19th Annual Meeting

April 15 - 18, 2015

Sheraton Vancouver Wall Centre
1088 Burrard Street, Vancouver, BC, Canada

Program and Abstracts

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Wednesday, April 15, 2015

3:30 pm to 5:30 pm	**Registration**
5:30 pm to 8:00 pm	**Reception**
7:00 pm to 8:00 pm	**Keynote Lecture: The International Burkholderia cepacia Working Group: 20 years and still going strong** Dr. David Speert, University of British Columbia

Thursday, April 16, 2015

8:00 am to 9:00 am	**Breakfast & Registration**
9:00 am to 9:10 am	Welcome Dr. Silvia Cardona, University of Manitoba and Dr. James Zlosnik, University of British Columbia
9:10 am to 10:30 am	**Session 1. Clinics and Epidemiology in a Genomic Era** Moderators: Dr. Valerie Waters and Dr. Pavel Drevinek
9:10 am to 9:30 am	1.01 James Zlosnik THE CANADIAN BURKHOLDERIA CEPACIA COMPLEX RESEARCH AND REFERRAL REPOSITORY: PAST, PRESENT AND FUTURE.
9:30 am to 9:50 am	1.02 Charlotte Peeters COMPARATIVE GENOMICS OF CLINICAL AND ENVIRONMENTAL B. MULTIVORANS ISOLATES
9:50 am to 10:10 am	1.03 Pavel Drevinek GENE EXPRESSION PROFILING OF THE BURKHOLDERIA CONTAMINANS BLOODSTREAM ISOLATE
10:10 am to 10:30 am	Conclusions by moderators and further discussions
10:30 am to 11:00 am	**Coffee Break**
11:00 am to 12:00 pm	**Conference: Burkholderia spp. analysis in an era with thousands of genomes** Dr. Fiona S. L. Brinkman, Simon Fraser University
12:00 pm to 12:50 pm	**Lunch**
12:50 pm to 3:00 pm	**Session 2. Virulence and Host Interactions** Moderators: Dr. Jonathan Dennis and Dr. Amal Amer
12:50 pm to 1:10 pm	2.04 Lucie Kalferstova IS THE TYPE III SECRETION SYSTEM OF BURKHOLDERIA CENOCEPACIA ST32 INVOLVED IN CEPACIA SYNDROME?

1:10 pm to 1:30 pm	2.05 Benjamin Kopp BURKHOLDERIA SPECIES SUBVERT THE OXIDATIVE BURST IN HUMAN CYSTIC FIBROSIS MACROPHAGES
1:30 pm to 1:50 pm	2.06 Margarida Castro Gomes MODELING THE ROLE OF THE GLOBAL REGULATOR SHVR OF BURKHOLDERIA CENOCEPACIA IN VIRULENCE USING ZEBRAFISH EMBRYOS
1:50 pm to 2:10 pm	2.07 Amal Amer EPIGENETIC MODIFICATION OF AUTOPHAGY MOLECULES BY *BURKHOLDERIA CENOCEPACIA*
2:10 pm to 2:30 pm	2.08 Matthew Schaefers THE BURKHOLDERIA FIX PATHWAY SENSES OXYGEN AND MEDIATES VIRULENCE AND BIOFILM FORMATION
2:30 pm to 2:50 pm	2.09 Jonathan Dennis GENERAL PROTEIN GLYCOSYLATION CONTROLS VIRULENCE IN BURKHOLDERIA CENOCEPACIA
2:50 pm to 3:00 pm	Conclusions by moderators and further discussions
3:00 pm to 3:30 pm	**Coffee Break**
3:30 pm to 5:30 pm	**Session 3. Physiology and Polymicrobial Interactions** Moderator: Dr. Vaughn Cooper and Dr. Leo Eberl
3:30 pm to 3:50 pm	3.10 Ines Silva GENETIC VARIATION ANALYSIS OF BURKHOLDERIA MULTIVORANS DURING LONG-TERM COLONIZATION OF A CYSTIC FIBROSIS PATIENT
3:50 pm to 4:10 pm	3.11 Elisabeth Steiner THE RPFFR QUORUM SENSING SYSTEM ATTENUATES C-DI-GMP-MEDIATED EXPRESSION OF THE BURKHOLDERIA CENOCEPACIA BCAM1330-1341 EXOPOLYSACCHARIDE GENE CLUSTER
4:10 pm to 4:30 pm	3.12 Sean Buskirk UNDER SELECTION: BIOFILM SELECTION PLACES YCIR AT THE INTERSECTION OF QUORUM SENSING AND C-DI-GMP METABOLISM
4:30 pm to 4:50 pm	3.13 Christian Jenul THE BIOSYNTHETIC ORIGIN OF A BIOACTIVE N-HYDROXY-N-NITROSOAMINO COMPOUND FROM BURKHOLDERIA CENOCEPACIA H111

4:50 pm to 5:10 pm	3.14 Nejc Stopnisek ABIOTIC AND BIOTIC FACTORS INFLUENCE SOIL BURKHOLDERIA
5:10 pm to 5:30 pm	Conclusions by moderators and further discussions
5:30 pm to 6:00 pm	Open Discussion: 20th and 21st IBCWG Meetings. Where and when?

Friday, April 17, 2015

8:00 am to 8:40 am	**Continental Breakfast**
8:40 am to 11:00 am	**Session 4. Antibiotic Resistance and Therapeutic Developments** Moderators: Dr. Silvia Cardona and Dr. Eshwar Mahenthiralingham
8:40 am to 9:00 am	4.15 Ruhi Bloodworth AN ELECTRON TRANSFER FLAVOPROTEIN (ETF) IS ESSENTIAL FOR VIABILITY AND DETERMINATION OF CELL SIZE IN BURKHOLDERIA CENOCEPACIA
9:00 am to 9:20 am	4.16 Annelien Everaert NONMEVALONATE PATHWAY FOR ISOPRENOID BIOSYNTHESIS AS NOVEL TARGET FOR ANTIBACTERIAL THERAPY AGAINST THE BCC.
9:20 am to 9:40 am	4.17 Heleen Van Acker THE ROLE OF REACTIVE OXYGEN SPECIES IN ANTIBIOTIC INDUCED CELL DEATH IN BURKHOLDERIA CEPACIA COMPLEX BACTERIA
9:40 am to 10:00 am	4.18 Cerith Jones GENETIC CHARACTERISATION OF BIOACTIVE COMPOUNDS PRODUCED BY BURKHOLDERIA GLADIOLI
10:00 am to 10:20 am	4.19 Victor Juarez-Perez BURKHOLDERIA SPP. RESISTANCE STRATEGIES. OSCN-/BLF (ALX-009), IS ITS MODE OF ACTION A BULWARK AGAINST BACTERIAL RESISTANCE?
10:20 am to 10:40 am	4.20 Sarah Kennedy INVESTIGATING THE ACTIVITY OF ANTIBIOTICS AT AEROSOLIZED CONCENTRATIONS AGAINST BURKHOLDERIA CEPACIA COMPLEX BIOFILMS
10:40 am to 11:00 am	Conclusions by moderators and further discussions
11:00 am to 11:30 am	**Coffee Break**
11:30 am to 1:00 pm	**Bioinformatics Analysis with the Expanded Burkholderia Genome Database Workshop** Geoff Winsor, Database Developer, Simon Fraser University

1:00 pm to 2:00 pm	**Lunch**
2:00 pm to 5:00 pm	**Vancouver City Tour** – Pick up / Drop off at the Sheraton Wall Centre Hotel
6:30 pm	**Group Dinner** – Blue Water Cafe 1095 Hamilton Street 604.688.8078

Saturday, April 18, 2015

8:00 am to 9:00 am	**Continental Breakfast**
9:00 am to 11:00 am	**Session 5. Systems Biology Approaches** Moderators: Dr. Tom Coenye and Dr. Leo Eberl
9:00 am to 9:20 am	5.21 Ruhi Bloodworth DRAFT GENOME SEQUENCES OF THE *BURKHOLDERIA CONTAMINANS* STRAINS LMG 23361 AND FFH2055
9:20 am to 9:40 am	5.22 Tom Coenye GENOME-WIDE TRANSCRIPTION START SITE PROFILING IN BIOFILM-GROWN BURKHOLDERIA CENOCEPACIA J2315
9:40 am to 10:00 am	5.23 Amy H. Lee GENOMIC EVOLUTION OF BURKHOLDERIA CENOCEPACIA SEQUENTIAL ISOLATES FROM CYSTIC FIBROSIS PATIENTS
10:00 am to 10:20 am	5.24 Kirsty Agnoli FOCUSING IN ON VIRULENCE: ANALYSIS OF PARTIAL PC3 DERIVATIVES AND BCC STRAINS WITH NON-NATIVE PC3S
10:20 am to 10:40 am	5.25 Gabriella Pessi σ54-DEPENDENT RESPONSE TO NITROGEN LIMITATION AND VIRULENCE IN B. CENOCEPACIA H111
10:40 am to 11:00 am	Conclusions by moderators and further discussions
11:00 am to 11:30 am	**Coffee Break**
11:30 am to 12:30 pm	**Research and Collaboration Workshops**
12:30 pm to 1:00 pm	**Final Remarks and Closing**
1:00 pm to 2:00 pm	**Lunch**

ABSTRACTS

1.01 THE CANADIAN *BURKHOLDERIA CEPACIA* COMPLEX RESEARCH AND REFERRAL REPOSITORY: PAST, PRESENT AND FUTURE.

James E. A. Zlosnik[1], Rebecca Hickman[1], Trevor J. Hird, Deborah A. Henry, Eshwar Mahenthiralingam and David P. Speert[1]

[1] Canadian *Burkholderia cepacia* complex Research and Referral Repository, Centre for Understanding and Preventing Infection in Children, Department of Pediatrics, Faculty of Medicine, University of British Columbia

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The Canadian *Burkholderia cepacia* complex Research and Referral Repository (CBCCRRR) has been operated in our laboratory at the University of British Columbia in Vancouver, British Columbia, Canada since 1994. During this time, the CBCCRRR has been the primary resource for identification and storage of BCC bacteria isolated from patients attending cystic fibrosis (CF) clinics across Canada. The CBCCRRR is a resource for both the clinical community as well as the research community. We offer species identification and strain-typing to Canadian CF clinics. Isolates from CF patients that are sent to us for identification are stored in our repository and are available, de-linked from their clinical information, to the CF research community.
Using isolates submitted to the CBCCRRR up until July 2000, we previously assessed the epidemiology of BCC in Canada and showed that 80% of patients were infected with *B. cenocepacia* (Speert *et al.,* 2002. Emerg. Inf. Dis.). Since this time, several studies, including our own recent comprehensive analysis of BCC in the Vancouver population (Zlosnik *et al.,* 2015 Annals ATS), have revealed that the epidemiology of BCC infection has changed substantially, with most new acquisitions being by *B. multivorans* or species/strains other than epidemic clones of *B. cenocepacia*. This shift in epidemiology is most likely due to infection control measures. Nevertheless, despite infection control measures, new BCC acquisitions in people with CF continue. Since July 2000 we have received and stored over 1000 isolates from more than 500 CF patients across Canada. Of the patients whose samples were positively identified as BCC or *B. gladioli*: 38% were *B. cenocepacia*, 42% were *B. multivorans*, 7% were *B. vietnamiensis*, 6% were *B. gladioli*, 7% were other BCC. We will review the recent temporal and geographic distribution as well available strain-tying data for BCC in Canada. Additionally, we will address the limitations of these data and outline our plans to provide a fully comprehensive BCC resource, for both CF clinics and the research community, for which we have recently received funding from Cystic Fibrosis Canada.

1.02 COMPARATIVE GENOMICS OF CLINICAL AND ENVIRONMENTAL *B. MULTIVORANS* ISOLATES

Charlotte Peeters[1], Vaughn Cooper[2] and Peter Vandamme[1]

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Epidemiological surveys reveal that *B. multivorans* is the most prevalent Bcc cystic fibrosis (CF) pathogen in many countries including Belgium, and the continued emergence of unique *B. multivorans* strains in CF patients suggests acquisition from nonhuman sources, such as the natural environment. Because environmental pressure can select for traits that confer virulence, soil and other natural environments could be sources of new pathogens.
The aim of the present study was to examine to which extent isolates with the same sequence type (ST) but from different origins (CF vs environment, ENV) differ in genetic potential. We therefore selected eight *B. multivorans* isolates for whole-genome sequencing, representing 4 STs (ST-180, ST-189, ST-287 and ST-650). For each ST, one CF and environmental isolate were sequenced using PacBio SMRT sequencing technology, and reads were assembled into high-quality genome assemblies (3-22 contigs per genome). Annotation was done using Prokka and ortholog families were identified using the reciprocal best-blast method with normalized bit score as threshold.
The multiple genome alignment showed an overall highly conserved genome structure, and a translocation of approximately 200 genes from chromosome 1 to 2 in one CF isolate. Detailed comparative analysis of gene content, ortholog families, gene synteny and functional COG categories is being performed and will be presented. The results show that CF and ENV isolates with the same ST are more closely related to each other than to other isolates from the same origin, demonstrating that genomic lineages are defined by ST.
This research was supported by the Special Research Council of Ghent University.

1.03 GENE EXPRESSION PROFILING OF THE *BURKHOLDERIA CONTAMINANS* BLOODSTREAM ISOLATE

Jaroslav Nunvar[1], Lucie Kalferstova[1], Michal Kolar[2], Jose Degrossi[3], Silvia Cardona[4], Ruhi A. M. Bloodworth[4] and Pavel Drevinek[1]

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Burkholderia cepacia complex (Bcc) bacteria pose a major threat to cystic fibrosis (CF) patients. These infections can result in an untreatable clinical condition called cepacia syndrome (CS) which is manifested with rapid deterioration of lung function and septicaemia. Pathogenic mechanisms underlying transition from the Bcc chronic infection to CS remain unknown.
To gain insight into processes involved in development of CS, we compared transcriptomes of a lung isolate (chronic infection) with a bloodstream isolate (CS) retrieved from an Argentine CF patient. Both isolates were of the same sequence type (ST872) and belonged to the *B. contaminans*, a predominant Bcc species in Argentine and Spainish CF populations.
Both isolates were cultivated in three growth media: sputum (~ natural habitat of the lung isolate), heat-inactivated human serum (~ natural habitat of the bloodstream isolate) and a control mineral medium, each in biological triplicates. RNA was extracted in mid-log growth phase, sequenced using RNA-Seq technology (Illumina) and converted to normalized transcript level values.
The expression of approximately 1,300 (18%) genes differed more than 3-fold between lung and bloodstream isolate. The bloodstream isolate showed markedly increased expression of quorum sensing-regulated pathogenicity determinants (motility, extracellular proteases, AidA, lectins, ornibactin, Flp pilus, type III and VI secretion systems). Furthermore, hypoxia-activated genes (*lxa* locus) and two antifungal compound biosynthetic clusters (occidiofungin and pyrrolnitrin) were rapidly expressed in the bloodstream isolate. Agar plate assays confirmed increased motility and proteolytic, haemolytic and antifungal activities of the bloodstream isolate. Assessing these phenotypes may help to monitor the progress of chronic infections caused with *B. contaminans* in CF patients.
This work was funded by grant from Ministry of Health of the Czech Republic NT12405-5.

2.04 IS THE TYPE III SECRETION SYSTEM OF *BURKHOLDERIA CENOCEPACIA* ST32 INVOLVED IN CEPACIA SYNDROME?

Lucie Kalferstova[1], Jolana Vavrova[1], Cristobal Mujica-Troncoso[2], Miguel Valvano[2] and Pavel Drevinek[1]

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How *B. cenocepacia* can spread systemically in cystic fibrosis (CF) patients undergoing cepacia syndrome is poorly understood. Comparative transcriptomic analysis of *Burkholderia cenocepacia* sputum and blood isolates from the strain ST32 lineage revealed that the blood isolates had significantly increased expression of a gene cluster encoding a Type III secretion system (T3SS). Increased expression was also demonstrated by quantitative RT-PCR in all ST32 blood isolates that were recovered from 8 CF patients before they succumbed to cepacia syndrome. We hypothesise that the T3SS is a virulence factor associated with the ability of *B. cenocepacia* to disseminate through blood.
To assess this hypothesis we constructed a mutant strain with a complete deletion of T3SS region (genes BCAM2040 to BCAM2057, as annotated in strain J2315) using the ST32 blood isolate (ID 7419). This was achieved by tri-parental mating using the well-established I-SceI method. *B. cenocepacia* ST32 isolates are highly resistant to nearly all antibiotics, making it difficult to perform genetic manipulations in these bacteria. Prior to mutagenesis we isolated tetracycline sensitive strains by using a negative selection procedure based on fusaric acid that favours the selection of tetracycline sensitive spontaneous mutants. One of the tetracycline sensitive ST32 isolates was used as the recipient for tri-parental mating and the procedure resulted in the isolation of a mutant strain with a deletion of the T3SS gene cluster, which was confirmed by PCR amplification and DNA sequencing.
The results of phenotypic assays comparing growth characteristics and biological behaviour of the T3SS mutant (ΔT3SS7419) with the wild type 7419, as well as sputum ST32 isolates will be discussed.
Supported by IGA MZ NT12405-5, GAUK 2120176, and COST action BM1005.

2.05 *BURKHOLDERIA* SPECIES SUBVERT THE OXIDATIVE BURST IN HUMAN CYSTIC FIBROSIS MACROPHAGES

Kaivon Assani[1], Amal Amer[2], Larry Schlesinger[2], and Benjamin Kopp[1]

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Burkholderia cepacia complex members cause fatal septicemia and rapid outbreaks in patients with cystic fibrosis (CF), and their characteristic multi-drug resistant phenotype renders antibiotic regimens ineffective. CF patients fail to clear *Burkholderia*, a major factor in the disease course, and this is predicated on the inability of macrophages to kill ingested bacteria. We demonstrated that this deficit in CF is partially due to deficient autophagy-mediated killing. The relationship between reactive oxygen species (ROS) and macrophage autophagy in CF is not known, although defective autophagy is potentially mediated through altered ROS pathways that result in aggregation of essential autophagy initiators. *Burkholderia* can scavenge ROS and affect the assembly of key oxidase components in non-CF cells. However, the production and function of ROS in CF macrophages is not clear. We therefore sought to determine whether *B. cenocepacia* and the increasingly prevalent *B. multivorans* effect ROS production in CF macrophages. We infected human peripheral blood monocyte derived macrophages (MDMs) with *B. cenocepacia* clinical isolate k56-2 and *B. multivorans* clinical isolate FC-445. ROS production was evaluated with a DCF assay using fluorescent units as a measurement of the cellular oxidative burst. Uninfected CF MDMs have a reduced oxidative burst in response to Phorbol 12-myristate 13-acetate (PMA), but not to opsonized zymosan compared to non-CF MDMs. Both CF and non-CF MDMs have a reduced response to PMA when first infected with *B. cenocepacia*. CF MDMs have a reduced oxidative burst in response to both *B. cenocepacia* and *B. multivorans* compared to non CF MDMs. However, the addition of IFN-γ prior to infection, or the use of paraformaldehyde-killed *Burkholderia* partially restores the oxidative burst in CF MDMs. CF MDMs also demonstrate decreased phosphorylation (activation) of NADPH oxidase components $p40^{phox}$ and $p47^{phox}$ during *B.* cenocepacia infection in comparison to non-CF MDMs. Together, these results suggest that there are deficits in the CF macrophage oxidative burst, which are further exaggerated by ingestion of *Burkholderia*. Deficits are likely due to decreased phosphorylation of cytosolic components of the NADPH oxidase and a subsequent reduction in NADPH oxidase activation in CF.

2.06 MODELING THE ROLE OF THE GLOBAL REGULATOR SHVR OF *BURKHOLDERIA CENOCEPACIA* IN VIRULENCE USING ZEBRAFISH EMBRYOS

Margarida Gomes[1,2], Sujatha Subramoni[3], Pamela Sokol[3] and Annette Vergunst[1,2]

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Burkholderia cenocepacia has been demonstrated to be capable of surviving and multiplying inside cells by evading host cell defense mechanisms. In our laboratory, we have developed a zebrafish model to study virulence caused by bacteria belonging to the *Burkholderia cepacia* complex, and have shown that macrophages provide an intracellular niche for Bcc *in vivo*. The model allowed us to distinguish strains that cause an acute, rapidly fatal infection, including the *B. cenocepacia* ET12 lineage strains, and strains that were persistent.
In this study zebrafish embryos were used to further understand a role in virulence for the LysR-type transcriptional regulator ShvR. Initially identified based on its role in colony morphology, previous infection experiments in alfalfa seedlings and rats have shown that this regulator is important for virulence, and inflammation in rat lungs. Interestingly, bacterial numbers were, paradoxically, sometimes higher in animals infected with the mutant compared to wildtype, suggesting the *shvR* mutant was highly persistent.
Here, we show, in agreement with these earlier results, that the *shvR* mutant is attenuated in virulence in zebrafish embryos, with a marked reduction in pro-inflammatory responses and tissue inflammation. Using real time non-invasive imaging, we observed that the *shvR* mutant persists in macrophages and, in contrast to the wildtype, is unable to disseminate from infected host cells. This persistent phenotype is reminiscent to those we described earlier for strains including *B. stabilis* LMG14294 and *B. vietnamiensis* LMG14942. We will present our results on the host phagocyte and immune response to infection with the *B. cenocepacia shvR* mutant, and further describe our recent efforts to show a role for this global regulator in persistent versus virulent infections.

2.07 EPIGENETIC MODIFICATION OF AUTOPHAGY MOLECULES BY *BURKHOLDERIA CENOCEPACIA*

Mia F. Tazi[1,2,4] , Duaa Dakhlallah[2,4], Kyle Caution[1,2,4], Ian Davis[3,4], Estelle Cormet-Boyaka[3,4], Clay Marsh[2,4] and Amal O. Amer[1,2,4]

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Autophagy is a fundamental conserved physiological degradation pathway by which cells target non-functional organelles and specific microbes to lysosomes for degradation. Typically, formation of autophagosomes is initiated upon starvation or stress and requires several essential autophagy molecules such as BECN1/Atg6, Atg12, Atg5 and LC3/Atg8 for completion. Compromised autophagy underlies many diseases conditions such as neurodegenerative disorders, Crohn's diseases and cystic fibrosis (CF). CF macrophages and epithelial cells exhibit weak autophagy activity and the underlying etiology is still obscure. CF is a fatal genetic disorder that is caused by malfunction of CFTR chloride channel. CF macrophages allow specific organisms such as *Burkholderia cenocepacia* to establish infection. Notably, CF macrophages express low levels of autophagy molecules due to unknown mechanisms. In addition, *B. cenocepacia* infection further reduces the expression of Atg5 and LC3 and deteriorates autophagy responses which allows bacterial persistence and permits uncontrollable inflammation. Given that DNA methylation occurs on at CpG islands and that autophagy genes are rich in CpG islands within their promoter regions, we investigated epigenetic modifications in CF macrophages before and after infection. We found that *B. cenocepacia* strongly methylates Atg5 and LC3 promoter regions and suppresses their expression and thus, evades clearance by autophagosomes. In addition, we found that this methylation process can be repressed by specific methyltransferases inhibitors, which restores autophagy. Therefore, our findings provide mechanistic insight into the connection between deterioration of autophagy activity upon *B. cenocepacia* infection and offer new treatment options for CF patients.

2.08 THE *BURKHOLDERIA FIX* PATHWAY SENSES OXYGEN AND MEDIATES VIRULENCE AND BIOFILM FORMATION

Matthew M. Schaefers[1,2,3], Roger Lu[1,2], Tami D. Lieberman[3], Roy Kishony[3,4], Gregory P. Priebe[1,2,3]

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Bacteria in the *Burkholderia cepacia* complex (BCC), including *B. dolosa*, cause chronic lung infections in patients with cystic fibrosis (CF). We have previously identified *fixL* (BDAG_01161) in *B. dolosa* as a gene that is under strong positive selection during chronic infection in CF patients. BDAG_01161 has homology to *fixL* of the FixL/FixJ two-component system of *Rhizobium* and *Caulobacter*. FixL in those species is a sensory histidine kinase that detects oxygen tension and phosphorylates the transcription factor FixJ under low oxygen conditions, leading to activation of the *fixK* gene. Our previous work found that *B. dolosa* FixLJ senses oxygen when expressed in *E. coli*. In the current study, we evaluated the role of the *fix* system in oxygen sensing in other BCC species and in both virulence and biofilm formation.
Using a beta-galactosidase reporter assay measuring transcription from the *B.dolosa fixK* promoter we found the activity of the *fix* system in *B. dolosa, B. cenocepacia,* and *B. multivorans* was induced 2-5 fold when grown in low-oxygen environment compared to growth in ambient oxygen conditions (p <0.0001 for each species). In a murine model of pneumonia, a *B. dolosa* clinical isolate containing a mutation within the predicted histidine kinase domain of FixL (A619T) was better able to survive within murine lung (p <0.01) than a closely related (although not isogenic) isolate that lacked the FixL A619T mutation. A *fixLJ* deletion mutant constructed in *B. dolosa* strain AU0158 produced 2-fold more biofilm on polyvinyl chloride plates than the wild-type strain (p <0.0001) despite having a slight growth defect (40% reduction in OD_{600} at 24 hours, p <0.0001). As expected, the *fixLJ* deletion mutant completely lost the ability to induce expression from the *fixK* promoter in the beta-galactosidase reporter assay. In conclusion, the *Burkholderia fix* system senses oxygen in multiple BCC species and is important for virulence in the lung *in vivo* and biofilm formation *in vitro*.
Funding: Cystic Fibrosis Foundation

2.09 GENERAL PROTEIN GLYCOSYLATION CONTROLS VIRULENCE IN *BURKHOLDERIA CENOCEPACIA*

Erin M. Dockery[1], Nichollas E. Scott[2], Leonard J. Foster[2], and Jonathan J. Dennis[1]

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General protein glycosylation is now recognized as a common mechanism within commensal and pathogenic bacteria [1]. Utilizing genetic analyses in combination with a proteomic approach, we were able to characterize a general protein O-glycosylation system in *Burkholderia cenocepacia* K56-2 based upon the $PglL_{Bc}$ *O*-oligosaccharyl transferase (*O*-OTase), encoded by the gene *bcal0960*. This enzyme is responsible for the glycosylation of 28 target proteins involved in diverse cellular processes, though none of these are known virulence factors. Somewhat surprisingly, disruption of *bcal0960* abolished glycosylation and resulted in greatly attenuated virulence towards plant and insect infection models [2]. This effect was independent of the presence of flagella, or the flagellar subunit glycosylation provided by BCAL0111 [3]. To further investigate the observed loss of virulence in K56-2 $\Delta PglL_{Bc}$, quantitative proteomics analyses was undertaken using dimethyl labeling and 2-D liquid chromatography coupled mass spectrometry. A total of 1249 proteins were identified of which 223 proteins were outside one log2 of wild-type abundance levels. 207 proteins showed a decrease in relative abundance as compared to wild-type levels, whereas 16 proteins showed an increase. Proteins with the greatest reduction in abundance corresponded to known Bcc virulence-related proteins such as AidA, ZmpA, and ZmpB. In combintion with these factors, other virulence determinants were altered to a lesser degree as compared to wild-type K56-2, such as components of the anti-fungal compound (*afc*) biosynthesis cluster, and the Type VI secretion system. The expression levels of several important genes shown to have altered protein abundance levels were confirmed using *lux* transcriptional reporter assays. Using advanced bioinformatic analyses, no $PglL_{Bc}$-glycosylated proteins were determined to be obvious or likely regulators of the genes encoding the unglycosylated virulence factors identified in the proteomic study. Genetic analyses of the genes encoding glycosylated target proteins demonstrate that several are involved in controlling *B. cenocepacia* virulence production. Overall, these data suggest that the absence of a general *O*-glycosylation system within K56-2 results in an alteration of glycosylated proteins that control the expression and abundance of a number of non-glycosylated virulence factors involved in Bcc pathogenesis.

1. IwashkiwJA.etal. MolMicrobiol89:14-28.
2. LithgowKV.etal. MolMicrobiol.92:116-37.
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3.10 GENETIC VARIATION ANALYSIS OF *BURKHOLDERIA MULTIVORANS* DURING LONG-TERM COLONIZATION OF A CYSTIC FIBROSIS PATIENT

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To get insights on the genome content evolution during long-term colonization of cystic fibrosis airways we have sequenced the genomes of 22 *Burkholderia multivorans* sequential isolates recovered from a CF patient within a 20 year period. Comparative genomics allowed the identification of 428 mutations, from which 351 were SNPs and 77 were indels, resulting in a mutation rate per year of 3.7 (R = 0.8). Phylogenetic analysis shows four distinct clades with 2-3 persisting at any given time. The distribution of synonymous and nonsynonymous mutations indicated that the majority are nonsynonymous, in particular those defining the different clades. A total of 9 genes, most of them involved in signaling/gene expression regulation and lipid metabolism acquired more than 3 mutations and are likely targets of selection. Early evolution involved mutations affecting genes involved in the biosynthesis of the lipopolysaccharide O-antigen, lipid metabolism and cyclic-di-GMP signaling. Consistent with mutations in genes directing the biosynthesis of the O-antigen, most of the isolates display a rough LPS while the first isolate shows a smooth one. Another early mutation was in the BDSF receptor RpfR (also known as YciR). Accordingly, those isolates have lower motility and increased c-di-GMP levels. Biofilm formation assays also correlate with the RpfR mutation (except for one of the clades), with isolates carrying that mutation producing thicker biofilm. Other phenotypic traits measured showed that overall, the antibiotic resistance and adhesion to cystic fibrosis epithelial cells CFBE41o- increases from the first to the last isolate. Altogether, these observations suggest that adaptation of *B. multivorans* to CF long-term colonization is mediated by metabolic changes, altered surface polysaccharide expression and remodeling of signaling pathways.

3.11 THE RPFFR QUORUM SENSING SYSTEM ATTENUATES C-DI-GMP-MEDIATED EXPRESSION OF THE *BURKHOLDERIA CENOCEPACIA* BCAM1330-1341 EXOPOLYSACCHARIDE GENE CLUSTER

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In *Burkholderia cenocepacia* H111, biofilm formation and the expression of virulence factors are under the control of two highly conserved quorum sensing (QS) systems: (i) the CepIR system, which depends on an *N*-acyl homoserine lactone (AHL) signal molecule and (ii) the RpfFR system, which depends on the *Burkholderia* diffusible signal factor *cis*-2-dodecenoic acid (BDSF) [1]. Whereas CepIR represents a classical QS system where the AHL receptor CepR binds to specific DNA sequences in the promoter region of target genes, little is still known about the signal transduction pathway downstream of the RpfFR QS system. The fatty acid signaling molecule BDSF is synthesized by RpfF [2] and the gene adjacent to *rpfF* encodes the BDSF receptor RpfR, a multidomain protein containing a PAS, a GGDEF, and an EAL domain [3]. Purified RpfR was shown to be capable of synthesizing as well as degrading c-di-GMP. However, upon binding of BDSF to the PAS domain of RpfR, the protein displayed net c-di-GMP phosphodiesterase activity. Inactivation of either *rpfF* or *rpfR* reduced swarming motility, biofilm formation, proteolytic activity, and virulence [3]. The observation that *rpfR* mutants display a wrinkly colony morphology prompted us to investigate factors responsible for this phenotype. Using gene inactivation studies, we were able to identify the product of the recently described *bcam1330-1341* exopolysaccharide (EPS) gene cluster [4] as essential for wrinkly colony morphology of *B. cenocepacia* H111 *rpfR* mutants, suggesting that RpfR is a negative regulator of this putative EPS cluster. Based on this finding, we constructed *bcam1331*::*lacZ* reporter strains to quantify the effect modified intracellular c-di-GMP levels have on this downstream target of RpfR. Taken together, our data suggest that RpfR is associated with a specific c-di-GMP-mediated response rather than modulation of the global c-di-GMP pool. The underlying molecular mechanism of the RpfFR signaling network still needs to be elucidated and is the focus of our current work.

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3.12 UNDER SELETION: BIOFILM SELECTION PLACES YCIR AT THE INTERSECTION OF QUORUM SENSING AND C-DI-GMP METABOLISM

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Biofilms are a ubiquitous lifestyle of bacteria during infections and represent a significant barrier to immune clearance and drug treatment. During the course of a chronic infection, the founding isolate replicates and evolves under host selection to yield a heterogeneous population capable of inhabiting different ecological niches within the host. One such pathogen, *Burkholderia cenocepacia*, appears to rely upon environmental cues to transition between planktonic and biofilm lifestyles during colonization and persistence within the lungs of individuals with cystic fibrosis. At the forefront of this transition are the quorum sensing systems that promote bacterial communication through perception of secreted factors.

We conducted a long-term experimental evolution with *B. cenocepacia* that selects for daily cycles of adherence and dispersal. Sequencing of replicate populations revealed the persistence of lineages with independent mutations in the *yciR* (*rpfR*) gene, which physically and enzymatically links the perception of the quorum sensing molecule BDSF with synthesis and degradation of c-di-GMP, a second messenger that induces biofilm formation. The evolved *yciR* mutant alleles persist throughout the duration of the experiment, result in ecological diversification, and associate with different patterns of biofilm formation. We hypothesize that the unique *yciR* alleles correspond to specific ecological strategies based on modification of BDSF perception and c-di-GMP synthesis/degradation.

To address how different *yciR* alleles alter lifestyle in a structured environment, we characterized a panel of isogenic mutants by quantifying biofilm formation as well as fitness in both planktonic and biofilm environments. Our findings demonstrate that mutations in *yciR* differentially influence fitness and productivity, and we suggest that this observation is a result of temporal variation of intracellular c-di-GMP. Furthermore, the positions of mutations in the *yciR* gene offer insight into how each functional domain affects lifestyle and ecology. Future efforts will be directed toward extracting and measuring intracellular pools of c-di-GMP from the isogenic mutants to further understand how modifications in *yciR* impact attachment and dispersal strategies.

3.13 THE BIOSYNTHETIC ORIGIN OF A BIOACTIVE *N*-HYDROXY-*N*-NITROSOAMINO COMPOUND FROM *BURKHOLDERIA CENOCEPACIA* H111

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Many *Burkholderia cepacia* complex (Bcc) strains are known for their ability to suppress fungal pathogens by the production of secondary metabolites. A previous study has shown that the antifungal activity in *Burkholderia cenocepacia* H111 is regulated by quorum sensing. The objective of the present study is to identify and characterise the genes involved in the biosynthesis of antifungal metabolites of this strain. Furthermore, we seek to elucidate the structure of the bioactive compound(s) and gain insight into their biosynthesis.
In silico analysis of quorum sensing regulated genes in *B. cenocepacia* H111 has identified a candidate gene cluster comprised of seven genes (H111 antifungal metabolite cluster, *ham* cluster) that may be involved in the biosynthesis of an uncharacterized secondary metabolite. Non-polar single gene deletions of each of the seven genes in the *ham* cluster abrogated antifungal activity. We subsequently extracted a compound from bacterial cultures that was present in supernatants of the wildtype strain but missing from *ham* mutant strains. NMR analysis and X-ray crystallography revealed that the active molecule is the *N*-hydroxy-*N*-nitrosoamino compound fragin. Bioactivity tests showed that fragin inhibits growth of several fungi as well as Gram-positive bacteria.
In our study we identify the *ham* gene cluster to be responsible for the biosynthesis of the *B. cenocepacia* H111 main antifungal metabolite fragin. This study represents the first report on the biosynthetic origin of an *N*-hydroxy-*N*-nitrosoamino compound.

3.14 ABIOTIC AND BIOTIC FACTORS INFLUENCE SOIL *BURKHOLDERIA*

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The genus *Burkholderia* consists of approx. 90 phylogenetically closely related species that are metabolically highly versatile. This enables them to be ubiquitously present in the environment as free-living bacteria as well as in antagonistic, mutualistic or symbiotic associations with plants, fungi and animals. Soil is the environment that contains the largest pool of *Burkholderia* diversity. Soil *Burkholderia* are involved in a large number of processes, such as decomposition of organic matter, detoxification/removal of pollutants or nitrogen fixation. Even though these processes are well understood, there is still a lack of knowledge regarding the ecology of *Burkholderia* in the soil.

To better understand the environmental parameters affecting *Burkholderia* distribution and abundance in the soil we investigated the relative abundance of the genus *Burkholderia* in the soil at a trans-continental and at a local scale and correlate the obtained abundances with environmental parameters. The results showed that *Burkholderia* relative abundance was significantly influenced by soil pH. In contrast to most bacteria, *Burkholderia* favored low pH environments and were undetectable in neutral and alkaline soils. However, while their relative abundance was influenced by the soil pH, their diversity was not. Moreover, in vitro growth experiments revealed that although they are undetectable in neutral or high pH soils, *Burkholderia* are able to grow at pH values as high as 9, suggesting that beside abiotic factors such as pH, biological interactions such as the presence of interacting partners might contribute to *Burkholderia* abundance and diversity. For that we investigated further the interactions of *Burkholderia* with fungi. Interactions between fungi and selected *Burkholderia* strains are described, but less is known about their relevance in explaining *Burkholderia's* biogeography and preference for low pH soils, or the mechanisms underlying such interactions. We used a proteomic approach to gain first insight into the physiological changes occurring in *Burkholderia glathei*, a common soil bacterium, while interacting with two fungi, *Alternaria alternata* or *Fusarium solani*. Interestingly, the proteome of *B. glathei* underwent similar changes with both fungi, and these changes revealed a highly beneficial effect for the bacterium, which apparently derived much of its carbon, nitrogen and phosphate from the fungi. Additionally, co-occurrence network analysis and growth experiments revealed that these associations between *Burkholderia* and fungi are very common in soils and occur with a broad range of fungal partners.

4.15 AN ELECTRON TRANSFER FLAVOPROTEIN (ETF) IS ESSENTIAL FOR VIABILITY AND DETERMINATION OF CELL SIZE IN BURKHOLDERIA CENOCEPACIA

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Essential gene studies often reveal novel essential functions for genes with dispensable homologs in other species. This is the case with the widespread family of electron transfer flavoproteins (ETFs), which are required for the metabolism of specific substrates or for symbiotic nitrogen fixation in some bacteria. Despite these non-essential functions several high throughput screens have identified ETFs as putatively essential in Burkholderia genomes. In this study, we constructed a conditional expression mutant of one of the ETFs in *Burkholderia cenocepacia* and demonstrate that its expression is essential for growth on both complex media and a variety of single carbon sources. We further demonstrate that the two subunits EtfA and EtfB interact with each other and that cells depleted of ETF lose viability and redox potential. These cells also transition from the short rods characteristic of *B. cenocepacia* to small spheres independently of MreB. The putative membrane partner ETF dehydrogenase was also essential in *B. cenocepacia* and induced the same rod to sphere change. We propose that the lack of aerobic respiration and small sphere phenotype are related with ETF coupling metabolic state and cell size and that the ETF of *B. cenocepacia* is a novel antibacterial target.

4.16 NONMEVALONATE PATHWAY FOR ISOPRENOID BIOSYNTHESIS AS NOVEL TARGET FOR ANTIBACTERIAL THERAPY AGAINST THE BCC.

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Isoprenoids play widely varying roles in physiological processes of all living organisms. Some bacteria use an alternative pathway for isoprenoid biosynthesis, the DOXP or nonmevalonate pathway, instead of the more common mevalonate pathway. Since this pathway is not present in human cells, it is a promising target for the development of antibacterial chemotherapy. Most species of the *Burkholderia cepacia* complex (Bcc) use the nonmevalonate pathway as sole pathway for isoprenoid biosynthesis. Fosmidomycin and FR900098 are effective inhibitors of Dxr (a key enzyme in the nonmevalonate pathway) and could be used for the treatment of the malaria parasite *Plasmodium falciparum*, but there is a paucity of research investigating the nonmevalonate pathway in bacteria. Our first goal is to evaluate the biological efficacy of inhibitors of several enzymes of the nonmevalonate pathway *in vitro*. Susceptibility of planktonic and sessile cells of *Burkholderia cenocepacia*, *Burkholderia cepacia, Burkholderia ambifaria* and *Burkholderia multivorans* was tested against clomazone and 5-ketoclomazone (Dxs-inhibitors), several fosmidomycin-derivatives (Dxr-inhibitors) and several thiazolopyrimidines (IspF-inhibitors). One of the thiazolopyrimidines shows promising activity on planktonic cultures of *B. cenocepacia* and *B. ambifaria* with MIC values of 31.25 – 62.5 µM. Unfortunately, this compound does not decrease growth of sessile cells of the *Bcc* species. All the other components show MIC values ≥ 250 µM on planktonic cells of all strains tested. Further experiments were carried out to investigate the importance of efflux and cell penetration on the *in vitro* activity of all components. Our second goal is to obtain a better insight in the nonmevalonate pathway in *B. cenocepacia*. Essentiality of the different DOXP genes was determined by constructing antisense overexpression mutants of certain genes. Furthermore, rhamnose-dependent conditional mutants of *B. cenocepacia* DOXP genes were generated by using plasmid pSC200, which delivers the rhamnose-inducible promotor into the chromosome to control the target gene expression. Our third goal is to identify different resistance mechanisms against inhibitors of the nonmevalonate pathway and to examine how to circumvent these mechanisms. These experiments are currently in progress.

4.17 THE ROLE OF REACTIVE OXYGEN SPECIES IN ANTIBIOTIC INDUCED CELL DEATH IN *BURKHOLDERIA CEPACIA* COMPLEX BACTERIA

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It was recently proposed that bactericidal antibiotics kill bacteria through a common mechanism involving the production of reactive oxygen species (ROS). However, this mechanisms has become the subject of debate. Since the contribution of ROS to antibiotic mediated killing most likely depends on the conditions, differences in experimental procedures are expected to be at the basis of the conflicting results reported in the literature.
In this study we compared different methods (ROS specific stainings, gene-expression analyses, genetic and phenotypic experiments, detection of carbonylation and DNA oxidation) to measure the production of ROS upon antibiotic treatment in *Burkholderia cepacia* complex (*Bcc*) bacteria. Antibiotics belonging to different classes were included and both planktonic and biofilm cultures were studied.
While some methods were not sensitive enough to measure antibiotic induced production of ROS, others were found to be useful only in some conditions. For example, an increase in the expression of OxyR was measured in *Burkholderia cenocepacia* K56-2 after treatment with ciprofloxacin both in biofilms and planktonic cultures but not after treatment with tobramycin. Since fluorescein based stainings were shown to be highly pH sensitive and most *Bcc* species, due to resistance need to be treated with antibiotics in high concentrations (which has a marked influence on the pH), these dyes cannot be added simultaneously with the antibiotics and control solutions should have the same pH as the antibiotic solutions tested. When planktonic cultures were treated with high concentrations of tobramycin we did observe an increase in fluorescence in several *Bcc* strains and as expected fluorescence was higher in catalase and quorum sensing deletion mutants. Overexpression of the mismatch repair enzyme MutS, which was shown to protect against antibiotic lethality in *Escherichia coli*, only slightly increased survival after treatment in planktonic *B. cenocepacia* J2315 cultures, and on the contrary led to a significant decrease in survival in biofilms.
Overall these results highlight some methodological key issues to be considered when evaluating the contribution of ROS in antibiotic mediated killing and although not one ideal method could be identified the different methods suggest that ROS is involved in antibiotic mediated killing in *Bcc* species.

4.18 GENETIC CHARACTERISATION OF BIOACTIVE COMPOUNDS PRODUCED BY *BURKHOLDERIA GLADIOLI*

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The genus *Burkholderia* encompasses diverse and versatile bacteria. Large, multireplicon genomes encoding an array of catabolic and anabolic functions contribute to the versatility of these organisms, allowing them to exist in a wide variety of environmental niches and generate an array of secondary metabolites. One such species, *Burkholderia gladioli*, responsible for both plant pathogenicity and lung infections in cystic fibrosis (CF), has been shown to produce compounds with potent antibacterial and antifungal activity. Growth of *B. gladioli* BCC0238 on solid media with glycerol as a sole carbon source and screening using a microbial overlay assay identified antagonism against clinically problematic Gram-positive and Gram-negative bacteria, as well as yeast. Extraction of the bioactive compounds, and further analysis using HPLC and LCMS techniques, uncovered a novel macrolide antibiotic, designated gladiolin.
The genetic basis of the antimicrobial activity of this *B. gladioli* isolate was investigated using Single Molecule Real Time (SMRT) sequencing to generate a high quality draft genome. Genome mining using the antiSMASH tool revealed a wealth of secondary metabolite biosynthetic gene clusters, including four polyketide synthetic clusters (PKS) and eight nonribosomal peptide synthetic clusters (NRPS). Of these, one PKS locus was consistent with the biosynthesis of gladiolin, and was confirmed using targeted insertional mutagenesis. A second locus, encoding hybrid NRPS/PKS machinery with homology to genes encoding the biosynthesis of the plant toxin syringomycin, was postulated to encode an additional bioactive compound that co-expressed and purified with gladiolin. Genetic analysis of this hybrid NRPS/PKS locus will be presented. Overall, a combination of genome mining, genetic engineering and analytical chemistry facilitates rapid analysis of the biosynthetic ability of *Burkholderia* and is providing promising hits for development as novel antibiotics.

4.19 *BURKHOLDERIA* SPP. RESISTANCE STRATEGIES. OSCN-/BLF (ALX-009), IS ITS MODE OF ACTION A BULWARK AGAINST BACTERIAL RESISTANCE?

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Burkholderia spp. are natural muti-resistant bacteria that have developed multifactorial resistance strategies to escape from antibiotics. In addition to the standard resistance mechanisms developed among most bacterial species, the *Burkholderia* spp also have the particular ability to modify their central metabolism and to activate, in parallel, several escape resistance pathways without apparent loss of fitness. This incredible genetic and physiological plasticity makes *Burkholderia* an excellent model to study bacterial adaptability under intense selective pressure.
ALX-009 is a drug candidate composed of the highly bactericidal hypothiocyanite ion ($OSCN^-$) and the bacteriostatic bovine lactoferrin (bLF). ALX-009 bactericidal capabilities have been demonstrated. At least160 *Burkholderia* isolates, mostly clinical, were tested but no naturally resistant isolate has been detected to date. Initial attempts to induce resistance in a clinical *B. cenocepacia* isolate to $OSCN^-$ alone or to ALX-009 failed. A non-significant increase in MIC levels to bLF was found.
Time kill curves suggest a putative mode of action by the drug at three different levels that could explain the lack of occurrence of resistant strains. In conjunction with the literature review, future research objectives will be presented.

4.20 INVESTIGATING THE ACTIVITY OF ANTIBIOTICS AT AEROSOLIZED CONCENTRATIONS AGAINST *BURKHOLDERIA CEPACIA* COMPLEX BIOFILMS

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Objectives: *Burkholderia cepacia* complex (Bcc) causes chronic pulmonary infections in patients with Cystic Fibrosis (CF). Bcc isolates are drug resistant and grow as biofilms *in vivo*, thus protecting themselves from host defences. Currently, there is no effective antimicrobial therapy for CF patients infected with Bcc. New aerosolized antimicrobials can deliver very high intrapulmonary drug concentrations that may be able to overcome this antimicrobial resistance. The objective of this study was to compare the activity of tobramycin at systemically achievable vs aerosolized concentrations on Bcc biofilms cultured under continuous flow in the novel microfluidic Bioflux system and in a static chamber slide model.
Methods: Susceptibility of *Burkholderia multivorans* biofilms to tobramycin concentrations achievable by systemic and aerosolized delivery methods were investigated. Biofilms were generated under continuous flow using the novel Bioflux system and in static chamber slides for 24 hours, then treated with tobramycin (Bioflux 8, 100 and 200 μg/ml: Chamber slide 8, 100, 200 and 1,000 μg/ml) for 24 hrs.
Results: Biofilm thickness (μm) in the Bioflux (0 μg/ml, 34 μm: 8 μg/ml, 38μm: 100 μg/ml, 45 μm; 200μg/ml, 35 μm) and chamber slide (0 μg/ml, 25 μm: 8 μg/ml, 20 μm: 100 μg/ml, 21 μm: 200 μg/ml, 22 μm: 1000 μg/ml, 20 μm) models were similar to the non-treated control biofilms in both systems. Analysis of Live/Dead staining in both models revealed that the number of live (green) cells in each condition (including the non-treated controls) were similar. However, as the concentration of tobramycin increased, the number of dead (red) cells increased.
Conclusions: In conclusion, these results demonstrate that while biofilms generated in Bioflux are thicker compared to the static chamber model, a similar pattern in Live/Dead staining was observed in both systems. Although tobramycin did not eradicate Bcc biofilms *in vitro*, there was increased killing at higher concentrations. Additional work is underway to determine effects of these treatments on mature 48 and 72 hour biofilms. Taken together, this and future work will provide better understanding of how Bcc biofilms can be eradicated in order to improve existing antibiotic therapies and develop novel treatments, for patients with CF.

5.21 DRAFT GENOME SEQUENCES OF THE *BURKHOLDERIA CONTAMINANS* STRAINS LMG 23361 AND FFH2055

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We report the draft genome sequences of *Burkholderia contaminans* LMG 23361, the type strain for the species*, B. contaminans* FFH2055, an isolate from the sputum of a CF patient in Buenos Aires, Argentina and an improved assembly of the draft genome of *B. cenocepacia* K56-2, a member of the highly transmissible ET12 linage. Genomic DNA was prepared and sequenced using the PacBio RS II system. Sequencing yielded 241,868 reads with a mean length of 6.7kb for K56-2, 237,907 reads with a mean length of 8.7kb for LMG23361 and 256,171 reads with a mean read length of 7kb for FFH2055. The reads were assembled using HGAP (PacBio SMRT Analysis software version 2.3), followed by polishing using Quiver (PacBio). The assemblies of LMG23361 and K56-2 both consisted of 17 contigs containing 9.2Mb and 7.8 Mb respectively, while FFH2055 contained 8.2 Mb organized into 4 contigs. Species in the genus *Burkholderia* are known for having large multipartite genomes and the sizes of our assemblies fell within the range of 7.4 to 9.73 Mb seen in previously sequenced genomes. Likewise, annotation of the assemblies with RAST, identified 8,674, 7,641 and 7,375 open reading frames in LMG23361, FFH2055 and K56-2 respectively, which also falls within the range seen in previously sequenced species. Both LMG23361 and K56-2 contained the complete core genome conserved across the order *Burkholderiales*, while FFH2055 was missing 8 conserved genes.

5.22 GENOME-WIDE TRANSCRIPTION START SITE PROFILING IN BIOFILM-GROWN *BURKHOLDERIA CENOCEPACIA* J2315

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Present information about the *B. cenocepacia* J2315 genome architecture does not include transcription start sites (TSS) and annotation of regulatory non-coding RNAs is incomplete. We applied the novel differential RNA sequencing approach, which is geared towards identifying TSS, to *B. cenocepacia* J2315 biofilms, with the aim to map TSS on a whole genome scale and thereby identify novel non-coding regulatory small RNAs. Using this approach, we mapped 4010 TSS and defined the primary TSS for 2089 genes expressed under biofilm condition. Based on TSS analysis, alternative start codons for 64 genes were proposed, making the annotated genes shorter or longer, and a yet un-annotated toxin-antitoxin system was discovered.
TSS not associated with an annotated gene start and producing a short transcript were screened for properties such as sequence conservation, presence of a rho-independent terminator and secondary structure formation. Additionally, they were compared to the Rfam database as well as to small RNAs of *B. cenocepacia* previously identified by RNA sequencing of strains AU1054 and HI2424 and by a sequencing RNAs co-purifying with Hfq protein.
15 short transcripts were homologous to small RNAs represented in the Rfam database or to previously identified small RNAs, showing that differential RNA sequencing is suitable for identifying novel small RNAs. Nine candidate novel small RNAs were confirmed by Northern blotting.
Plausible candidates for novel small RNAs with high expression in biofilms were further analysed, by comparing expression levels in biofilms to planktonic cultures, and by *in silico* screening of putative target mRNAs. Several RNAs induced under biofilm condition and with partial sequence complementary to mRNAs in regions important for ribosome binding were then selected for deletion and silencing, because they might constitute small trans-acting regulatory RNAs with a role in biofilm formation. First results indicate that these selected small RNAs have a role in growth rate control, carbon metabolism and response to oxidative stress.

5.23 GENOMIC EVOLUTION OF *BURKHOLDERIA CENOCEPACIA* SEQUENTIAL ISOLATES FROM CYSTIC FIBROSIS PATIENTS

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Bacterial pathogens from the *Burkholderia cepacia* complex (*Bcc*) are a serious health concern for cystic fibrosis (CF) patients, because *Bcc* can establish chronic infections in the lungs and are highly resistant to antibiotics. Recent advances in genome sequencing allow a high-resolution view of the genetic changes of *Bcc* pathogens as they adapt to the CF lung environment. To date, most studies have focused on genome evolution of a single clonal lineage. To compare evolutionary changes between genotypically different strains from the same species, we are examining if *Bcc* pathogens with distinct genetic backgrounds take similar or distinct pathways to adapt to the CF lungs. We sequenced the genomes of 40 *B. cenocepacia* clinical isolates from six CF patients to ~50X coverage using the Illumina technology. These 40 sequential isolates of *B. cenocepacia* have previously been genotyped by randomly amplified polymorphic DNA (RAPD) PCR, and belong to RAPD genotypes 01, 02 or 06. We found that 18% of sequence reads from RAPD 01 and 12% from RAPD 06 do not map to the *B. cenocepacia J2315* reference sequence, indicating the large genome diversity between the different RAPD types. We used the programs SPADES for de novo assembly and Prokka for annotating the 40 draft genomes. To improve our draft assembly and generate a reference sequence for RAPD 01, we used Pacific Biosciences long-read sequencing. Comparative analyses of sequential RAPD 01 isolates from one CF patient provided evidence of large genome reduction in chromosomes 2 and 3 of later isolates, compared to the initial isolate from eight years ago. In contrast, RAPD 02 isolates do not show evidence of such large genomic changes; most of the differences are single nucleotide polymorphisms or small insertions/ deletions. We are currently sequencing an additional 200 *B. cenocepacia* clinical isolates of different RAPD genotypes (04, 09, 15 and 44) to identify genomic changes, and potentially different pathoadaptation strategies, between the different *B. cenocepacia* RAPD types.

5.24 FOCUSING IN ON VIRULENCE: ANALYSIS OF PARTIAL PC3 DERIVATIVES AND BCC STRAINS WITH NON-NATIVE PC3S

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We have previously reported that the third replicon of the Bcc, pC3, is important for various Bcc phenotypes, including virulence, antifungal activity and proteolytic activity. Bioinformatic analysis of sequenced Bcc strains has shown that approximately half of *B. cenocepacia* H111 pC3 is well-conserved throughout sequenced Bcc members, while the other half is poorly conserved. To allow further investigations of the importance of these conserved and poorly conserved halves, two partial pC3 derivatives were constructed using FLP-*FRT* recombination. Each consisted of one of these two regions, including the pC3 origin of replication in both cases. It was found that the presence of the conserved region of pC3 results in full virulence of H111 in the *C. elegans* and *G. mellonella* models. The presence of the non-conserved part gave a similar level of virulence to that seen after pC3 loss. To allow better localisation of the region of H111 pC3 responsible for virulence, two further partial derivatives were constructed, each containing approximately half of the conserved region. It was found that the part of pC3 containing *aidA* conferred full pathogenicity against *C. elegans*, while antifungal activity was unexpectedly dependent upon the other part of the conserved region, and not that carrying the *afc* cluster. Proteolytic activity was dependent on the non-conserved part of pC3, as expected given that this part carries a number of protease-encoding genes, including *zmpA*.
To investigate to what degree the pathogenicity of a Bcc strain is dependent upon its resident pC3, we integrated an origin of transfer into pC3 from *B. cenocepacia* K56-2, and then transferred this replicon to *B. cenocepacia* H111, HI2424 and MCO-3 via conjugation, for analysis by pathogenicity assay.

5.25 σ^{54}-DEPENDENT RESPONSE TO NITROGEN LIMITATION AND VIRULENCE IN *B. CENOCEPACIA* H111

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Members of the genus *Burkholderia* are versatile bacteria capable of colonizing highly diverse environmental niches. In this study, we investigated the global response of the opportunistic pathogen *Burkholderia cenocepacia* H111 to nitrogen limitation at the transcript and protein expression level. In addition to a classical response to nitrogen starvation, including the activation of glutamine synthetase, PII proteins and the two component regulatory system NtrBC, *B. cenocepacia* H111 also up-regulated polyhydroxybutyrate (PHB) accumulation and exopolysaccharide (EPS) production in response to nitrogen shortage. A search for consensus sequences in promoter regions of nitrogen responsive genes showed an enrichment of σ^{54} boxes, underlining an important role of this alternative sigma factor in the response of *B. cenocepacia* to nitrogen limitation. Mutation of σ^{54} in *B. cenocepacia* H111 and mapping of the σ^{54}-dependent regulon revealed an important role for this sigma factor not only in the control of nitrogen assimilation genes but also in the control of genes involved in EPS production, biofilm formation, motility and virulence.

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