A1

BURKHOLDERIA CEPACIA COMPLEX INFECTIONS IN VANCOUVER AND BRITISH COLUMBIA FROM 1981 TO 2010

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Our laboratory has been collecting clinical isolates from patients attending the paediatric and adult cystic fibrosis clinics in Vancouver, BC, Canada since 1981. Since 1994 we have also housed the Canadian *Burkholderia cepacia* complex referral and research repository (CBCCRRR). During this period the taxonomy of *Burkholderia cepacia* complex species has changed considerably, as have approaches to treatment. When our collection was first started all the bacteria now known as the BCC were classified as *Pseudomonas cepacia* and the severity of the infections they could cause was not fully appreciated. Subsequent advances in taxonomy and the understanding of the potential for patient to patient spread has led to the isolation of all patients that become infected with any members of the BCC. Here we describe the current picture of BCC infections in CF patients attending the Vancouver clinics and how this has evolved over the past 30 years, including rates of new acquisition, species and strain prevalence and survival following infection. Additionally we will provide an update on the picture across Canada as represented by isolates sent to us for the CBCCRRR.

A2

INFECTION WITH *BURKHOLDERIA* SPECIES IN PATIENTS WITH CYSTIC FIBROSIS: FRENCH NETWORK SURVEY 2002-2009.

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In the 1990's, the epidemiology of *Burkholderia cepacia* complex (Bcc) infections in French CF patients was dominated by the occurrence of outbreaks due to 5 major clones: 1 of *B. cenocepacia* IIIA, 2 of *B. cenocepacia* IIIB and 2 of *B. multivorans*. In contrast with other countries where *B. cenocepacia* was the main species involved in outbreaks, *B. multivorans* accounted for 50% of Bcc infections. Most colonisations (≈75%) were persistent. Following the implementation of infection control measures, the incidence of cross-transmisssion dramatically decreased in the late 1990's. We report prospective data of the national surveillance network from 2002 to 2009. Of the 152 primary colonisations observed during this 8-year survey, 124 (82%) were due to Bcc organisms (56 *B. multivorans*, 48 *B. cenocepacia* and 20 other species), and 28 (18%) to *B gladioli*. Within the 104 *B. cenocepacia*/*B. multivorans* colonisations, 24 (23%) were still due to major epidemic clones (13 *B. multivorans* and 11 *B. cenocepacia*). Only 42% of colonisations with Bcc, and 54 % of colonisations with *B. gladioli* were persistent. The occurrence of a cepacia syndrome was reported in 5% of Bcc colonised patients. During the same period, 29 patients colonised with Bcc (12 *B. cenocepacia*, 12 *B. multivorans*, and 5 other species) and 2 patients colonised with *B. gladioli* underwent lung transplantation. Post-transplant BSI occurred in 11 of the 31 patients (35%): 5 *B. cenocepacia*, 3 *B. multivorans*, 1 *B. vietnamiensis* and 2 *B. gladioli* colonisations, and death within 6 months post-transplant in 10 (32%): 5 *B. cenocepacia*, 2 *B. multivorans*, 1 *B. vietnamiensis*, 1 *B. dolosa* and 1 *B. pyrrocinia* colonisations). Though *B. cenocepacia* seems to be more frequently associated to BSI and short survival than *B. multivorans*, these differences do not reach statistical significance. The Observatoire cepacia network is supported by Vaincre la Mucoviscidose.

A3
THE FATE OF EPIDEMIC STRAIN *BURKHOLDERIA CENOCEPACIA* ST-32 IN THE CYSTIC FIBROSIS CENTRE IN PRAGUE

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Burkholderia cenocepacia is arguably one of the most serious respiratory pathogens in patients with cystic fibrosis (CF). Its presence in a CF population is associated not only with worse life prognosis for infected patients, but also with the risk of transmission and subsequent outbreak formation. Indeed, many CF centers over the world including the Prague one have faced epidemic spread of *B. cenocepacia* within their CF populations.
A story of epidemic strain *B. cenocepacia* ST-32 in the CF centre in Prague started in 1990s with first detection of the microorganism by culture. Additional diagnostic tools implemented in 2001– *recA* PCR and Bcc selective agar – revealed cumulative Bcc incidence to be ca. 30% in 2000s.
Between 1994 and 2002, 2 to 18 patients were newly diagnosed with ST-32 every year. This resulted in having 45 individuals infected with ST-32 (out of 196 CF patients examined) by the end of 2003. On the contrary, following years only 2 patients got infected with ST-32 (in March 2004 and in May 2007). As 21 patients with ST-32 infection died, today we take care of twenty six ST-32 positive patients out of 323 patients attending the Center. ST-32 now represents 52% of all Bcc strains found in the Czech CF population (vs. 78% in 2003).
Based on the data we can conclude that the outbreak caused with *B. cenocepacia* ST-32 in the CF Center in Prague is over. Key attributes that enhanced efficacy of the infection control were very likely an early and specific Bcc detection by PCR, along with understanding of and full compliance with strict infection control rules by both patients and healthcare workers.
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A4
DISTRIBUTION OF *BURKHOLDERIA CEPACIA* COMPLEX SPECIES ISOLATED FROM PATIENTS, HOSPITALS AND INDUSTRIAL SETTINGS OF ARGENTINA

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The *Burkholderia cepacia* complex (Bcc) is a group of closely related, non-fermenting, Gam-negative species that are opportunistic pathogens in cystic fibrosis (CF) and inmunocompromised patients. Members of the Bcc are also frequent contaminants in industrial products.
The correct identification of Bcc members is fundamental in the treatment of patients, in infection and contamination control measures, and for epidemiological studies. The aim of this work was to identify the species of isolates obtained from patients, hospitals and industrial settings, and to have knowledge about the Bcc distribution in Argentina.
Using amplification and sequence analysis of the *recA* gene, we assigned the species to 130 Bcc strains isolated between 2004 and 2010 from CF and non-CF patients, hospital and industrial products, purified water and one hospital air sample.

B. contaminans was the predominant Bcc member isolated from CF and non-CF patients (66% of 108 isolates). More than 50% of the strains analyzed from each health centre (four pediatric and one adult centre) were identified as *B. contaminans*. Strains isolated from the air sample and hospital products were also identified as *B. contaminans*. *B. cenocepacia* (14%) was the second frequent species isolated from patients. Among other species isolated, ranging from 0,9 to 5% , we identified *B. cepacia*, *B. multivorans*, *B. stabilis*, *B. seminalis*, *B. arboris* and *B. ambifaria*.

Concerning to industrial isolates (22 strains from pharmaceutics, cosmetic, house cleaners industries and one purified water supplier); *B. contaminans* and *B. lata* were the most frequent species (36% each one). *B. vietnamiensis* (18%), *B. cepacia* (5%) and *B. cenocepacia* (5%) were also identified.

The exposed results are the first approach for understanding the epidemiology of Bcc in Argentina, which seems to be different from those reported in other countries; and will be helpful for establishing more effective infection control measures.

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A5
CEPACIA SYNDROME IN *BURKHOLDERIA CENOCEPACIA* ST-32 STRAIN: 10 YEARS OF EXPERIENCE

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Introduction. Cepacia syndrome (cep sy) is the most unfavourable clinical condition caused by *B. cepacia* complex (BCC) in cystic fibrosis (CF) patients. Due to the past outbreak appr. 40% of Czech adult CF population harbor BCC epidemic strain ST-32. In this study, we evaluated the incidence of cep sy and clinical characteristics in the adult population attending the Prague CF centre.

Methods. All BCC ST-32 infected CF patients during 2001-2010 were included into study. To identify individuals with cep sy we reviewed all hospital records with in-patient treatments for pulmonary exacerbation. Confirmed cep sy (cCS) was defined as follows: white blood cell count (WBC) $>25\text{x}10^9$/L and/or C-reactive protein (CRP) >100 mg/L, CXR with new multifocal infiltrates and positive blood cultures. If no BCC was cultured from blood, the condition was concluded as probable cep sy (pCS).

Results. We evaluated 286.5 patient-years and 275 hospital records in 55 CF patients. We identified 33 cases of cep sy (13 cCS and 20 pCS) in 27 patients, which represented 11.5% prevalence of cep sy per one patient-year. CRP concentration was >100 mg/L in all cep sy cases and WBC count was $>25\text{x}10^9$/l in 78.8% of cep sy cases (100% in cCS). Mortality rate from cep sy was 57.6% (76.9% in cCS). Median (range) CRP concentration and WBC count was 224.4 (106.7-410.5) mg/L and 34.3 (13.6-64.0) $\text{x}10^9$/L, respectively. In 17 cases of cep sy (8 of cCS), procalcitonin (PCT) concentrations were also measured. PCT concentrations >0.5 μg/L were found in 47.1% of cep sy cases (50.0% of cCS). Median (range) time from acquisition of BCC to first episode of cep sy was 8.92 (1.50-16.34) years. Median (range) best FEV_1 and BMI value in one year before the first episode of cep sy was 39 (24-80)% pred. and 19.7 (15.8-23.2) kg/m^2, respectively. Simultaneous airway colonization with *P. aeruginosa* was present in 22.2% of patients. In two months before cep sy episode, pneumothorax and

significant haemoptysis developed in one and three patients, respectively. Pancreatic insufficiency, CF-related diabetes and CF-liver disease were present in 96.3%, 40.7% and 25.9% of patients, respectively.
Conclusion. Patients with ST-32 frequently experience cep sy with high mortality rates. Very high CRP concentrations and WBC counts (but not PCT concentrations) are both present in cep sy cases.
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A6
OCCURRENCE AND BIODIVERSITY OF *BURKHOLDERIA* SPP. IN NATURAL ENVIRONMENTS OR NON-TILLAGE SOILS OF ARGENTINA

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The *Burkholderia* genus is a ubiquitous bacterial group occupying diverse niches in the environment, such as soils, plants or water sources, and displaying a wide range of biological features. Some of these activities include phytopathogenicity, plant growth promoting, endophytic or symbiotic nitrogen fixation, biological control of fungi and siderophore production (1). Although the clinical relevance of some strains is associated to the *Burkholderia cepacia* complex (*Bcc)*, species comprise in this group showed relevant biological properties for plant health and growth. In the context of the BIOSPAS global project (2), a survey to recognize different *Burkholderia* strains in soils with different agricultural management within the main agricultural production area of Argentina was performed. Cultivable *Burkholderia* spp. on PCAT medium were identified by both Random Fragment Length Polymorphism (RFLP) with *HaeIII* digestion of an 869 bp *recA* amplicon and sequencing of a 384 bp *recA* fragment, according to Payne *et al* (3). Most of the isolates belonged to species from the Bcc, mainly *B. ambifaria* (55%) and *B. cepacia* (14%) strains. RFLP dendograms showed different patterns for *Burkholderia* isolates that belong to the same species, suggesting gene polymorphism or, alternatively the presence of new species that require a more detailed characterization. Although the quantification did not show any statistical difference among sites or treatments (reaching ca. 10^6 per gram of soil irrespective of soils types or agricultural managements) we observed differences in biodiversity indexes (Rarefaction, Simpson evenness Index), especially when comparing non-cultivated vs. cultivated soils. Further studies about the *Burkholderia* evolution in these soils will provide insight into the impact of agricultural management on the biodiversity of this genus.
This work was supported by Agencia de Promoción Científica y Tecnológica-PAE36976-PID 52.
1- Compant, S.; Nowak, J.; Coenye, T.; Clement, C.; Ait Barka, E. (2008). Diversity and occurrence of *Burkholderia spp*. in the natural environment. FEMS Microbiol Rev (607-626)
2- http://www.biospas.org/en/
3- Payne, G.W.; Vandamme,P.; Morgan,S.H.; LiPuma,J.J., Coenye,T, Weightman,A.J.; Jones,T; and Mahenthiralingam, E. (2005). Development of a *recA* Gene-Based Identification Approach for the Entire *Burkholderia* Genus. Applied and Environmental Microbiology (3917–3927).

A7
MOLECULAR EPIDEMIOLOGY OF *BURKHOLDERIA CEPACIA* COMPLEX BACTERIA: CAN RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TYPING SHOW INTER-LABORATORY STABILITY?

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The application of molecular epidemiology methods to the study of *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) infection in individuals with cystic fibrosis (CF) has had a massive impact on survival, quality of life and clinical care practices. Surveillance for transmissible and virulent strains of these species is ongoing in many countries and frequently carried out by CF microbiology reference laboratories. The bacterial strain typing methods applied vary considerably; methods such as macrorestriction and pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) typing, repetitive element or BOX-PCR, variable number tandem repeat (VNTR) PCR and multilocus sequence typing (MLST) have been applied with great success in a number of studies and reference centres. Of the latter methods, PCR fingerprinting techniques such as RAPD, VNTR and BOX-PCR, are among the fastest and easiest to apply, but may suffer from problems of reproducibility across centres and hence have not been used for global strain tracking. Given the successful use of RAPD typing for over 15 years of CF molecular epidemiology, we set out to establish if an updated method could be applied reproducibly at two laboratories, taking advantage of modern colony-based DNA extraction procedures to speed up processing and the Agilent BioAnalyser microfluidics platform as a high resolution means to separate the amplified polymorphisms. To test inter-laboratory reproducibility of this enhanced RAPD typing approach, a collection of 65 *Burkholderia cepacia* complex strains was assembled. The strains had been MLST typed as a gold standard comparator and spanned all the formally named species groups as well as novel groups. The strain collection was RAPD typed at two locations, Cardiff and Prague, with only the DNA extraction (by CHELEX resin), PCR reagents (Qiagen), PCR amplification cycling (not the thermal cycler), and Agilent BioAnalyser, standardised at each location. RAPD profiles from each location were clustered using GelCompar II at Cardiff. Greater than 97% reproducibility between the two centres was obtained for RAPD typing with primer 270 with only 3 of the 65 Bcc failing to cluster correctly across the laboratories. In comparison to MLST, RAPD was also able to correctly discriminate 41 of the 59 (69.5% effective) sequence types (STs). Bcc clonal complexes such as the multiple ST *B. cenoecpacia* ET12 lineage were not discriminated individually but were clustered as a single group. Separation of the RAPD profiles on standard agarose gels, as opposed to PCR amplification anomalies was shown to account for major variation in typing results. Overall, an RAPD typing scheme with excellent inter-laboratory stability was established for the Bcc, opening up the potential to apply this rapid PCR typing method to other CF pathogens across multiple research centres.

A8
BCC CHROMOSOME 3: A VIRULENCE PLASMID!

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Following the discovery that 3 mini-Tn*5* insertion mutants showing reduced pathogenicity in a *Caenorhabditis elegans* infection model had lost chromosome 3 (c3), the role of this chromosome in *B. cenocepacia* growth and pathogenicity was investigated. A pEX18-based vector was constructed bearing the origin of replication and adjacent genes, encoding proteins required for replication of *B. cenocepacia* H111 c3. This 'mini c3' was introduced into a range of BCC members, resulting in c3 loss owing to plasmid incompatibility in most cases. The resultant strains were subjected to a series of phenotypic tests. Loss of c3 correlated with significantly decreased virulence in the *Caenorhabditis elegans* and *Galleria mellonella* models in the majority of strains tested.

Loss of c3 also resulted in the decrease or abrogation of antifungal activity against *Rhizoctonia solani* and *Fusarium solani* in all strains that originally exhibited such activity.
A *lacZ* gene was cloned into c3 to facilitate investigation of the frequency of c3 loss in *B. cenocepacia* H111 under different conditions. Hence, the ratio of blue (c3 positive) to white (c3 negative) colonies on agar plates containing X-gal could be calculated. The frequency of c3 loss from *B. cenocepacia* H111 was found to be surprisingly high (~1 in 1,000 on LB medium).

A9

HIGH CONFIDENCE PREDICTION OF ESSENTIAL GENES IN THE GENUS *BURKHOLDERIA*

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Essential genes, considered to be the foundation of life, are absolutely required for the survival of the organism. Determination of a minimal set of essential genes needed to sustain a life form, besides having importance as one of the most fundamental questions of science, also plays an important role in the emerging field of synthetic biology. Furthermore, due to their indispensability for bacterial cell's survival, essential genes are considered to be the best targets of novel antimicrobials. A set of 620 putative essential genes has been identified which consists mainly of the open reading frames of the core genome of *Burkholderias*. The vast majority of the essential genes identified in our study are located on the chromosome 1, thus underlying the importance of this chromosome for the storage of the essential "house-keeping" functions. Besides known homologues from other bacteria, the set of genes identified in our study harbors a number of unknown essential genes. The essentiality of several of the genes of this gene set, namely *infB*, *engA*, *gyrB*, *uniB*, *valS*, *BCAL1882*, *BCAL3142* and *BCAL3369* has been confirmed experimentally by the construction of the rhamnose-dependent inducible mutants. The constructed conditional mutants were viable only in the presence of rhamnose in the medium, as expected for mutants with an essential gene under the control of the rhamnose-inducible promoter. The essential genes identified in our analysis could be exploited as promising targets of novel antibiotics against *Burkholderia* and more detailed study, particularly of those less characterized, may shed new light on the functions required to support cellular life.

A10

GENE EXPRESSION IN *BURKHOLDERIA CENOCEPACIA* AND *BURKHOLDERIA MULTIVORANS*: THE GLOBAL TRANSCRIPTOMIC RESPONSE TO DIFFERENT GROWTH CONDITIONS ENCOUNTERED IN THE CYSTIC FIBROSIS LUNG

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Several *B. cepacia* complex (Bcc) genomes have been recently completed and facilitated the implementation of post-genomic approaches to study CF infection. Microarrays have been designed to the genome of several BCC

strains, among them *B. cenocepacia* strain J2315, representative of a highly virulent and transmissible CF strain, and *B. multivorans* ATCC17616, representative of the other dominant Bcc species infecting CF patients. These arrays have provided an ideal opportunity to explore the global transcriptomic response to changes in environmental conditions likely to occur when Bcc bacteria from the environment come into contact with the CF lung. We have investigated global gene expression during shifts in nutritional status, oxygen and iron concentration, pH, temperature and the response to oxidative stress in *B. cenocepacia* J2315. We then repeated the experiments with *B. multivorans* with a selection of these conditions, focussing on low oxygen concentration, entry into stationary phase, low pH and low iron concentration. Using this approach we aimed to identify the genes expressed and adaptations made by Bcc bacteria that allow them to infect and persist in the CF lung. Particularly entry into stationary phase and exposure to low oxygen concentration revealed numerous upregulated genes and gene clusters that might be necessary to survive conditions of growth arrest. Among these are genes shared between both strains investigated as well as genes unique to either strain. Likewise some highly regulated genes with orthologs in both strains show the same expression pattern in both strains; however there were also examples of orthologs demonstrating differential regulation in the two species. This demonstrates once more the versatility and plasticity within the genus *Burkholderia*. The studies presented will provide valuable preliminary information on gene expression and a reference guide for future studies on the molecular pathogenesis of the Bcc.

A11

ADAPTATION OF *BURKHOLDERIA CENOCEPACIA* DURING CHRONIC INFECTION OF THE CYSTIC FIBROSIS LUNG

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Within the cystic fibrosis (CF) lung, pathogens coexist with the host for many years. To understand how *Burkholderia* species adapt to this environment, we are performing phenotypic and genotypic studies of sequential *B. cenocepacia* and *B. multivorans* isolates from individual CF patients. Here we present initial findings based on our studies of the adaptation of *B. cenocepacia* J2315 during chronic infection of the CF lung. *B. cenocepacia* J2315, the UK index case of the highly transmissible ET-12 lineage, was isolated from a CF patient in Edinburgh in 1989. In the present study, we have sequenced the genome of *B. cenocepacia* C4452, which was isolated from the same patient 14 years later. The genome sequence of C4452 was compared to that of J2315 (sequenced previously by the Sanger Institute [Holden *et al*. 2009. J. Bacteriol. 191, 261-277]), enabling the identification of single nucleotide polymorphisms (SNPs) and insertion-deletion events (Indels) that had accumulated during the 14 year period. The timecourse of the emergence of these SNPs and Indels was ascertained by analysis of intermediate isolates from the same patient. Forty non-synonomous SNPs were identified, over 50% of which were predicted to affect protein function on the basis of bioinformatic analysis. These included SNPs in genes associated with auxotrophy, antibiotic resistance, iron uptake and gene regulation (particularly two-component systems). The functional significance of these SNPs and Indels is being investigated through analysis of the sequential isolates, and also by the targeted inactivation and subsequent complementation of the genes of interest. The analysis of these natural gene polymorphisms is shedding light on the critical determinants of protein function, as well as enhancing our understanding of how *Burkholderia* species adapt to the unique environment of the CF lung.

A12

ADAPTIVE DEVELOPMENTS OF *BURKHOLDERIA MULTIVORANS* CLINICAL ISOLATES DURING LUNG COLONIZATION– A TRANSCRIPTOMIC AND PHENOTYPIC APPROACH

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Bacteria from the *Burkholderia cepacia* complex (Bcc) have emerged as important opportunistic pathogens in cystic fibrosis patients. Amongst other virulence factors, the production of exopolysaccharide (EPS) has been linked to virulence of Bcc bacteria, and all of the 17 Bcc species contain strains that are able to produce EPS (1, 2). However, some of the most virulent Bcc strains are non EPS producers. Variation from mucoid to nonmucoid phenotype has being reported to occur in Bcc clinical isolates during the course of infection (2). The significance of mucoid variation in chronic infections as well as its regulation and relationship to other virulence traits displayed by Bcc bacteria need to be assessed. Transcriptomic profiling of two isogenic mucoid and nonmucoid sequential clinical isolates – *B. multivorans* D2095 and *B. multivorans* D2214 – was performed and genes involved in EPS biosynthesis, cell wall biogenesis, virulence traits, central metabolism, transcription, and signal transduction showed a down-regulation in the nonmucoid clinical isolate. These changes indicate a shift toward decreased expression of genes involved in metabolism and may suggest a lower metabolic activity of the nonmucoid clinical isolate. A phenotypic characterization was also performed and whilst several phenotypic assays showed similarities between two strains (growth rates, siderophore production, ability to oxidize 95 compounds and resistance to oxidative stress conditions), differences were found in motility, antibiotic resistance, biofilm formation and virulence in *G. mellonella*. The obtained results may evidence an adaptation of *B. multivorans* to the depleted lung environment. Further work to understand the regulatory mechanisms underlying mucoid phenotype switch and to identify the conditions that trigger the mucoid to nonmucoid variation are already being carried out.

1) Ferreira *et al.*, *Appl Environ Microbiol*, 76 (2): 441-50, 2010
2) Zlosnik *et al.*, *J Clin Microbiol*, 46 (4): 1470-3, 2008

A13
GENOMIC EVOLUTION OF A BACTERIAL PATHOGEN WITHIN HUMAN HOSTS

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Bacterial pathogens evolve during the infection of human hosts, but little is known about the mutations underlying adaptation. Here we systematically track the genomic evolution of a pathogenic strain during the

long-term infection of multiple patients. We sequenced the genomes of 112 Burkholderia dolosa isolates recovered from 14 people with cystic fibrosis, part of a 39-patient epidemic spanning almost two decades. Phylogenetic analysis suggests a likely transmission network between patients. Within patients, we find evidence for the transfer of multiple bacterial clones from the lungs to the bloodstream. While most mutated genes evolve under purifying selection, independent occurrences of mutations in multiple patients identifies 17 genes under strong positive selection. These genes illuminate the genetic basis of known pathogenic phenotypes and implicate novel pathways in bacterial pathogenesis. Sequencing the genomes of spreading microbial pathogens can help trace infection networks and identify evolutionary pressures relevant to therapy.

A14

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

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Burkholderia cenocepacia is a multi-drug resistant opportunistic pathogen that can establish chronic infection in cystic fibrosis (CF) patients, contributing to increased morbidity and mortality. While the mechanisms by which *B. cenocepacia* causes disease are poorly understood, survival within host phagocytic cells may contribute to persistent infection and bacterial dissemination. Previous research in our laboratory has shown that *B. cenocepacia* is able to delay phagosomal maturation and assembly of the NADPH oxidase, and alter the actin cytoskeleton of macrophages. Viable bacteria are required to modify macrophage activity. Thus, we hypothesize that *B. cenocepacia* alters its gene expression within macrophages. Using a combination of microarrays and "selective capture of transcribed sequences" (SCOTS), optimized for *B. cenocepacia*, we have identified genes differentially expressed by intracellular versus extracellular bacteria. Adaptation to the phagosomal environment requires differential expression of genes involved in transcriptional regulation, signal transduction, and metabolic adaptation, as well as a high proportion of unannotated genes. Previous studies in our laboratory have indicated *in vivo* relevance for a number of these SCOTS-identified genes, providing external validation of our findings. Our results are further validated via reverse transcription real-time PCR quantification of intracellular gene expression. We deleted several SCOTS-identified genes in gentamicin-sensitive *B. cenocepacia* and identified alterations in the ability of the bacteria to infect and replicate within macrophages. The SCOTS technique provides valuable information on the intracellular gene expression of *B. cenocepacia*, perhaps explaining the intracellular behaviour of this bacterium within murine macrophages.

A15

THE EVOLUTIONARY DYNAMICS OF PLASMIDS AND CHROMOSOMES INHABITING THE SAME BACTERIAL CYTOPLASM: THE *BURKHOLDERIA VIETNAMIENSIS* G4 CASE

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Despite the key-role of plasmids in the horizontal spreading of genetic information within the prokaryotic community, their evolutionary dynamics has been poorly explored. Particularly interesting from an evolutionary

viewpoint is the cross-talk existing between the different DNA molecules (plasmids and chromosomes) inhabiting the same cytoplasm. In order to shed some ligth on this issue, we performed a detailed analysis of the genome of *Burkholderia vietnamiensis* G4, a β-proteobacterium belonging to the *Burkholderia* genus. We focused the attention on this micro-organism since its genome consisted of five plasmids and three chromosomes of different size; hence, strain G4 can be considered as a model microorganism to analyze the molecular mechanisms responsible of the gene rearrangements occurring between different DNA molecules of the same cell. To this purpose, we adopted the pipeline Blast2Network (B2N). This software allows the visualization of evolutionary relationships among a datasets of sequences through the building up of identity-based similarity networks.
The aims of our work were: i) the identification and the analysis of the possible evolutionary relationships existing within and among the five *B. vietnamiensis G4* plasmids at both intra- and intermolecular level, ii) the analysis of the gene flow (if any) existing between the chromosomes and plasmids of different size inhabiting the same cytoplasm. Data obtained revealed: i) the existence of intra-molecular rearrangements mainly due to *duplication events* that occurred in different stages of plasmids evolution; ii) an extensive gene flow between both different plasmids and plasmids and chromosomes; iii) these genetic rearrangements involved single genes, multiple genes, operons and/or gene clusters; iv) as might be expected, *mobile genetic elements* have played and are still playing a key-role in the recombination events occurring between different molecules; v) complex evolutionary pathways leading to the structure of the G4 plasmids.

A16
IDENTIFICATION OF *BURKHOLDERIA CENOCEPACIA* H111 VIRULENCE FACTORS BY USING MULTIPLE INFECTION HOSTS

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Species of the *Burkholderia cepacia* complex (Bcc) can cause disease in plants, and can establish opportunistic infections in animals and in humans affected by immunodeficiency, chronic granulomatous disease or cystic fibrosis. However virulence mechanisms are poorly understood.
Recent studies have demonstrated that non-mammalian infection models are higly valuable in the identification of virulance factors. In the case of Bcc strains, infection models using *Caenorhabditis elegans*, *Galleria mellonella* and *Drosophila melanogaster* have been described. In this study, approximately 6000 mutants from a *B. cenocepacia* H111 random mini-Tn*5* insertion library were screened by using the *C. elegans* pathogenicity model. Around 25 mutants were found to be attenuated in this infection model. To our surprise none of the mutated genes coded for the biosynthesis of classical virulence factors like proteases or siderophores. Instead we found several mutants with deletions in metabolic and regulatory pathways. The attenuated mutants were tested with the *D. melanogaster* pricking assay, representing an acute infection model. To exclude host-specific factors, all mutants which showed an attenuation in both of the models were also tested using the *G. mellonella* infection assay.
We found that several mutants which were strongly attenuated in the *C. elegans* model were also less virulent in the *D. melanogaster* pricking model. Mutants strongly attenuated in the *G. mellonella* model were generally also attenuated in the *D. melanogaster* pricking model.

A17
ROLE OF THE TYROSINE KINASE BceF IN *BURKHOLDERIA* PHYSIOLOGY

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Production of the exopolysaccharide (EPS) cepacian by *Burkholderia* is a common feature among environmental and clinical strains. Its role in the interaction with the host immune system, in biofilm formation and protection against several stress conditions has being described. The proteins involved in cepacian biosynthesis are well known and among them, BceF, a BY-kinase protein from *B. cepacia* IST408, was previously described as required for cepacian biosynthesis (1). Further studies on biofilm formation assays and virulence indicate that BceF kinase has a broader role in cell physiology, besides EPS production. Indeed, phenotypic studies on *bceF* and *bceE* insertion mutants, both unable to produce cepacian, showed a much stronger reduction of biofilm size and virulence in *Galleria mellonela* in the absence of the BY-kinase protein than in the mutant on the gene encoding the outer membrane auxiliary protein, BceE. The ability of BY-kinases to interact with other proteins, promoting their phosphorylation and thereby controlling their activity, has been described in BceF homologues. The BY-kinases substrates include other proteins that are not involved in EPS production and indicate their involvement in broader regulatory systems. To evaluate whether this was also the case of BceF protein, we have used a *Burkholderia* Affymetrix custom microarrays to study global gene expression changes promoted by *bceF* and *bceE* mutations in *B. cepacia* IST408. The results indicate that while *bceE* mutation induced changes of about 30 genes, most of them related with carbon metabolism, *bceF* mutant induced the change of more than 200 genes, including genes involved in transcription, signal transduction and transport. Down-regulated genes include many porins and transport systems, suggesting a reduction of proteins and virulence factors export in *bceF* mutant. Taken together the data obtained indicates that BceF protein has an important and broader role in *Burkholderia*, not only in EPS biosynthesis, but also contributing to virulence.

(1) Ferreira *et al.* (2007) *Appl Environ Microbiol* 73: 524–534

A18
ROLE OF IRON ACQUISITION IN *B. CENOCEPACIA* PATHOGENESIS

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Burkholderia cenocepacia in common with many bacterial pathogens, produces low molecular weight compounds known as siderophores to scavenge iron from the host environment and to facilitate survival *in vivo*. We were interested in examining the siderophore response of *B. cenocepacia* and its potential significance in the pathogenesis of this organism. Analysis of siderophore production and gene expression in response to altered iron concentrations, demonstrated that *B. cenocepacia* secreted significant siderophore levels below 3uM iron, with significant upregulation of five genes involved in siderophore production (*orbA, orbF, pvdA, pchD* and *fptA*). In particular, there was a 10 fold increase in the three ornibactin related genes (*orbA*, *orbF* and *pvdA*) relative to that of pyochelin related genes (*pchD* and *fptA*). When transferred from iron replete to minimal iron media, the *B. cenocepacia* siderophore response was rapid, with all three ornibactin related genes detectable in 15 minutes reaching maximal levels within 45 minutes. In combined cultures with *P. aeruginosa*, the expression of *pvdA* (ornibactin biosynthesis) by *B. cenocepacia* was significantly upregulated compared to control *B. cenocepacia* cultures and further analysis of the effect of competing pathogens on *B. cenocepacia* iron acquisition is ongoing. The role of iron availability on bacterial internalization by epithelial cells was examined. The addition of recombinant ornibactin to *B. cenocepacia* infected epithelial cultures, resulted in significantly increased internalization of bacteria by CFTR negative but not CFTR positive epithelial cells. Bacterial internalization by CFTR negative cells was also significantly greater in iron replete conditions compared to minimal iron conditions. Proteomics are underway on *B. cenocepacia* under low and high iron conditions in an effort to identify the bacterial responses that facilitate the enhanced uptake. These data confirm that the *B. cenocepacia* siderophore response in iron depleted conditions is ornibactin dominated, and also demonstrate that ornibactin production by *B. cenocepacia* is rapidly activated, is sensitive to the presence of co-colonising pathogens, and may play a role in *B. cenocepacia* epithelial internalization in the CF lung.

A19
ROLE OF ADHESINS IN *BURKHOLDERIA MULTIVORANS* VIRULENCE

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Recent shifts in the epidemiology of the *Burkholderia cepacia* complex (Bcc) place *Burkholderia multivorans* as the dominant species in Bcc respiratory infections in patients with cystic fibrosis (CF). In contrast to *B. cenocepacia*, we know very little about the mechanisms of virulence in *B. multivorans*. From studies to assess the impact of mucoidy within the Bcc, we found that exopolysaccharide (EPS) production positively correlated with the ability of *B. multivorans* ATCC17616 (an environmental strain) to adhere to mucin. Interestingly, the ability of the CF isolate *B. multivorans* C1576 to adhere to mucin was EPS independent. From whole genome sequencing of C1576, we identified putative adhesins that were absent from ATCC17616, namely a fimbrial locus and two distinct adhesins of the filamentous haemagglutinin (FHA) family. We hypothesized that the presence of these putative adhesins in C1576 could explain the observed strain variation in adhesion. This study aimed to investigate the role of these adhesins in *B. multivorans*, and examine their distribution amongst *Burkholderia* species. The three adhesins were knocked-out (individually) in C1576 by insertional inactivation. Results show that all three adhesin mutants are impaired in their ability to bind the extracellular matrix protein fibronectin, and form significantly less biofilm than the wild-type C1576. Based on analysis of available genome sequences, all three adhesins show a restricted species distribution within the Bcc, being largely confined to clinical isolates of *B. multivorans*. Adhesins play an important role in host-pathogen interactions in many gram-negative bacteria and our characterisation of adhesins associated with clinical strains of *B. multivorans* provides new insight into the virulence factors within this species.

A20
THE *pobA* GENE OF *BURKHOLDERIA CENOCEPACIA* ENCODES A GROUP I SFP-TYPE PHOSPHOPANTETHEINYL TRANSFERASE REQUIRED FOR BIOSYNTHESIS OF THE SIDEROPHORES ORNIBACTIN AND PYOCHELIN

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Burkholderia cenocepacia, produces the siderophores ornibactin and pyochelin under iron restricted conditions. Biosynthesis of both siderophores requires the involvement of non-ribosomal peptide synthetases (NRPSs). Using a transposon containing the *lacZ* reporter gene, two *B. cenocepacia* mutants were isolated which were deficient in siderophore production. Mutant IW10 was shown to produce normal amounts of ornibactin but only trace amounts of pyochelin, whereas synthesis of both siderophores was abolished in AHA27. Growth of AHA27, but not IW10, was inhibited under iron restricted conditions. In both mutants, the transposon had integrated into the *pobA* gene, encoding a polypeptide exhibiting similarity to the Sfp-type phosphopantetheinyl

transferases (PPTases). These enzymes are responsible for activation of NRPSs by the covalent attachment of the 4'-phosphopantetheine (P-pant) moiety of coenzyme A. Previously characterised PPTase genes from other bacteria were shown to efficiently complement both mutants for siderophore production when provided in *trans*. The *pobA* gene was also able to efficiently complement an *E. coli entD* mutant for production of the siderophore enterobactin. Analysis of PPTase amino acid sequences confirms that Sfp-type bacterial PPTases can be subdivided into two distinct groups and reveals that PobA is a member of Group I. The *pobA* mutant is a useful tool for analysing xenosiderophore utilisation by *B. cenocepacia*. Measurement of *lacZ* reporter gene expression demonstrated that *pobA* is not appreciably iron regulated.

A21
SERUM RESISTANCE AND MOTILITY IN CLINICAL ISOLATES OF *BURKHOLDERIA CEPACIA* COMPLEX BACTERIA

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Infection with *Burkholderia cepacia* complex bacteria in cystic fibrosis is associated with an unpredictable clinical outcome. We have previously demonstrated an inverse association between the mucoid phenotype and outcome in a patient population. Here we examine two other bacterial factors that could contribute towards virulence in in cystic fibrosis: resistance to the inhibitory effects of human complement proteins and bacterial motility. Traditional methods of assessing serum resistance in bacteria involve a laborious assessment of colony forming units before and after exposure to serum. To permit a higher throughput assessment of serum resistance, we have established a growth assay using a BioScreen growth curve machine. Using both this and traditional viability based serum exposure assays we have determined that there is no link between the capacity of an isolate to elaborate EPS and serum resistance. Furthermore, an assessment of pulmonary isolates of BCC from the late-stage of CF infections that subsequently progressed to 'cepacia-syndrome' showed that many of these isolates were serum sensitive, indicating that resistance to serum is not an indicator for subsequent 'cepacia syndrome'. To assess the significance of motility in clinical isolates of BCC we analyzed the swimming motility phenotype of 507 isolates from 78 patients infected with either *B. cenocepacia* or *B. multivorans*. In our population of BCC isolates, motile bacteria were consistently isolated from the majority of patients (48/78). Of the remaining cases, nonmotile isolates were consistently isolated from 17. We also observed a small population of cases (13) where sequential isolates switched from motile to nonmotile during chronic infection-10 *B. cenocepacia* and 7 *B. multivorans*. Western blotting demonstrated that the loss of motility corresponded to a loss of detectable flagella. Therefore, while motility appears to be a common feature of BCC recovered from CF patients, the phenotype does not appear to be associated with severity of disease. The phenotypic switching of BCC from motile to nonmotile, in some cases, mirrors the well described switch in *P. aeruginosa*. Unlike *P. aeruginosa*, in which loss of motility can be caused by multiple factors, in BCC it appears that this phenotypic switch is accompanied by a loss in detectable flagellin protein.

A22
BURKHOLDERIA CENOCEPACIA SHVR REGULATED GENES THAT INFLUENCE COLONY MORPHOLOGY, BIOFILM FORMATION AND VIRULENCE

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Burkholderia cenocepacia is an opportunistic pathogen that primarily infects cystic fibrosis (CF) patients. Previously, we reported isolation of spontaneously occurring shiny variant (shv) mutants that were altered in virulence on plant and animal models of infection, biofilm formation, *N*-acyl homoserine lactone production, protease activity and motility. Several shv had mutations in *shvR*, a LysR regulator that influences colony morphology, virulence and biofilm formation (Bernier et al. 2008). Comparison of gene expression profiles of a K56-2 *shvR* mutant to wild type revealed that *shvR* is a global transcriptional regulator influencing expression of over 1000 genes including quorum sensing, type II secretion, zinc metalloprotease, and an adjacent 24-kb genomic region encoding twenty four genes (*afc* genes; O'Grady et al. 2011). In this study we report the functional characterization of selected genes in this region. A Tn5 mutant with shiny colony morphology was identified with a polar mutation in BCAS0208, predicted to encode an acyl-coenzyme A dehydrogenase. Mutagenesis of BCAS0208 and complementation analyses revealed that BCAS0208 is required for rough colony morphology, biofilm formation and virulence on alfalfa seedlings. A mutation in the catalytic site of BCAS0208 resulted in a lack of complementation. BCAS0201, encoding a putative FAD-dependent oxidoreductase and BCAS0207, encoding a putative citrate synthase do not influence colony morphology but are required for optimum levels of biofilm formation and virulence. Both BCAS0208 and BCAS0201 contribute to pellicle formation although individual mutations in each of these genes had no appreciable effect on pellicle formation. A mutant with a polar insertion in BCAS0208 was significantly less virulent in a rat model of chronic lung infection as well as the alfalfa model. Genes in this region were shown to influence utilization of branched chain fatty acids, tricarboxylic acid cycle substrates, L-arabinose and branched chain amino acids, respectively. Together, our data show that the ShvR regulated genes from BCAS0208-S0201 are required for the rough colony morphotype, biofilm and pellicle formation and virulence in *B. cenocepacia*.

A23
NEUTROPHIL AND PRO-INFLAMMATORY RESPONSE IN ZEBRAFISH EMBRYOS AFTER INFECTION WITH *BURKHOLDERIA CENOCEPACIA*.

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Bacteria belonging to the '*Burkholderia cepacia* complex' (Bcc) have emerged as opportunistic pathogens in immunocompromised patients, particularly those with cystic fibrosis (CF) and chronic granulomatous disease. After an unpredictable period of colonisation, the bacteria can cause acute, fatal necrotizing pneumonia and septicaemia, known as 'cepacia syndrome'. These Gram negative bacteria can adopt an intracellular life style, but little is know about the underlying molecular mechanisms.
Previously, we developed a zebrafish embryo model to study Bcc infection. *B. cenocepacia* isolates belonging to the epidemic ET12 lineage were highly virulent for the embryos. However, the young embryos could control infection with other less virulent Bcc strains, such as *B. vietnamiensis* FC441 and *B. stabilis* LMG14294. Using real time analysis with DSRed expressing bacteria, kinetics studies and survival assays, we showed that Bcc bacteria are mainly phagocytosed by, and survive and multiply in macrophages of zebrafish embryos. After about 15 hours, bacteria seemed to escape from infected macrophages by non-lytic release resulting first in local dissemination, followed later by systemic infection.
Lung infection in CF is characterized by a neutrophil-predominated inflammation and patients show a high level of the potent neutrophil chemoattractant Il-8. *B. cenocepacia* has been shown to be a potent inducer of IL-8 secretion in several infection models. We used neutrophil specific GFP-reporter fish to show that, although neutrophils are recruited to injection wound sites, they are only marginally involved in phagocytosis of bacteria injected in the blood circulation. However, early inflammation sites showed massive neutrophil recruitment, and, interestingly, the fate of neutrophils differed between fatal and persistent infections. Infection with *B.*

cenocepacia K56-2 and J2315, but not with a K56-2 quorum sensing mutant, induced an increase in IL-8 expression, correlating with the observed massive neutrophil recruitment. Similarly, the expression of other pro-inflammatory response genes was up-regulated. We will report and discuss our recent findings.

A24
ACTIVATION OF MMP-9 BY HUMAN LUNG EPITHELIAL CELLS IN RESPONSE TO *BURKHOLDERIA CENOCEPACIA* INFECTION DELAYED WOUND REPAIR

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Matrix metalloproteinases (MMPs) are a group of related enzymes which regulate various inflammatory and repair processes. MMPs are upregulated in CF patients and are elevated in response to infection. The aim of this work was to examine the role of MMPs in the pathogenesis of *Burkholderia cepacia* complex (Bcc), which has not been explored to date.
Real-time PCR analysis showed that *B. cenocepacia* infection resulted in up-regulation of MMP-2 and MMP-9 genes in CF lung cell line, CFBE41o- and in non-CF lung cell line, 16HBE14o-. *B. multivorans* infection upregulated the MMP-2 gene in 16HBE14o- cells only. No alteration in MMP-9 gene expression was observed in response to either *B. multivorans* or to *P. aeruginosa* in either cell line. Conditioned cell media (CCM) from both cell lines also showed increased MMP-9 activation following *B. cenocepacia* infection and to a lesser degree, following exposure to *B. multivorans*. In polarized lung epithelia, this activation was basolateral only. To examine the potential effect of MMP-9 activation on lung cells, confluent lung cell monolayers were wounded and exposed to CCM from cells infected with *B. cenocepacia*, *B. multivorans* or *P. aeruginosa*. CCM from *B. cenocepacia* infected cells significantly reduced the rate of wound healing in confluent lung epithelia ($p<0.05$), in contrast to CCM from *P. aeruginosa* infected cells, which showed predominant MMP-2 activation. CCM from infected with *B. multivorans* also significantly reduced the rate of wound repair ($p<0.05$), but to a lesser extent. Wound repair was not delayed in wounded monolayers treated with CCM from *B. cenocepacia* infected cells in the presence of specific MMP-9 inhibitor. This demonstrates that MMP-9 plays a role in the reduced epithelial repair observed in response to *B. cenocepacia* infection. It implies that MMP-9 activation following *B. cenocepacia* infection contributes to the pathogenesis of this virulent pathogen.
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A25
QUORUM SENSING-CONTROLLED GENES IN A *BURKHOLDERIA AMBIFARIA* CLINICAL STRAIN

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The *Burkholderia cepacia* complex (Bcc) is a group of closely related species that are widespread in the environment. They display a remarkable ability to colonize diverse niches, leading to various lifestyles [1]. One characteristic feature of Bcc species is that they can be isolated from both the environment and immunocompromised patients, especially those suffering from cystic fibrosis (CF).
Burkholderia ambifaria, formerly *B. cepacia* genomovar VII, well illustrates this trait. This species mainly inhabits the rhizosphere, where it can positively influence plants growth, thanks to the secretion of antifungal compounds; these properties have even been commercially exploited. Unfortunately, even if it is a rather rare occurrence, *B. ambifaria* can also be isolated from the lungs of CF patients [2].

We have previously demonstrated that clinical isolates of *B. ambifaria*, including strain HSJ1, produce phase variants displaying phenotypes close to environmental strains [3]. While these distinctive phenotypes are mostly known to be controlled by quorum sensing (QS), the variants do not show significant differences in terms of C_8-homoserine lactone (HSL) production. These intriguing results lead us to investigate in more details which genes are under QS regulation in the HSJ1 clinical strain. Using a *cepI* mutant deficient in C_8-HSL production as a background for random whole-genome transposon-insertion mutagenesis, we have identified several not previously described QS-controlled genes. Most of them are implicated in metabolism, some others in signal transduction or in virulence. For instance, our results show that even in a clinical strain, the production of antifungal compounds is an important trait controlled by QS. Several other phenotypes associated with virulence, such as extracellular proteases production, haemolytic activity and siderophores secretion, appear to be QS-controlled as well in the HSJ1 strain. We have also further confirmed the link between AHL-mediated QS and the production of 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs), a family of molecules to which also belongs the *Pseudomonas* Quinolone Signal (PQS). Our results will contribute to a better understanding of QS regulation in the Bcc.

1. Vial L, Chapalain A, Groleau MC, Déziel E (2011) The various lifestyles of the Burkholderia cepacia complex species: a tribute to adaptation. Environ Microbiol 13: 1-12.
2. LiPuma JJ, Spilker T, Gill LH, Campbell PW, 3rd, Liu L, et al. (2001) Disproportionate distribution of Burkholderia cepacia complex species and transmissibility markers in cystic fibrosis. Am J Respir Crit Care Med 164: 92-96.
3. Vial L, Groleau MC, Lamarche MG, Filion G, Castonguay-Vanier J, et al. (2010) Phase variation has a role in Burkholderia ambifaria niche adaptation. ISME J 4: 49-60.

A26

BDSF (*CIS*-2-DODECENOIC ACID) SIGNALLING AND VIRULENCE IN THE CYSTIC FIBROSIS RESPIRATORY PATHOGEN *BURKHOLDERIA CENOCEPACIA*

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Many bacteria use cell-cell communication mediated by diffusible signal molecules to monitor their population density or confinement to niches and to modulate their behaviour in response to these aspects of their environment. *Burkholderia cenocepacia* is an opportunistic human pathogen that uses *cis*-2-dodecenoic acid (BDSF) as a quorum-sensing signal to control expression of biofilm formation and virulence factors. BDSF is a signal molecule of the diffusible signal factor (DSF) family that was first described in the plant pathogen *Xanthomonas campestris*. The mechanism of perception of this signal and the range of functions regulated in *B. cenocepacia* are however unknown. A screen for transposon mutants unable to respond addition of the exogenous signal identified BCAM0227 as a potential BDSF sensor. BCAM0227 is a histidine sensor kinase with an input domain unrelated to that of RpfC, the DSF sensor found in xanthomonads. Transcriptome profiling established the scope of the BDSF regulon and demonstrated that the sensor controls expression of a subset of these genes. A chimaeric sensor kinase in which the input domain of BCAM0227 replaced the input domain of RpfC was active in BDSF signal perception when expressed in *X. campestris*. Mutation of BCAM0227 gave rise to reduced cytotoxicity to CHO cells and reduced virulence to wax moth (*Galleria mellonella)* larvae and in the agar-bead mouse model of pulmonary infection. In conclusion, we have shown that BCAM0227 is a sensor kinase that is an element of the BDSF mediated cell-cell signalling pathway of *B. cenocepacia* that contributes to the virulence of this organism. In the context of the global rise of anti-microbial resistance and the slower development of novel antibiotics, cell-cell signalling pathways are being evaluated as potential drug targets. Our findings make a contribution towards the greater understanding of the mechanisms of signal generation and perception that will be important for the development of novel therapeutic strategies to combat bacterial infections.

A27
FACTORS LINKING QUORUM SENSING AND BIOFILM FORMATION IN *BURKHOLDERIA CENOCEPACIA* H111

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Burkholderia cenocepacia H111, a CF isolate, employs the CepIR quorum-sensing (QS) system to control the expression of virulence factors as well as the formation of biofilms. To date, however, very little is known about the factors that link QS and biofilm formation. In this study, we have used a combined transcriptomic and proteomic approach to precisely define the QS regulon in *B. cenocepacia* H111. Among the QS-regulated loci, we focused on three gene clusters with potential impact on biofilm formation: i) the *bclACB* lectin operon, ii) a gene cluster coding for a *fimA* homologue and a putative chaperon/usher pathway and iii) a locus comprising the large surface protein BapA and an ABC transporter. By means of transcriptional fusions of the respective promoters to *lacZ*, we could confirm the QS-dependent expression of all the three loci. Analyses of defined mutants revealed that the type 1 pilus showed little, if any, effect on biofilm formation in both a static microtiter-dish based assay as well as in flow-through cells. Inactivation of the *bclACB* lectins resulted in equal amount of biomass compared to the wild-type strain in the static biofilm assay, but revealed characteristic hollow microcolonies when biofilms were cultured in flow-chambers, suggesting that the lectins are important for the structural development of a biofilm. Inactivation of *bapA* leads to a significant decrease of biofilm formation in both assays, indicating that BapA plays a major role in biofilm formation on abiotic surfaces.

A28
QUORUM SENSING IN THE EMERGING GROUP OF PLANT ASSOCIATED *BURKHOLDERIA*

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The genus *Burkholderia* comprises more than 60 species isolated from a wide range of niches. Although *Burkholderia* species have shown to be diverse and ubiquitously distributed, most of the studies have thus far focused on the pathogenic species due to their clinical importance. Recently, the growing number of described species associated to plants or to the environment has evidenced an emerging sub-group comprising at least 31 non-pathogenic species, which in most cases have been found to be associated with plants. Several species from this beneficial plant-associated group share characteristics such as a conserved quorum sensing (QS) system, the presence of nitrogen fixation and nodulation genes and the aromatic compound degrading ability. Our laboratory is interested in studying the role of cell-cell communication in this group of *Burkholderia* and initial studies revealed the presence of a conserved QS system in all species and with some having two QS systems. The presence of a well conserved and distinct AHL QS system among this group of *Burkholderia* is a further indication that (i) they are closely related, and (ii) that this system might play an important and conserved role in the life style of this emerging group of species. Our current studies are focused on the role of QS in plant-bacteria interaction.

A29
CLINICAL AND ENVIRONMENTAL *B. CENOCEPACIA* AND *P. AERUGINOSA*: DUAL-SPECIES INTERACTION IN BIOFILM AND IN NON-CF AND CF MICE

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In the lung of patients with cystic fibrosis (CF) the persistent bacterial infection not only attests to the complex interaction between the pathogen and the host, but also to the communication between pathogenic microorganisms. Since *P. aeruginosa* (PA) and *B. cenocepacia* (BC) inhabit the same environmental niches and can also form mixed biofilms in CF lung, it is very likely that the two organisms interact with each other. Therefore, exploring the interactions between the different pathogens that may have synergic effects on their respective virulence is fundamental to understand the pathogenesis of the CF infection. Since natural environment represents a “reservoir” of PA and BC potential pathogenic strains for CF patients, both clinical and environmental strains were examined as concerns: (i) population dynamics in single and dual co-cultures, (ii) mutual influence on planktonic and biofilm growth, (iii) mutual effects of extracellular compounds on each species growth and biofilm formation, and (iv) competition in non-CF and CF mice and host inflammatory response.
Results revealed that PA planktonic growth was not affected by the presence of BC, while a negative effect of PA over BC planktonic growth was observed in exponential and stationary phases liquid culture. Mixed cultures of clinical BC and PA strains resulted in a significant increase in biofilm formation compared to their pure culture counterparts. Both physical interactions between cells and extracellular secreted factors contribute to dual-species biofilm formation. *In-vivo* experiments revealed that PA and BC co-infection did not enhance mortality of non-CF and CF mice in comparison with single infection. Both pathogens were present in all vital organs of both non-CF and CF mice after 2-3 days of infection at a similar bacterial load, suggesting the same level of bacteremia, whereas after 13 days of co-infection mice were mainly infected by PA, indicating that BC lost its ability to establish chronic infection. The co-presence of the two bacterial species caused an altered lung inflammatory response in both non-CF and CF mice in comparison with that produced by single infections. In conclusion, our *in vitro* and *in vivo* data indicate that the two bacterial species affect each other’s virulence and physiology when coexisting. These results provide an interesting example of interspecies communication and point out the impact of dynamic interactions between immune response and unrelated microrganisms on disease outcomes.
Supported by the Italian Cystic Fibrosis Research Foundation, grant FFC#7/2008, with the contribution of “Associazione siciliana FC”.

A30
EXOPOLYSACCHARIDES PRODUCED BY SPECIES OF THE *BURKHOLDERIA CEPACIA* COMPLEX.

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Exopolysaccharides (EPS) have been recognised as factors favouring both set up and maintenance of lung infections in CF patients. The production of an extracellular matrix by bacteria contributes to cells protection against external threats, including the immune system. In addition, EPS contribute to the formation of biofilms which increase the resistance of bacterial cell and, probably, influence the inflammatory processes.
To better characterise the potentiality of species of the *Burkholderia cepacia* Complex (BCC) in terms of EPS production, 10 species of the complex were grown on YEM solid medium, which stimulates abundant EPS production. The species investigated were: *B cepacia, B. multivorans, B. cenocepacia, B. ambifaria, B. dolosa, B. stabilis, B. lata, B. anthina, B. vietnamensis, B. gladioli*.
The structural analysis of the purified EPS showed that the BCC is able to synthesise at least eight different polymers. The majority of the species investigated produces cepacian, whose chemical structure was elucidated years ago, thus resulting the EPS typical of this group of bacteria. Besides cepacian, some strains belonging to different species produce EPS with different structures, either as sole polysaccharide or in mixtures with cepacian or other EPS. These EPS are: i) an EPS composed of galactoses and Kdo (with one galactose substituted with one acetyl group); ii) levan which is a →6)-β-D-Fru*f*-(2→ polymer; iii) dextran, a →6)-α-D-Glc*p*-(1→ homopolysaccharide; iv) PS-I constituted of glucose and galactose, the latter substituted with a pyruvic acid; v) a 1,2-linked glucan with occasional branching at position 3. In addition, a strain of *B. vietnamensis* produces a novel EPS which includes fucose in its primary structure, a sugar never found before. Furthermore, most of the NMR spectra of purified cepacian, produced by different species of the BCC, showed the presence of minor amounts of a second EPS whose structure was not yet unravelled, but preliminary data strongly indicated that it might be a cepacian-like EPS. A modified cepacian structure was also described by other authors[1] in Brazil investigating a strain of *B. kururiensis*. It is worth mentioning that the production of the above described EPS is usually medium dependant.
In conclusion, when investigating the biological effect of EPS in bacterial infections, the quite large ability of strains of species of the BCC to produce different EPS makes important to define which EPS is produced and in which conditions.

1) Hallack LF, Passos DS, Mattos KA, Agrellos OA, Jones C, Mendoça-Previato L, Previato JO, Todeschini AR. (2010) Glycobiology 30:338-347

A31
POLYSACCHARIDES OF BIOFILMS PRODUCED BY BURKHOLDERIA CEPACIA COMPLEX BACTERIA

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Biofilms are communities of bacteria that live surrounded by a complex polymeric matrix which is composed of polysaccharides, proteins and nucleic acids[1], and are associated with a surface. This matrix protects bacterial from external threats, therefore helping pathogens to escape antibacterial agents. After determining the structureof exopolysaccharides (EPS) produced by *Burkholderia cepacia* complex (BCC) bacteria in planktonic conditions, we focused on the characterization of EPS in biofilm matrices. Our attention was captured by literature data for biofilms of *P. aeruginosa* where it was reported[2] that "the relationship between biofilm formation by nonmucoid strains, and those in which conversion to mucoidy has occurred, does not appear to be a change in the alginate levels in the biofilm matrix, but a fundamental change in its carbohydrate constituents". Moreover, a polysaccharide present in *P. aeruginosa* biofilms was fully characterised and resulted to be deeply different from alginate, being neutral and composed of mannose, rhamnose and glucose. Therefore, the assumption that the EPS produced in planktonic conditions are the same of those present in biofilms might not be always correct.

The purpose of our investigation is to define the polysaccharides present in the biofilms matrix produced by BCC bacteria. The strain *B. cenocepacia* BTS2b was isolated from a CF patient and it was cultured in polystyrene plates in the biofilm mode of growth, using different media. The biofilm matrix was scraped off the plates, and the polysaccharidic component was purified. The ^{1}H-NMR spectra recorded were typical of polysaccharides, and all identical, regardless of the growth medium used. They were also very different from those of polysaccharides obtained using the same type of media, but growing the cells in planktonic mode. Methylation analysis and NMR data showed (1-4)-β-linked Gal, and various arabinose residues differing for the glycosidic linkages, the most abundant being terminal Ara and 5-linked Ara. It is remarkable that Ara has never been found before in polysaccharides from BCC bacteria. These results showed that the major polysaccharide component produced by BTS2b in the biofilm matrix is neither cepacian nor any other known polysaccharides produced by BCC bacteria grown in liquid or solid media, but a completely novel polymer.

1. R. E. Steinberger, P. A. Holden, *Biofilms* (2004) 1: 37–47
2. C. Ryder, M. Byrd, D. J. Wozniak, *Curr. Opin. Microbiol*. (2007) 10(6): 644–648.
3. M. S. Byrd, I. Sadovskaya, E. Vinogradov, H. Lu, A. B. Sprinkle, Stephen H. Richardson, L. Ma, B. Ralston, M. R. Parsek, E. M. Anderson, J. S. Lam, D. J. Wozniak, *Mol. Microbiol.* (2009) 73(4): 622–638.

A32
NEW POTENTIAL ANTIBIOTIC SOURCES FOR *BURKHOLDERIA CEPACIA*

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Bacteria belonging to the *Burkholderia cepacia* complex (Bcc) are significant pathogens in Cystic Fibrosis (CF) patients and are resistant to a plethora of antibiotics. In this context microorganisms from Antarctica are interesting since they produce antimicrobial compounds inhibiting the growth of other bacteria. This is particularly true for bacteria isolated from Antarctic sponges.The aim of this work was to check bacterial communities isolated from three Antarctic sponges for their ability to produce new natural drugs that could be exploited in the control of infections in CF patients by Bcc bacteria. Hence, 132 bacterial strains affiliated to different genera (e.g., *Pseudoalteromonas*, *Arthrobacter* and *Psychrobacter*) were tested (through the cross-streaking method) for their ability to inhibit the growth of 23 Bcc strains and some other human pathogens. Most of these bacteria completely inhibited the growth only of all the Bcc strains, suggesting a highly specific activity toward Bcc strains. The PCR amplification of genes coding for multienzyme complexes (PKS or NRPS) from the Antarctic bacteria revealed that only in few cases an amplicon of the expected size was obtained. Besides, the antimicrobial compounds are small volatile organic compounds (VOCs), and are constitutively produced *via* an unknown pathway. These data highlight the potentiality of Antarctic bacteria as novel sources of antibacterial substances to face Bcc infections in CF patients

A33
THE BACTERIAL CYTOSKELETON – A NEW ANTIMICROBIAL TARGET IN CYSTIC FIBROSIS PATHOGENS?

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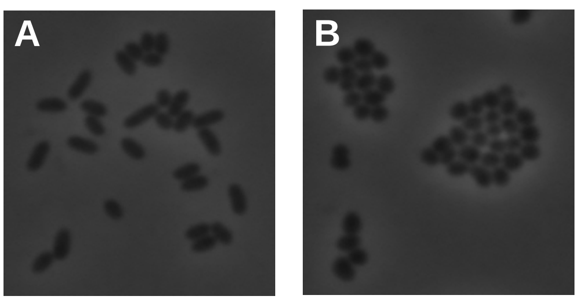
Background *Burkholderia cepacia* complex (BCC) bacteria are opportunistic pathogens which cause severe lung infections in cystic fibrosis (CF) patients. Treatment of BCC infections is difficult due to the inherent multidrug resistance of BCC. There is a pressing need to find new bacterial targets for antimicrobials that provide functions essential for cell growth & replication. A major component of the bacterial cytoskeleton is the actin homolog MreB. MreB maintains bacterial cell shape by forming filaments under the bacterial inner membrane. A22 is a cell permeable compound that disrupts MreB, destabilizing the bacterial cytoskeleton and altering the bacterial shape.
Aims To investigate the MreB bacterial cytoskeleton as a novel target for antimicrobials.
Methods We have tested a synthetic library of A22-related compounds and identified compound Q22 as a potential antimicrobial of interest against BCC and *Pseudomonas aeruginosa* strains. BCC bacteria have been grown in the presence of Q22 and a number of phenotypic changes observed.
Results Q22 inhibited growth of all 9 BCC species tested, including *B. cenocepacia*. A reduction in growth rate and cell morphology changes were also observed (Fig.1). Higher concentrations of Q22 were required to exert *B. cenocepacia* growth effects (30μg/ml Q22) when compared to *P. aeruginosa* (3μg/ml Q22), probably due to the presence of two *mreB* genes in the *B. cenocepacia* genome. BCC bacteria lipopolysaccharide (LPS) is known to play an important role during infection. We analysed the LPS profile of BCC bacteria grown in the presence of Q22 and selected strains show profile differences when compared to untreated bacteria. The cytokine responses of BCC whole cell lysates grown in the presence or absence of Q22 for 24hr were analysed in differentiated THP-1 cells. A species-specific increased response in pro inflammatory activity was observed for cytokines TNFα and IL-1β. The basis of this response is under investigation. The influence of Q22 treatment on bacterial motility and Type 3 secretion, a virulence associated secretion system, was assessed. However, growth inhibition masked motility analysis and differences observed in secreted protein profiles could not be attributed to Type 3 secretion. The growth conditions required for induction of Type 3 secretion *in vitro* remain undefined.



A, *B. cenocepacia* J2315 grown in LB with no additives;
B, *B. cenocepacia* J2315 grown in LB containing 30ug/ml Q22

Conclusions *in vitro* MreB is an attractive new target for novel antimicrobials. Further analysis of current observations and additional phenotypic analysis will be required to dissect the nature of Q22-induced changes.
Work supported by Newcastle-Upon-Tyne Hospitals Special Trustees & Italian CF Research Foundation (FFC).

A34
DEVELOPMENT OF AN ENHANCED LETHALITY ASSAY FOR TARGET IDENTIFICATION OF NOVEL GROWTH INHIBITORS IN *B. CENOCEPACIA*

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One of the most significant obstacles to developing novel molecules with antibacterial properties is the lack of understanding of their mechanism of action. We are creating a genomic library of rhamnose conditional growth mutants of *B. cenocepacia* (Bce) essential genes as a starting point for new antibacterial drug development for Bcc. We hypothesize that large scale exploration of growth inhibition will identify the molecular targets of novel antibiotics by enhanced lethality of these compounds against particular clones of a conditional growth mutant library. Our goal is to develop a highly sensitive, high throughput method where hyper-sensitized conditional growth mutant pools are grown in the presence of novel growth inhibitors and specific enhanced lethality is detected by multiplex PCR and next generation sequencing. Based on essentiality studies in other microbial genomes a library of 200-350 conditional-growth mutants should represent the totality of essential gene-containing operons in Bce. To construct a genomic library of conditional growth mutants, we developed a large-scale transposon-based delivery of a rhamnose inducible promoter where an estimated 500,000 clones need to be screened to achieve 5X genomic coverage. In addition, we predicted a hit frequency of 0.033 %, taking into account an organism with 300 essential genes, the Bce genome size and the experimental average distance from transposon insertion site to operon start for our mutants. Indeed, we are isolating mutants at a higher frequency (0.066%). This difference is probably due to insertion sites located in intragenic regions, upstream of essential genes, which also result in conditional growth mutants. Currently, our library consists of 84 clones that contain insertions upstream well-known essential genes (29), well-characterized genes not previously described as essential (14) and novel essential genes of unknown function (2). The remaining clones are either redundant (15) or we have not been successful at localizing the insertion site (24). The next step involves phenotypic characterization of library clones to find conditions where hypersensitivity is observed. Two parameters are being considered: the minimal inducing rhamnose concentration for wild type-like growth (MIRC) at which the clones are assumed to be hypersensitive and the growth profile where library clones fall into four different categories according to the growth inhibition phenotype. Finally, we challenged library growth in enhanced lethality assays with ciprofloxacin or colistin as examples of single target and nonspecific antibiotics. This allowed us to adjust conditions for which enhanced lethality of specific growth inhibitor-target pairs is observed.

A35

COMBINING TOBRAMYCIN WITH QUORUM SENSING INHIBITORS RESULTS IN INCREASED EFFICACY *IN VITRO* AND *IN VIVO*: EXPLORING THE POTENTIAL OF COMBINATION THERAPY TO TREAT INFECTIONS CAUSED BY *BURKHOLDERIA CEPACIA* COMPLEX ORGANISMS

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Biofilm-associated infections of *Burkholderia cepacia* complex (Bcc) organisms are often very difficult to treat with conventional antibiotics and novel targets are needed to combat these infections. One of these novel targets could be the bacterial communication system (quorum sensing, QS). Bacteria coordinate their cell-density dependent gene expression by excreting small signalling molecules. QS plays a role in various stages of biofilm formation, maturation and dispersal in Bcc and for this reason QS inhibitors (QSI) are predicted to be potential anti-biofilm agents. In the present study, we investigated whether cinnamaldehyde and baicalin hydrate, QSI targeting the acyl homoserine lactone QS system, enhance the susceptibility of *Burkholderia multivorans* and *Burkholderia cenocepacia* biofilms towards tobramycin. The effect of tobramycin, alone or in combination with QSI was evaluated in various *in vitro* and *in vivo* biofilm model systems, including two invertebrate models and one BALB/c mouse pulmonary infection model. *In vitro* the combined use of tobramycin and a QSI generally resulted in increased killing compared to killing by tobramycin alone. In addition, QS inhibition during biofilm formation also resulted in an increased susceptibility of these sessile cells towards tobramycin. A significantly higher fraction of infected *Galleria mellonella* and *Caenorhabditis elegans* survived infection following combined treatment, compared to treatment with tobramycin alone. Finally, the combined use of tobramycin and baicalin hydrate reduced the microbial load in the lungs of BALB/c mice infected with *B. cenocepacia* more than tobramycin treatment alone. Our data suggest that QSI may increase the efficacy of tobramycin in *B. multivorans* and *B. cenocepacia* biofilm-related infections.

A36
TRANSCRIPTOME ANALYSIS OF PERSISTER CELLS IN *BURKHOLDERIA CEPACIA* COMPLEX BIOFILMS

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Burkholderia cepacia complex bacteria are opportunistic pathogens which are highly resistant against many antimicrobials. A possible explanation for this resistance is the occurrence of persisters, cells that have entered a dormant multidrug-tolerant state. The aim of this study was to obtain a global picture of the gene expression patterns in persisters isolated from *Burkholderia cenocepacia* J2315 biofilms.
Biofilms were exposed to tobramycin (4 times MIC = 1024 µg/ml) for 24 hours. RNA of both treated and untreated samples was extracted and linearly amplified prior to the actual microarray analysis. Experiments were performed using custom made Agilent microarrays.
Approximately 0.1% of the sessile cells survived the tobramycin treatment; these were designated as persisters. 669 (9.4%) protein-coding genes were upregulated, while 651 (9.1%) were downregulated (2-fold difference; $p<0.05$). Several of the differentially expressed genes are likely to play a role in resistance and/or persistence. The upregulated genes included toxin/antitoxin modules, genes involved in glycerol-3-phosphate metabolism and the gene encoding isocitrate lyase. It has previously been proposed that these genes play a role in persistence. Several genes involved in the production of reactive oxygen species (ROS) (citrate synthase, succinic dehydrogenase, oxoglutarate dehydrogenase, NADH dehydrogenase, ferredoxin reductase) were downregulated, suggesting that tobramycin induces intracellular oxidative stress and that persisters survive because they downregulate genes involved in the production of ROS. The induction of ROS by tobramycin was confirmed by staining treated and untreated biofims with the ROS-specific fluorescent dye DCHFDA, followed by quantification of labeled cells by flow cytometry. 72% of the treated sessile cells demonstrated high intracellular ROS levels, compared to only 21% of the untreated sessile cells. In addition, the number of surviving cells after treatment was 10 times higher when the antioxidant cysteine was added and more than 10 times lower after addition of the superoxide dismutase inhibitor diethyldithiocarbamate.
These results indicate that a reduction of the levels of ROS produced in response to tobramycin treatment results in increased survival of persisters in *B. cenocepacia* biofilms.

A37
ANTIBIOTIC RESISTANCE OF *BURKHOLDERIA CEPACIA* COMPLEX STRAINS ISOLATED FROM SPUTUM OF CYSTIC FIBROSIS PATIENTS

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The *Burkholderia cepacia complex* (Bcc) is a group of closely related bacteria that are opportunistic pathogens for cystic fibrosis (CF) patients and inmunocompromised individuals. Infection of CF patient with Bcc members often has important negative consequences over patient's health. Bcc members are resistant to a great number of antimicrobials, so antibiotic therapy not always result effective.
When Bcc is detected in CF patient sputum, usually one strain is isolated and the antibiotic resistance profile of this strain is studied. We observed Bcc strains collected at different periods of time from the same chronic infected CF patient that belong to the same Bcc species but have not the same antibiotic resistance profiles. Then we decided to study the antibiotic resistance of different Bcc strains isolated from the same sputum sample. Five Bcc chronic infected CF patients were selected and 12 colonies grown on *Burkholderia cepacia* selective agar from the sputum of each patient were collected. Three to four different antibiotic resistance profiles were detected in each patient. One strain belonging to each resistance profile was identified using amplification and sequence analysis of the *recA* gene. In three patients all the strains studied were *B. contaminans,* in one patient all the strains were *B. cenocepacia*, and the last patient was infected both with *B. contaminans* and *B. cenocepacia*. Ongoing studies will allow a phenotypic and genotypic characterization of the isolated strains. The co-infection of CF patients with Bcc strains that have different resistance to antibiotics observed in this study adds complexity to antimicrobial therapy against these bacteria.

A38
PRESERVATIVE SUSCEPTIBILITY OF *BURKHOLDERIA CEPACIA* COMPLEX BACTERIA

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Burkholderia cepacia complex (Bcc) bacteria are ubiquitous in nature, isolated from soil and water. Bcc may also occur as opportunistic human pathogens in cystic fibrosis individuals and other vulnerable patients. In addition, they may be occasionally isolated as contaminants of industrial processes. With the use of techniques such as multilocus sequence typing (MLST), Bcc species diversity has been considerably expanded. We examined if Bcc species diversity was related to preservative resistance and industrial contamination. A collection of 67 isolates representative of industrial sources was examined by MLST. In addition, the preservative susceptibility of a panel of 93 bacterial isolates (83 Bcc strains and 10 non-Bcc species) was determined. The MIC of 9 preservatives was measured by broth and agar dilution methods. Bacterial growth dynamics were evaluated using a Bioscreen growth analyser. Passage of *B. lata* strain 383 on agar containing preservatives was used to examine adaptive resistance. MLST analysis of the industrial isolates revealed the following species diversity: *B. lata* (n = 17), *B. cenocepacia* (n = 11), Novel Bcc (n = 9), *B. vietnamiensis* (n = 8), *B. arboris* (n = 7), B. *stabilis* (n = 6), *B. cepacia* (n = 3), *B. multivorans* (n = 3), *B. contaminans* (n = 2), and *B. ambifaria* (n = 1). MIC values varied between and within Bcc species groups, however, the industrial isolates possessed a higher average MIC for DMDM hydantoin. Stable adaptive resistance of *B. lata* 383 was promoted to Isothiazolinone and Benzethonium chloride preservatives. The genetic basis for the innate and adaptive preservative resistance seen in *B. lata* strain 383 was investigated using transposon mutagenesis and global gene expression analysis. In summary, a range of Bcc species are encountered as process contaminants with *B. lata* being one of the more frequently isolated species. Bcc species show varied susceptibility to preservatives and species such as *B. lata* may develop stable adaptive resistance to certain preservative groups. A greater understanding of preservative susceptibility in Bcc bacteria will facilitate the design of novel strategies to target these organisms and prevent contamination.

A39
THE ROLE OF RND EFFLUX TRANSPORTERS IN *BURKHOLDERIA CENOCEPACIA* LIFE

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Burkholderia cenocepacia is representative of a problematic group of cystic fibrosis pathogens. Eradication of *B. cenocepacia* is very difficult being the antimicrobial therapy ineffective due to its high resistance to clinically relevant antimicrobial agents. RND (Resistance-Nodulation-Cell Division) efflux pumps are known to be mediators of multidrug resistance in Gram-negative bacteria. Since the significance of the RND efflux systems in *B. cenocepacia* has been partially determined, the aim of this work was to analyze mutants of *B. cenocepacia* J2315 impaired in BCAL2820-22 (RND-4) and BCAM1945-47 (RND-9) pumps, and assess their role in the efflux of toxic compounds. The transcriptomes of mutants deleted individually in RND-4 and RND-9 (named D4 and D9), and a double-mutant in both efflux pumps (named D4-D9), were compared to that of the wild-type strain using microarray analysis. Microarray data were confirmed by qRT-PCR, phenotypic experiments, and by Phenotype MicroArray analysis. The data revealed that RND-4 made a significant contribution to the antibiotic resistance of *B. cenocepacia, whereas RND-9 was only marginally involved in this process. The double mutant D4-D9 showed a phenotype and an* expression profile *similar to D4.* The microarray data showed that motility and chemotaxis-related genes appeared to be up-regulated in both D4 and D4-D9 strains. In contrast, these gene sets were down-regulated or expressed at levels similar to J2315 in the D9 mutant. Biofilm production was enhanced in all mutants. These results indicate that in *B. cenocepacia* RND pumps play a wider role than just in drug resistance, influencing phenotypic traits important for pathogenesis.

A40
THE ROLE OF HOPANOIDS IN THE ANTIMICROBIAL RESISTANCE AND INTRAMACROPHAGE SURVIVAL OF *BURKHOLDERIA CENOCEPACIA*

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Most members of the *Burkholderia* species, including members of the *Burkholderia cepacia* complex, produce hopanoids. These pentacyclic triterpenoids are hypothesized to be bacterial surrogates of eukaryotic sterols, acting to stabilize membranes and regulate fluidity. Little is known concerning the function of hopanoids in bacterial species and they may play an important role in regulating membrane permeability, contributing to *B. cenocepacia*'s resistance to antibiotics and antimicrobial peptides as well as facilitating bacterial survival in the acidic lysosomal environment during infection. The two copies of the gene encoding the squalene-hopene cyclase protein (Shc), an essential component of hopanoid synthesis, were deleted to create a mutant strain

incapable of producing polycyclic triterpenoids. This strain was able to grow in neutral and basic conditions but was unable to grow at pH4.5, indicating that the Δ*shc* mutant is highly sensitive to acidic conditions such as those found in the macrophage phagolysosome. The Δ*shc* mutant was also sensitive to the detergent SDS, the antibiotics novobiocin, erythromycin and rifampicin and the antimicrobial peptide polymixin B, indicating increased permeability likely due to decreased membrane integrity. However, the hopanoid mutant showed negligible sensitivity to oxidative, osmotic and high temperature stresses. To test the intramacrophage survival of the Δ*shc* mutant, RAW264.7 macrophages were infected with the Δ*shc* strain and the bacteria were able to survive and replicate within the macrophages at levels similar to wild type. This suggests that although hopanoids may be required to decrease membrane permeability, they are not essential for intramacrophage survival.

A41
HOPANOIDS CONTRIBUTE TO CATIONIC PEPTIDE RESISTANCE IN *BURKHOLDERIA MULTIVORANS*

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A major challenge to clinical therapy of *Burkholderia cepacia* complex (Bcc) infection is their innate resistance to disinfectants and a broad range of antimicrobials including polycationic agents such as aminoglycosides, polymyxins and cationic peptides. Resistance to polycatonic antimicrobials is due in part to outer membrane permeability. Hopanoids are sterol analogues present in a sub-set of bacteria that are presumed to be involved in membrane stability and barrier function. Bcc hopanoid biosynthesis was identified as a contributing factor to polymyxin B reisistance, as identified by a transposon mutant screen in *B. multivorans* ATTCC 17616. Genetic analysis of mutant 26D6 indicated the transposon inserted in a cluster of hopanoid biosynthesis genes. Mutant 26D7 exhibited increased susceptibility to polymyxin B, colistin and the cationic peptide CP26. Complementation of 26D7 *in trans* with *hpnI,* a hopanoid biosynthesis associated glycosyl transferase, restored the polymyxin B resistance phenotype. The outer membrane permeability of mutant 26D7 was assessed by uptake of 1-*N*-phenylnaphthylamine (NPN), a hydrophobic compound that fluoresces once in the hydrophobic periplasmic space. The mutant and parent were exposed to increasing concentrations of polymyxin B in the presence of NPN. Mutant 26D7 showed increased permeability to NPN in the presence of 1.6 μg/ml of polymyxin while the parental strain remained impermeable up to 50 μg/ml, indicating that the mutation in the hopanoid biosynthesis cluster altered outer membrane permeability, leading to increased susceptibility to cationic antimicrobials. A site-directed mutant in *hpnI* was constructed to confirm the role of *hpnI* in resistance to cationic antimicrobials. Like mutant 26D7, the Δ*hpnI* strain exhibited increased susceptibility to polymyxinB. Studies are ongoing to determine how hopanoids further contribute to the multiple resistance arsenal in the Bcc.

A42
HYPERMUTABILITY OF *BURKHOLDERIA CENOCEPACIA* ISOLATES FROM INDIVIDUALS WITH CYSTIC FIBROSIS (CF)

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Hypermutability (HM), the presence of a higher than normal mutation rate, has been identified in several CF pathogens, including *P. aeruginosa, S. aureus* and *H. influenzae*. However, the mutator (mut) phenotype has not previously been reported among *B. cepacia* complex (*BCC)* isolates. HM is most often the result of mutations in the DNA mismatch repair (MMR) systems and might be expected among all species associated with CF as the unique environment of the CF airway, including frequent exposure to antibiotics, applies selective pressure to maintain the mut phenotype. We have examined the presence of mutators among CF isolates of *B. cenocepacia* and have begun to investigate the mechanism of HM. A total of 149 isolates from 92 subjects have been examined thus far. Using the Luria Delbruck fluctuation test, HM to ceftazidime (CTZ) and ciprofloxacin (CIP) was tested among the most recent isolates available (late). Isolates resistant at baseline could not be tested. Using a mutation frequency of $< 10^{-7}$, HM was 55% among CTZ susceptible isolates and 59% of CIP susceptible isolates. The first isolates (early) from 43 subjects were also evaluated for HM. In this group, HM was 35% among 40 CTZ susceptible isolates and 10% among 21 CIP susceptible isolates. Comparisons were made between initial and most recent isolates to look for discordant pairs (early non-mutator, later HM). Seven discordant pairs were identified for CTZ and 4 for CIP. Evaluation of the early/late pairs for the 3 known MMR genes has begun. Amplifying *uvrD* by colony PCR and cutting with *HaeIII* identified a 550 bp band that was present in 21 late isolates and 9 early. There were no early isolates that had the band without its occurrence also in the late isolate of the pair. Only two pairs lacked the band entirely. The other 2 MMR genes, *mutL* and *mutS*, have not yet been examined. These data suggest that HM emerges over time among CF isolates of *B. cenocepacia* and alterations in MMR genes may be associated with HM.

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