkholderia cepacia complex* (BCC) isolates have long been known to have a high degree of genetic diversity, attributed to the large size of the genome and to the presence of many insertion sequences. However, cystic fibrosis (CF) isolates of BCC appear to have an even more diverse phenotype than either environmental or non-CF clinical isolates. Like *P. aeruginosa*, BCC is able to adapt to the environment in the CF airway; the source of this adaptability has not been identified. An increased mutation frequency would result in an efficient and rapid adaptation to the very unique niche occupied by BCC in CF airway infections and has been seen in other CF pathogens including *P. aeruginosa*, *S. aureus*, and *H. influenzae*. Hypermutability, the presence of a higher than normal genetic mutation rate, has been found to be caused by alteration in genes that are components of the DNA error avoidance systems, particularly those involved in mismatch repair systems. Among CF pathogens the prevalence of hypermutability may be as high as 36% compared with other bacterial populations, such as *E. coli* and *Salmonella*, where subpopulations of both pathogenic and commensal strains have a reported prevalence between 1.4% and 6.7%. The methyl-directed mismatch repair gene, *mutS*, has been most commonly implicated in CF isolates. Using the Luria DelBrook fluctuation assay, we examined 71 environmental and clinical isolates of BCC for evidence of hypermutability. Mutation frequencies ranged from  $1.9 \times 10^{-5}$  to  $3.5 \times 10^{-9}$  with a mean of  $6.8 \times 10^{-7}$  and a median of  $7.30 \times 10^{-8}$ . For other organisms, hypermutability has been defined as greater than  $10^{-7}$ . There were no mutators identified among the environmental or non-CF clinical isolates (41 total isolates). However, among the 30 BCC isolates from CF patients, 4 isolates were identified as hypermutable. Three of the mutator strains were *B. cenocepacia* and further studies focused on them. Among the 11 *B. cenocepacia* tested, 3 of 4 CF isolates and 0 of 7 non-CF isolates were hypermutable. All of the hypermutable strains were ET12 lineage, but from discrete sources and with distinct PFGE patterns. No obvious differences were identified between mutator and non-mutator *B. cenocepacia* isolates with regard to antibiotic susceptibility, biofilm formation, or cellular invasion; however, mutators were much more inflammatory than non-mutators in a murine model of chronic infection. In summary, hypermutability appears to be common among CF isolates of *B. cenocepacia* and has the potential to contribute to increased virulence.

## V-f

### SPECIES-ASSOCIATED OUTCOMES OF *B. CEPACIA* COMPLEX INFECTION IN CF

Mary Corey<sup>1</sup>, Jeffery Charbeneau<sup>2</sup>, Susan Murray<sup>3</sup> and John J. LiPuma<sup>4</sup>

<sup>1</sup>Child Health Evaluative Sciences, Hospital for Sick Children, University of Toronto, Canada

<sup>2</sup>Department of Health Studies, University of Chicago, USA

<sup>3</sup>Department of Biostatistics and <sup>4</sup>Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, USA

Contact: Mary Corey, mary.corey@sickkids.ca

Several distinct species can be identified among bacteria collectively referred to as the “*B. cepacia* complex” (Bcc). These species are phylogenetically very closely related and phenotypically nearly indistinguishable, but differ significantly in their ecology and epidemiology. They also show a marked difference in the frequency with which they infect persons with cystic fibrosis (CF), with two of the ten species in the complex accounting for approximately 80-85% of infection. Genotyping studies further indicate that at a sub-species level, some specific strains are particularly common among CF patients. Limited observations suggest that Bcc species and strains also differ in their virulence (and therefore clinical impact) during infection in CF patients. We tested the hypothesis that Bcc species and/or strains differ in their clinical impact on the health status of infected CF patients. New cases of Bcc infection in the period 1997 to 2007 were identified and clinical data was recorded from two years before infection to the end of 2008, using microbiologic data from the CFF *Burkholderia cepacia* Research Laboratory and Repository and clinical data from the US CFF National Patient Registry. Bcc isolates were genotyped using BOX-PCR. Clinical outcome was assessed by Cox proportional hazards regression analysis of survival and by mixed model analysis of longitudinal measures of Forced Expiratory Volume in 1 second (FEV<sub>1</sub>). There were 550 patients newly infected with Bcc identified from the Repository during this interval. One historically important strain (*B. cenocepacia* ET12) was not seen, so patients from Toronto, where this strain has been endemic, were included in the analysis. Overall, *B. multivorans* (n=242) was the most common species isolated over this time period, representing a shift from the previously most prevalent *B. cenocepacia* (n=174). Among the *B. cenocepacia* infected patients, were several infected with previously described ‘epidemic’ strains, including the PHDC (n=46), Midwest (n=25), and ET12 (n=11) strains; the remaining 92 *B. cenocepacia*-infected patients had ‘unique’ strains. Also identified were 29 patients infected with *B. dolosa* strain SLC6. The set also included 13 patients infected with *B. cepacia* and 77 infected with one of the remaining Bcc species. Twenty-six cases of *B. gladioli* infection were also identified and included in the analysis. Survival curves, adjusted for gender and age and FEV<sub>1</sub> at infection, showed the poorest survival for patients infected with *B. cenocepacia* ET12 (p=0.049) and *B. cepacia* (p=0.085), compared to *B. multivorans*, with similarity among other strains. Lung function decline was similar across species and strains except

for patients infected with *B. dolosa* SLC6, who had the best lung function at time of infection and the greatest rate of decline over the next 5 years. Overall the health impact of different species/strains of Bcc was remarkably similar. Poorer survival with ET12 infection is confirmed, but less dramatic than in early reports. The rapid decline in lung function associated with an outbreak of *B. dolosa* SLC6 validates the continued vigilance to avoid the spread of infectious organisms in the CF community.

**VI-a**  
**THE *BURKHOLDERIA* GENOME DATABASE:  
FACILITATING THE COMPARISON AND  
VISUALIZATION OF UPDATED GENOME DATA AND  
ANALYSES.**

Geoffrey L. Winsor, Raymond C. Lo, Bhav Khaira, Thea Van Rossum, Matthew D. Whiteside and Fiona S.L. Brinkman  
Department of Molecular Biology and Biochemistry, Simon Fraser University, Greater Vancouver, BC, Canada.  
Contact: Fiona Brinkman, brinkman@sfu.ca

The *Burkholderia* Genome Database is a new resource for peer-reviewed, continually-updated annotations for selected *Burkholderia cepacia* complex genomes, coupled with comparative analyses functionality applied to all *Burkholderia* genome sequences. We present a practical overview of what this database can do to aid *Burkholderia* research and describe what functionalities are planned for the future. In order to facilitate better cross-strain and cross-species genome comparisons, we have developed or are incorporating methods to improve the identification of orthologs and identify genes undergoing unusual selection. A choice of simple and more flexible user-friendly advanced search features allows researchers to search and compare annotations within or between the genomes of *Burkholderia* strains, returning annotations from multiple genomes or gene families suitable for simultaneous viewing and downstream analyses. We are also incorporating new, very accurate protein subcellular localization predictions for the deduced proteome from each of these genomes - predictions that can aid the identification of new cell surface or secreted therapeutic targets or vaccine component candidates. We welcome suggestions on the types of analyses and database functionalities that should be added in the future. Regardless, continually updated annotation information should ensure that this resource continues to be of value to the *Burkholderia cepacia* complex research community. Further comparative analyses with other newly-sequenced, related strains should provide insight into strain-specific features that may be exploited to better understand the virulence and antimicrobial resistance exhibited by these cystic fibrosis-relevant pathogens.

**VI-b**  
**MULTILOCUS SEQUENCE TYPING OF *B. CEPACIA*  
COMPLEX WITHOUT A NEED  
OF BACTERIAL CULTURE**

Pavel Drevinek, Sarka Vosahlikova and Ondrej Cinek  
Dept of Pediatrics, 2<sup>nd</sup> Medical School, Charles University, Prague, Czech Republic  
Contact : Pavel Drevinek, pavel.drevinek@Lfmotol.cuni.cz

Highly sensitive detection of the *Burkholderia cepacia* complex (Bcc) organisms in sputum of cystic fibrosis patients has been achieved by using nested PCR. This method allows to diagnose infection in its early stage, often when the culture-based examination is still negative. Therefore, it is considered an invaluable tool in assessment of microbiological status in all presumably Bcc negative patients. However, samples which had been found positive only by PCR were not analyzable for epidemiological purposes any further since traditional fingerprinting methods such as PFGE or RAPD require a pure culture. Also, Multilocus Sequence Typing (MLST) was originally supposed to work only with DNA extracted from grown bacteria. Nevertheless, its principle based on DNA sequencing of several genes does not rule out its applicability in situations where a bacterial culture is not available.

To evaluate whether strain identification is possible directly from clinical material, we carried out a pilot study with samples which differed by (i) intensity of Bcc positivity (round 1 vs. round 2 of nested PCR), (ii) Bcc species (*B. cenocepacia* vs. *B. multivorans* vs. *B. stabilis*), and (iii) co-infection with other pathogens (none vs. *S. aureus*). We took an advantage of MLST scheme developed for the Bcc whose primers were designed separately for gene amplification and for sequencing. As the latter primers formed the inner pair, in addition to sequencing they seemed suitable also for use in a second round of nested PCR. Similarly to PCR-based Bcc detection from sputa, pre-amplification of MLST genes was expected to enormously increase the method sensitivity and to allow a typing of bacteria which were present in a sample in a non-cultivable amount.

All samples examined were successfully amplified in all seven MLST genes, with some of them being positive only after a second PCR round. Unambiguous sequencing results leading to identification of strain sequence type were obtained for each of three Bcc species analyzed. No interference with DNA of either human or other bacterial origin was detected.

This proof-of-principle study shows that strain identification of Bcc bacteria is feasible from clinical samples, even from those where cultivation fails due to a low bacterial load.

Supported by grants of Czech Ministry of Education MSM0021620812 and VZ642036405.

## VI-c

### MAPPING THE *BURKHOLDERIA CENOCEPACIA* NICHE RESPONSE VIA HIGH-THROUGHPUT SEQUENCING

D.R. Yoder-Himes<sup>1</sup>, P.S.G. Chain<sup>1,2,3</sup>, Y. Zhu<sup>2</sup>, O. Wurtzel<sup>4</sup>, E.M. Rubin<sup>2,5</sup>, J.M. Tiedje<sup>1,\*</sup>, and R. Sorek<sup>4,\*</sup>

<sup>1</sup> Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824

<sup>2</sup> Microbial Program, DOE Joint Genome Institute, Walnut Creek, CA 94598

<sup>3</sup> Biosciences & Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

<sup>4</sup> Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

<sup>5</sup> Genomics Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720

\* These authors contributed equally

Contact : Deborah Yoder-Himes, yoderdeb@msu.edu

We used RNA-seq to quantitatively assess the transcriptional response of the bacterial opportunistic cystic fibrosis (CF) pathogen and endemic soil dweller, *Burkholderia cenocepacia*, in conditions mimicking these two environments. By sequencing 762 million bases of cDNA from two closely related *B. cenocepacia* strains [one isolated from a CF patient (AU1054) and one from soil (HI2424)], we identified a number of potential virulence factors expressed under CF sputum-like conditions, while genes involved in nitrogen scavenging and two-component sensing were among those induced under soil-like conditions. With this technique, we also discovered 13 differentially regulated putative non-coding RNAs. We detected a large number of regulatory differences between the two strains, despite their high genomic similarity, which may represent adaptations to their respective niches. The soil strain shows a stronger global gene expression response to its environment which is consistent with the need for a more dynamic reaction to the heterogeneous conditions of the soil environment

**VII-a**  
**VISUALIZATION OF THE INTRACELLULAR LIFE STYLE OF *BURKHOLDERIA CEPACIA* COMPLEX STRAINS IN ZEBRAFISH EMBRYOS**

Annette Vergunst and David O'Callaghan  
INSERM ESPRI 26, UFR Médecine, Avenue Kennedy, 30908  
Nîmes, France  
Contact: Annette Vergunst, Annette.vergunst@univ-montp1.fr

We further exploited the advantages of the zebrafish embryo to study virulence of *Burkholderia cepacia complex* strains in more detail. The number of studies using zebrafish as an infection model is growing rapidly, due to several important features including a fully developed immune system with significant similarities to that of humans and the possibility to follow infections in real time. Using real time live visualization, bacterial replication assays and survival studies we found that *B. cenocepacia* isolates belonging to the epidemic ET12 lineage are extremely virulent for zebrafish embryos; intravenous injection of 5 to 10 bacteria of K56-2 was sufficient to cause embryo mortality within three days. Micro injected bacteria are taken up by macrophages, and after an initial survival phase of 5 to 6 hours the bacteria replicate intracellularly. We will show in more detail the way bacteria seem to disseminate to neighbouring cells. Later, the infection became bacteraemic and bacteria started replicating extracellularly. Differences in virulence based on clinical observations of strains both within one species (*B. cenocepacia*) and between different Bcc species were reflected in the zebrafish model. Interestingly, a K56-2 *cepR* quorum sensing mutant was highly attenuated and greatly reduced in its ability to replicate and spread to neighbouring cells, suggesting that the CepIR quorum sensing system is required for effective bacterial replication and dissemination. Our new findings further validate the zebrafish embryo as a novel tool to study in detail the molecular basis of the intracellular life style of this bacterium in an animal model, as well as the response of the host's immune system on infection.

**VII-b**  
**DIFFERENTIAL MODULATION OF HUMAN DENDRITIC CELL FUNCTIONS BY *BURKHOLDERIA CENOCEPACIA* AND *BURKHOLDERIA MULTIVORANS***

Kelly L. MacDonald<sup>1</sup> and David P. Speert<sup>1,2</sup>  
<sup>1</sup>Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, CANADA  
<sup>2</sup>Department of Pediatrics, University of British Columbia, Vancouver, BC, CANADA.  
Contact : Kelly MacDonald, klmd@interchange.ubc.ca

*Burkholderia cepacia complex* (BCC) bacteria cause pulmonary infections which can progress into fatal, overwhelming septicemia in chronic granulomatous disease (CGD) or cystic fibrosis (CF) patients. *B. cenocepacia* and *B. multivorans* cause the majority of BCC infections in CF patients, but they differ dramatically in apparent virulence: *B. cenocepacia* induces greater inflammation and toxicity than *B. multivorans* in a murine pulmonary infection model, and the former is associated with a much poorer prognosis in CF patients. Dendritic cells (DCs), one of the resident lung mononuclear phagocytes, are crucial cells linking innate and adaptive immunity and may serve as systemic vectors for this putative intracellular pathogen. We hypothesize that *B. cenocepacia* and *B. multivorans* modulate the normal functions of primary human monocyte-derived DCs. DCs were challenged with live *B. multivorans* or *B. cenocepacia* at a multiplicity of infection of 0.3:1. After 2 hours, 8 µg/ml of meropenem was added and the DCs were re-incubated up to 24 hours. Effects of the bacteria on DC maturation were determined using flow cytometry. *B. cenocepacia*, but not *B. multivorans*, impaired DC maturation; DCs co-incubated for 24 hours with *B. cenocepacia*, but not *B. multivorans*, had reduced expression of co-stimulatory surface receptors when compared with BCC lipopolysaccharide-matured DCs. Cytokine enzyme linked absorbent assays revealed that *B. multivorans* and *B. cenocepacia* induced DCs to secrete similar amounts of IL-6, TNF-alpha, IL-10 and IL-12 after 24 hours. *B. cenocepacia*, but not *B. multivorans*, also induced significant necrosis in dendritic cells after 12-24 hours of co-incubation, as determined by flow cytometry following annexin V and propidium iodide staining. DC necrosis only occurred after phagocytosis of live *B. cenocepacia*; DCs exposed to heat-killed bacteria, bacterial supernatant, or those pre-treated with cytochalasin D (to prevent phagocytosis) then exposed to live bacteria remained viable. We also examined necrosis induction by a panel of ten *B. multivorans* and *B. cenocepacia* clinical isolates. None of the five *B. multivorans* isolates initiated significant DC necrosis after 24 h, whereas four out of five *B. cenocepacia* isolates stimulated DC cell death. In conclusion, the ability of *B. cenocepacia* to interfere with DC maturation and induce necrosis may contribute to its pathogenicity in CF and CGD patients.

**VII-c**  
**ELUCIDATION OF INTRACELLULAR GENE**  
**EXPRESSION BY *BURKHOLDERIA***  
***CENOCEPACIA* WITHIN MURINE MACROPHAGES**

Jennifer S. Tolman and Miguel A. Valvano  
Department of Microbiology & Immunology, Schulich School  
of Medicine & Dentistry, University of Western Ontario,  
London, Ontario, Canada.  
Contact: Jennifer Tolman, jtolman2@uwo.ca

*Burkholderia cenocepacia* is a multi-drug resistant opportunistic pathogen affecting immunocompromised individuals, particularly those with cystic fibrosis (CF). *B. cenocepacia* is able to establish a persistent infection, and in a proportion of CF patients causes “cepacia syndrome”, a fatal, acute, necrotizing pneumonia. The mechanisms by which *B. cenocepacia* causes disease are poorly understood. It is thought that the ability of *B. cenocepacia* to survive within host phagocytic cells contributes to the establishment of a persistent infection and the dissemination of the bacteria throughout the body. Previous research in our lab has shown that *B. cenocepacia* is able to delay phagosomal maturation in macrophages, slowing delivery of the bacteria to the lysosome; *B. cenocepacia* is also able to delay assembly of the NADPH oxidase and alter the actin cytoskeleton of the macrophage. To further characterize this intracellular behaviour we are utilizing a genetic approach.

To delay phagosomal maturation, intracellular *B. cenocepacia* must be viable; thus, we hypothesize that *B. cenocepacia* alters its gene expression to cause this delay. Using “selective capture of transcribed sequences” (SCOTS), optimized for *B. cenocepacia*, we have identified genes expressed solely within the context of the phagosome. Thus far, we have identified a number of genes expressed during infection of the macrophage, including those encoding transcriptional regulators, transport proteins, metabolic enzymes, and elements of the Type VI secretion system. Previous studies in our laboratory have indicated *in vivo* relevance for a number of these genes, providing external validation. The SCOTS technique is providing valuable information on the intracellular gene expression of *B. cenocepacia*, which may contribute to an explanation of the intracellular behaviour of *B. cenocepacia* within murine macrophages.

**VII-d**  
**THE ACTIVATION OF THE PHOSPHATIDYL-**  
**INOSITOL-3 KINASE (PI3K) PATHWAY IN**  
**RESPONSE TO THE *BURKHOLDERIA CEPACIA***  
**COMPLEX**

Billie Velapatiño<sup>1</sup>, and David P. Speert<sup>2</sup>  
<sup>1</sup>Department of Pathology and Laboratory Medicine,  
University of British Columbia, Vancouver, British Columbia,  
Canada.  
<sup>2</sup>Division of Infectious and Immunological Diseases,  
Department of Pediatrics, University of British Columbia and  
Centre for Understanding and Preventing Infections in  
Children, Vancouver, British Columbia, Canada.  
Contact: David P. Speert, dspeert@cw.bc.ca.

*Introduction:* Cystic fibrosis (CF) is the most common fatal inherited disease in Canada, and bacterial infection is the leading cause of death in patients with CF. Among the different bacteria that can cause infection in patients with CF, *Burkholderia cepacia* complex (*Bcc*) is the most serious, and infection with microbes from this group can result in rapid decline in lung function and death. Within the *Bcc*, *B. cenocepacia* (*Bc*) has caused about 80% of CF infections in Canada and *B. multivorans* (*Bm*) most of the remainder (Speert, 2002). *Bc* has the capacity to cause a much more serious infection than *Bm*, but a good explanation for this difference in virulence remains unidentified. Furthermore, *Bm* can persist in a benign form in macrophages or epithelial cells for extended periods, but *Bc* is readily cleared or kills the murine host (Chu, 2004). The phosphatidylinositol-3 kinase (PI3K/AKT) signaling pathway contributes to the regulation of a variety of biological processes, and it has been shown to play a role in host immune responses to microbial invasion (Hazeki, 2007). We investigated the signaling events, specifically in the PI3K/AKT signaling pathway, after bacterial-host interaction to determine if measures of cellular activation (phosphorylation and the release of inflammatory cytokines) are associated with the differential virulence and persistence of these bacteria in infected CF patients.

*Objective:* This study explored the signaling events after bacterial-host interaction to determine the influence of the PI3K/AKT pathway on *Bcc*-induced cellular inflammatory responses.

*Experimental approach:* Macrophages and bronchial epithelial CF cell lines were infected with *Bc* and *Bm* and optimal conditions were determined. Lysates from uninfected and infected cells were analyzed by Western blot or by immunoprecipitation using the appropriate antibodies.

*Results:* Our preliminary data suggest that the activation of the PI3K/AKT pathway occurred in CF bronchial epithelial cell line IB3-1, in THP-1 derived macrophages and in human monocyte-derived macrophages after infection with live *Bc*. PI3K/AKT was not activated by *Bc*-LPS in IB3-1 cell line but only with live *Bc*. Activation lasted at least three hours. Moreover, PI3K/AKT activation required live bacteria in THP-1 derived macrophages and this activation started 30 min after infection. Future experiments will investigate the influence of *Bm* on this pathway as well as the release of

cytokines after stimulation with live *Bcc* and by using PI3K inhibitors.

#### VII-e

### **BURKHOLDERIA CENOCEPACIA INDUCED LOSS OF CORTICAL ACTIN IN RAW264.7 MEMBRANES AND ACCUMULATION OF ACTIN AND RHO AT THE BACTERIAL-CONTAINING VACUOLAR**

<sup>1</sup>Jonathan Plumb, <sup>1,2</sup>Kassidy Huynh, <sup>1</sup>Iskra Peltekova, <sup>3,4</sup>Miguel Valvano and <sup>1</sup>Sergio Grinstein

<sup>1</sup>The Programme in Cell Biology, Hospital for Sick Children, Toronto, Canada; <sup>2</sup>Department of Biochemistry, University of Toronto, Toronto, Canada; <sup>3</sup>Department of Microbiology and Immunology, University of Western Ontario, London, Canada; <sup>4</sup>Department of Medicine, University of Western Ontario, London, Canada.

Contact: Sergio Grinstein, sga@sickkids.ca

Cystic fibrosis patients can succumb to life-threatening infections from *Burkholderia cenocepacia*, an opportunistic gram-negative bacterium. A clinically-relevant strain of *B. cenocepacia* (J2315) was used to investigate the mechanisms by which these bacteria survive inside macrophages. As early as 60 minutes into infection, macrophages invaded by live but not dead bacteria became rounded and displayed ball-on-a-string-like structures. We utilized phalloidin to visualize the actin cytoskeleton. Remarkably, after 60 minutes, cells infected with live bacteria lacked cortical F-actin and accumulated F-actin around the engulfed bacteria, whereas with dead bacteria, no changes in cortical actin were documented. Because actin was drastically altered, we examined the participation of Rho family GTPases that are known to regulate actin dynamics. Our results showed that Rac and Cdc42 were active in the plasma membrane of uninfected RAW264.7 cells, or cells infected with dead bacteria. By contrast, Rac and Cdc42 activity at the membrane was greatly diminished in cells infected by live *B. cenocepacia*. RhoA was associated with the vacuole containing both live and also dead bacteria. Consistent with disruptions in actin polymerization, phagocytic and macropinocytic indices were both lower in cells infected with live as compared to dead bacteria. Furthermore, we saw a distinct loss of vinculin-positive focal adhesions in cells infected with live *B. cenocepacia*. Together, these data suggest that *B. cenocepacia* significantly altered actin assembly at the plasma membrane and induced accumulation of actin and Rho at the vacuolar membrane.

#### VII-f

### **BcsK, A PUTATIVE TRANSGLYCOSYLASE CRITICAL FOR THE TYPE 6 SECRETION SYSTEM ACTIVITY OF BURKHOLDERIA CENOCEPACIA**

Daniel Aubert<sup>1</sup>, Douglas K. McDonald<sup>1</sup> and Miguel A. Valvano<sup>1,2</sup>

<sup>1</sup>Department of Microbiology & Immunology, <sup>2</sup> Schulich School of Medicine, University of Western Ontario, London, Ontario, Canada.

Contact : Daniel Aubert, daubert@uwo.ca

Type 6 secretion systems (T6SSs) are found in numerous Gram-negative pathogens or symbiotes that closely interact with eukaryotic cells. Beside the relevance of the T6SS in the pathogenicity of many bacteria including *Burkholderia cenocepacia*, little is known about the structure and organization of the T6S apparatus. Most of the genes of the T6SS clusters are of unknown function and need to be characterized in more detail.

Many macromolecule transport secretion systems clusters (bacteriophage entry, type II secretion, type III secretion and type IV secretion) often encode their own lytic transglycosylase (LT). LTs (also called “space making” enzymes) are hypothesized to locally degrade the glycan chains of the peptidoglycan to allow the efficient assembly of protein complexes in the cell envelope.

We looked for the presence of genes encoding putative LTs within the T6SS cluster of *B. cenocepacia* and identified *bcsK*. Expression of BcsK in *Escherichia coli* leads to cell lysis only when BcsK gains access to the periplasmic space suggesting that BcsK might act indeed as a peptidoglycan-degrading enzyme. Secretion and macrophage infection assays revealed that BcsK is critical for the T6SS activity in *B. cenocepacia* K56-2. Experiments employing bacterial two-hybrid system demonstrated that the N-terminus of BcsK is required for interaction with BcsL, another component of the T6SS also conserved in T6SSs homologues of other Gram-negative bacteria. We found that BcsK is a periplasmic protein and that its secretion to the periplasm requires the N-terminus of BcsK. Together these results suggest that interaction between BcsL and BcsK is required to target BcsK to the periplasmic space.

## VII-g

### **PSEUDOMONAS AERUGINOSA ALGINATE PROMOTES BURKHOLDERIA CENOCEPACIA INFECTION BY INTERFERING WITH HOST INNATE DEFENSE MECHANISM**

Umadevi Sajjan<sup>1\*</sup>, Rachana Murthy<sup>1</sup>, Joanna B. Goldberg<sup>3</sup>, Marc B. Hersenson<sup>1,2</sup>

<sup>1</sup>Department of Pediatrics and Communicable Diseases, <sup>2</sup>Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109. <sup>3</sup>Department of Microbiology, University of Virginia Health System, Charlottesville, VA 22908

Polymicrobial infection is an important factor in the pathogenesis of acute and chronic respiratory disorders including cystic fibrosis (CF). *P. aeruginosa* (PA), a major respiratory pathogen in CF, can promote colonization of other opportunistic pathogens. *Burkholderia cenocepacia* (BC), which normally infects adolescent patients encounters alginate elaborated by mucoid PA. To determine whether alginate promotes BC infection, C57BL/6 mice were infected with BC suspended in either PBS or PA alginate and monitored for bacterial load, and inflammation. While mice infected with BC/PBS cleared all the bacteria within 3 days and resolved inflammation by 5 days, BC/alginate group showed persistence of bacteria, increased cytokines levels and inflammation up to 5 days. Further, alginate attenuated the phagocytosis of BC by macrophages and neutrophils, and proinflammatory response from macrophages and airway epithelial cells. Finally, PA alginate also enhanced bacterial persistence in CF mice leading to pneumonic consolidation and bacteremia. The observed effects are specific to PA alginate, because enzymatically degraded alginate or other polyuronic acids did not facilitate bacterial persistence. These observations suggest that PA alginate facilitates BC infection by interfering with host innate defense mechanisms, and that CF mice are more susceptible to BC infection than normal mice.

Supported by American Cystic Fibrosis Foundation, SAJJAN0610

