PROGRAMME and ABSTRACTS

This Meeting has been made possible by the generous contribution of

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FFC - Fondazione per la Ricerca sulla Fibrosi Cistica
“International *Burkholderia cepacia* Working Group (IBCWG)”
14-17 April 2008
Ca’ Tron di Roncade (Treviso), Italy

Supported by

PRELIMINARY PROGRAMME

MONDAY, 14 April

Arrivals

19.30 Informal reception, Hotel Villa Fiorita (incl. acc. persons)

22.00 Transfer from Hotel Villa Fiorita to Ca’ Tron for ICGEB guests

TUESDAY, 15 April

8.00 Bus transfer from Hotel Villa Fiorita to Ca’ Tron

8.30 Registration

9.30 **Welcome address**

Decio Ripandelli
ICGEB, Trieste, Italy

Rector
University of Trieste, Italy

Antonio Zamboni
Fondazione Cassamarca, Treviso, Italy

10.00 **Introduction to the Meeting**

Vittorio Venturi
ICGEB, Trieste, Italy

10.15 **Keynote lecture**

*Burkholderia: past, present and future*

John R.W. Govan

11.00 Coffee-break
11.30-12.45  Session 1: Taxonomy and genomics
Chairman: Vittorio Venturi

11.30  (1A)  Comparative genomics of Burkholderia species using the MaGe_BurkholScope genome browser
Dalila Mil-Homens, Aurélie Lajus, Claudine Médigue, Arsenio M Fialho

11.45  (1B)  Is elements, genomic islands and diversification of Burkholderia cenocepacia
Arnault Graindorge, Aymeric Menard, Benoit Cournoyer

12.00  (1C)  Multi locus sequence typing (MLST) of Burkholderia cepacia complex isolates that elude recA–based identification
Silvia Cesarini, Annamaria Bevivino, Silvia Tabacchioni, Luigi Chiarini, Adam Baldwin, Claudia Dalmastri

12.15  (1D)  Identification of Burkholderia cepacia complex species by snupe and lamp analysis of histidine biosynthetic genes
Maria Cristina Papaleo, Marco Fondi, Elena Perrin, Isabel Maida, Barbara Tarabella, Renato Fani

12.30  (1E)  Speciation and evolution of the Burkholderia cepacia complex
Adam Baldwin, Esh Mahenthiralingam, Elke Vanlaere, Peter Vandamme, Chris Dowson

13.00  Buffet lunch at Ca’Tron

14.30-15.45  Session 2: Antibiotic resistance and clinical studies
Chairman: Tom Coenye

14.30  (2A)  Changes in gene expression of B. cenocepacia J2315 in response to different classes of antibiotics
Andrea Sass, Pavel Drevinek, Eshwar Mahenthiralingam

14.45  (2B)  Insights into the mechanisms underlying the acquisition of antimicrobial resistance in Burkholderia cenocepacia during chronic lung infection of a cystic fibrosis patient: an expression proteomics analysis
Pedro M. Santos, Ana Pinto-de-Oliveira, Mónica Cunha, Andreia Madeira, Isabel Sá-Correia

15.00  (2C)  Antibiotic resistance in planktonic and sessile Burkholderia cepacia complex organisms
Peeters E., Nelis H.J., Coenye T.

15.15  (2D)  Outcome post-lung transplant in cystic fibrosis patients infected with non-B. cenocepacia organisms. Toronto experience
Cecilia Chaparro, Elizabeth Tullis

15.30  (2E)  Survey of Burkholderia multivorans susceptibility in a pediatric clinic population over 3 years
Jane L. Burns, Xuan Qin, Anne Marie Buccat

15.45  Coffee-break

16.15-17.15  Session 3: Quorum sensing
Chairperson: Pamela Sokol

16.15  (3A)  Identification and characterisation of a novel orphan LuxR homologue in Burkholderia cenocepacia
Eoin P. O’Grady, Rebecca J. Malott, Pamela A. Sokol

16.30  (3B)  Production of the antifungal compound pyrrolnitrin is quorum sensing-regulated in members of the Burkholderia cepacia complex
Silvia Schmidt, Susan Schönmann, Judith Blom, Gabriele Berg, Leo Eberl
16.45 (3C) The new group of plant-associated nitrogen-fixing \textit{Burkholderia} spp. share a conserved quorum sensing system which is stringently regulated by the RsaL repressor

Zulma Rocío Suarez Moreno, Jesús Caballero-Mellado, Vittorio Venturi

17.00 (3D) Studies on the role of quorum sensing in \textit{Burkholderia plantarii} and \textit{Burkholderia glumae} virulence

Laura Cabrio, Giuliano Degrassi, Giulia Devescovi, Susanne Uehlinger, Paul Williams, Leo Eberl, Vittorio Venturi

17.15 Free time for discussion

18.00 Bus transfer to Hotel Villa Fiorita

19.30 Buffet dinner at Hotel Villa Fiorita (incl. acc. persons)

22.00 Transfer from Hotel Villa Fiorita to Ca’Tron for ICGEB guests

WEDNESDAY, 16 April

8.30 Bus transfer from Hotel Villa Fiorita to Ca’ Tron

9.00-10.00 Session 4: Polysaccharides
Chairman: Roberto Rizzo

9.00 (4A) Conditional mutations in the aminoarabinose biosynthesis and transfer (arn) gene cluster of \textit{B. cenocepacia} K56-2 induce changes in phospholipids and lipid A-core oligosaccharide

Ximena P. Ortega, Miguel A. Valvano

9.15 (4B) Plant pathogen \textit{Burkholderia} lipopolysaccharides

Antonio Molinaro

9.30 (4C) Differential mucoid exopolysaccharide production in \textit{Burkholderia cepacia} complex

James E. A. Zlosnik, Deborah A. Henry, Trevor J. Hird, Monica C. Fraenkel, Leonilde M. Moreira, David P. Speert

9.45 (4D) Functional characterization of BceC, an UDP-glucose dehydrogenase from \textit{Burkholderia cepacia} required for cepacian polysaccharide synthesis

Alma O. Popescu, Ana Teresa Granja, Arsenio M. Fialho

10.00 Coffee-break

10.45-11.45 Polysaccharides (cont’d)

10.45 (4E) Extension of the \textit{bce} cluster of genes involved in the biosynthesis of cepacian by the genus \textit{Burkholderia}

Ana S. Ferreira, Jorge Leitão, Silvia A. Sousa, Leonilde Moreira

11.00 (4F) \textit{Burkholderia cenocepacia pcaC}, a gene involved in the synthesis of the polysaccharide poly-n-acetyl-glucosamine (PNAG), is required for virulence in \textit{C. elegans}

Sara K. Bondi, Michael R. Davis, Jr., Akshay Bhatnagar, Dennis Benedetti, Gregory P. Priebe, Costi D. Sifri, Joanna B. Goldberg

11.15 (4G) Interaction of BCC exopolysaccharides with component of the innate immune system: bacterial escape from host defence mechanisms

Paola Cescutti, Michela Foschiatti, Bruno Cuzzi, Roberto Rizzo
11.30 (4H) Virulence factors of the *Burkholderia cepacia* complex; studies of the EPS and LPS saccharides


12.00 End of session - Bus transfer to the boat departure point

13.00 Guided boat trip to Venice – packed lunch served on board (incl. acc. persons)

15.30 Arrival in Venice - guided tours and/or free time

19.30 End of guided tours in Piazza San Marco, Venice

20.00 Dinner

22.30 Bus departure from Tronchetto (Venice) – transfer to Hotel Villa Fiorita (and Ca’Tron for ICGEB guests)

THURSDAY, 17 April

8.30 Transfer from Hotel Villa Fiorita to Ca’ Tron

9.00-10.45 Session 5: Pathogenesis

Chairman: Miguel Valvano

9.00 (5A) Characterization of MgtC, an inner membrane protein required for intracellular survival of *Burkholderia cenocepacia*

Kendra E. Maloney, Miguel A. Valvano

9.15 (5B) Invasion of *Burkholderia cepacia* complex into lung epithelial cells involves glycolipid receptors

Tracy Mullen, Mairé Callaghan, Siobhán McClean

9.30 (5C) Structure and pro-inflammatory activity of endotoxin from the *Burkholderia cepacia* complex

Alba Silipo

9.45 (5D) Effects of lactoferrin iron saturation on the ability of *Burkholderia cepacia* complex (Bcc) to invade lung epithelial cells and form biofilms

Emma Caraher, Kiranmai Gumulapurapu, Siobhán McLean, Mairé Callaghan

10.00 (5E) *Burkholderia cenocepacia* ZmpA and ZmpB metalloproteases degrade host cytokines and antimicrobial peptides

Cora Kooi, Pamela A. Sokol

10.15 (5F) The acyl carrier protein, involved in the virulence of *Burkholderia cenocepacia* J2315, is conserved within the *Burkholderia* genus

Silvia A. Sousa, Christian G. Ramos, Jorge H. Leitão

10.30 (5G) Characterization of a novel 1-cys peroxiredoxin of *Burkholderia cenocepacia*

Alan R Brown, David J Clarke, Ximena P Ortega, Miguel A Valvano, Dominic J Campopiano, John R W Govan

10.45 Coffee-break
11.15-12.30  Session 6: Virulence and models
Chairman: Leo Eberl

11.15 (6A)  Characterization of a lysogenic *Burkholderia cepacia* complex phage with potential for use in phage therapy
Amanda D. Goudie, Karlene H. Lynch, Jonathan J. Dennis

11.30 (6B)  Antibiotic susceptibility, virulence and taxonomic status of *Burkholderia cepacia*-complex multiresistant strains isolated from Italian cystic fibrosis patients
P. Cocchi, N. Ravenni, F. Favari, G. Taccetti, S. Campana

11.45 (6C)  Zebra fish as a novel virulence model for *Burkholderia cenocepacia*
Annette Vergunst, David O’Callaghan

12.00 (6D)  Investigations on the pathogenicity of *Burkholderia cepacia* complex strains in two different virulence models
Susanne Uehlinger, Stephan Schwager, Leo Eberl

12.15 (6E)  Pathogenicity of environmental *Burkholderia cenocepacia* strains
Moira Paroni, Luisa Pirone, Claudia Dalmastri, Fiorentina Ascenzioni, Alessandra Bragonzi, Annamaria Bevivino

12.30  Conclusions

12.45  Buffet lunch at Ca’Tron

14.00  Departures
COMPARATIVE GENOMICS OF BURKHOLDERIA SPECIES USING THE MAGE_BURKHOLSCEPE GE GENOME BROWSER

Dalila Mil-Homens¹, Aurélie Lajus², Claudine Médigue²³, Arsenio M Fialho¹

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Members of the genus Burkholderia are versatile organisms that occupy a surprisingly wide range of ecological niches. Most members of the genus are known to be plant pathogens and non-pathogenic soil bacteria. B. mallei and B. pseudomallei are well-recognised as pathogenic for humans and animals, but virtually all Burkholderia can be opportunistic pathogens. Indeed, bacteria of the Burkholderia cepacia complex (Bcc) have emerged as important opportunistic pathogen, establishing lung infections in patients with cystic fibrosis or immunocompromised patients. Despite the identification of some virulence factors, many details of Burkholderia pathogenicity mechanisms remain to be clarified. Comparative genomic analysis between the available Burkholderia genomes is believed to be a useful tool to understand the genetic basis of differences in the biology of clinical and environmental isolates. We have used MaGe_BurkholScope (https://www.genoscope.cns.fr/agc/mage) [1], a graph-based suite of programs developed at Genoscope (Evry, France), that allows the visualization of the synteny map between five Burkholderia and related bacterial species. In total, our analysis revealed the presence of 39 Burkholderia proteins with no orthologs in other microorganisms. Among these, three classes could be distinguished: those present in Burkholderia genus (class A), those present in Bcc members (class B), and those restricted to B. cenocepa (Genomovar III) (class C). We assigned the proteins to different functional categories; a special attention was devoted to surface-exposed proteins as potential immunogens and, thus, represent targets for future experimental analysis. Primers for the selected genes were constructed, and PCRs were evaluated with various genomic DNAs for the specific identification of Bcc members. Overall, the target genes/proteins identified in this study will be a valuable resource to develop new tests for specific diagnosis of Burkholderia infection and may enhance our understanding of the pathogenesis of the Bcc bacteria.

IS ELEMENTS, GENOMIC ISLANDS AND DIVERSIFICATION OF BURKHOLDERIA CENOCEPACIA

Arnault Graindorge, Aymeric Menard, and Benoit Cournoyer

Research group on Bacterial Opportunistic Pathogens and Environment, Université de Lyon, UMR 5557 Ecologie Microbienne, CNRS, Université Lyon 1, and Ecole Nationale Vétérinaire de Lyon, 69 622 Villeurbanne Cedex.

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IS (Insertion Sequences) elements are small mobile genetic entities able to insert into genomic DNA sequences of bacteria. These elements can play a role in genetic rearrangements and duplication processes by favoring recombination between chromosomes and/or plasmids. They can also inactivate gene sequences or modify their expression profiles by inserting into a promoter region. Three naturally occurring and active IS elements, IS402, IS407, and IS1416, have been reported in the Burkholderia cepacia complex (1). These elements were found broadly distributed in this bacterial complex, and to vary significantly in copy number from one strain to another. High copy numbers of IS407 were reported in the ET12 clone of B. cenocepacia, which can lead to the cepacia syndrome among individuals with cystic fibrosis (CF) (2). IS402 was found responsible of the inactivation of the O antigen lipopolysaccharide biosynthesis cluster in several strains of the ET12 clone (3). This latter IS was also suggested to have a significantly higher copy number in clinical rather than environmental strains of Bcc (2). Similarly, another element, named IS1363, was found to have a distribution mainly restricted to the PHDC and ET12 epidemic clones of B. cenocepacia (4). These observations suggest a possible relationship between IS elements and the diversification of B. cenocepacia into infra-specific lineages, including a role in the adaptation of B. cenocepacia to CF lungs.

An analysis of the distribution of IS elements among the ET12 J2315 genome showed regions with high numbers (5). These regions were termed IS hot spots. In this talk, these hot spots and their respective IS will be presented in detail. All IS were classified into their respective family. The IS insertion sites were identified and compared. Flanking DNA sequences were analysed by Blast in order to infer their function, and characterize the interrupted coding sequence. Signatures indicative of recent acquisition were searched among these regions. Some of these IS hot spots were found to match genomic islands (GI). These observations led us to investigate the distribution of the identified IS and GI among the ET12 clone, and the closely related Bourg-en-Bresse (B&B) clone (6). All ET12 and B&B strains harboured a high number of IS elements but an important instability was observed. Most if not all tested GI found among the J2315 genome were found conserved among the selected ET12 strains. However, only two of these GI were found conserved among the B&B clone. A summary of these data will be presented.

MULTI LOCUS SEQUENCE TYPING (MLST) OF BURKHOLDERIA CEPACIA COMPLEX ISOLATES THAT ELUDE recA–BASED IDENTIFICATION

Silvia Cesarini¹, Annamaria Bevivino¹, Silvia Tabacchioni¹, Luigi Chiarini¹, Adam Baldwin², Claudia Dalmastri¹

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Infections due to new Burkholderia cepacia complex (Bcc) strains, independently acquired from the environment, represent an emerging problem in cystic fibrosis (CF). In fact, implementation of stringent control measures has reduced the epidemic spread but not eliminated Bcc infections, and genetic identity observed among environmental and clinical isolates from both CF and non-CF patients supports the role of the environment as a reservoir of pathogenic strains. Therefore, to promptly detect, identify and type environmental Bcc isolates is fundamental to individuate the strains infecting CF patients and to manage infections by new acquisitions. Indeed, characterization of Bcc isolates is particularly difficult, given taxonomic complexity of Bcc, which comprises at least ten species, and mis-identification can occur with the most widely used identification methods, recA-RFLP and species-specific PCR. Since Multilocus Sequence Typing (MLST) validated for both species and strain discrimination in Bcc allows lab-to-lab data comparison and large scale epidemiological studies, we used this method to identify and type some Bcc strains that were not allowed to be discriminated at the species level by means of recA polymorphism based analysis.

Twenty two environmental (from river sediments and water, and maize rhizosphere) and 6 CF strains were analysed. Among these 28 strains, 24 were successfully sequenced at all the loci, resulting in many new sequences (alleles) and a total of 23 STs, all but one new. Of 15 strains with the RFLP profile I -originally characteristic of B. cenocepacia IIIB-, 7 were assigned to the B. cenocepacia IIIB species, and 6 to the BCC5 group. Four out of 8 isolates positive to the B. cenocepacia IIIA specific PCR in spite having RFLP profiles not characteristic of this lineage were assigned to BCC group K and 2 were assigned to BCC3 group. One false positive to B. cenocepacia IIIB specific PCR resulted to belong to BCC group K. Two out of 3 isolates with RFLP profiles not previously recovered among Bcc reference strains were assigned to B. cenocepacia IIIB and one to BCC5. A B. cenocepacia IIIA environmental strain was found to have a unique position in the concatenated MLST tree. A taxonomic position was also assessed for a few strains which failed to amplify at some loci. In conclusion, 24 isolates were clearly identified (86%) and 3 were assigned to the closest species/group by using MLST, some cases of mis-identification with recA and RFLP specific primers were put in evidence, and some improvements in MLST method are suggested.
(1D)
IDENTIFICATION OF BURKHOLDERIA CEPACIA COMPLEX SPECIES BY SNUPE AND LAMP ANALYSIS OF HISTIDINE BIOSYNTHETIC GENES

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Respiratory infections are the main cause of morbidity and mortality in cystic fibrosis, and bacteria belonging to the Burkholderia cepacia complex (Bcc) are important agents of infection due to their high-level intrinsic antibiotic resistance and easy patient-to-patient spread. Bcc comprises nine species that are characterized by a high-degree of DNA-homology, and thus are difficult to distinguish in routine clinical analysis. Nowadays a combination of molecular techniques is required for a correct identification of Bcc species. The aim of this study was to set up diagnostic methods based on SNUPE (Single Nucleotide Primer Extension) and LAMP (Loop-mediated Isothermal Amplification). SNuPE allows detecting and highlighting a single nucleotide polymorphism on a DNA target. It also allows to reduce misinterpretation in Bcc species identification; furthermore, this method requires less time than the combination of techniques actually in use. LAMP is a very important tool in clinical fields since it requires simple equipments available in all laboratories. Fifty-six representative strains both from environmental and clinical source used in this study were used in this work.

We focused the attention on some genes of the histidine (his) biosynthetic operon. Thus, a 4800 bp DNA fragment harboring the following genes: hisB, marC, hisH, hisA, hisF, hisI and hisE was PCR amplified from the DNA of each of the 56 Bcc strains. The nucleotide sequence of each fragment was determined and analyzed for the presence of both highly conserved or polymorphic sites. The comparative analysis of sequences obtained and those available in databases allowed to design a set of primers of different length for the SNuPE technique. These primers were used either in simplex or multiplex SNuPE reactions using the DNA of the 56 Bcc strains, representatives of all the Bcc species. Data obtained revealed that the SNuPE profiles allowed the easy identification of isolates belonging to the different Bcc species. In addition to this, a set of primers was also designed for LAMP reactions and used to specifically amplify a his region from some Bcc reference strains. Data obtained showed the high grade of applicability of both methods on Bcc members identification, so that they could be used in clinical routine as new procedures.

This work was supported by the Italian Cystic Fibrosis Research Foundation (grant FFC# 9/2003) and with the contribution of “For Me s.r.l.” (grant FFC# 13/2006).
SPECIATION AND EVOLUTION OF THE *BURKHOLDERIA CEPACIA* COMPLEX

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*Burkholderia cepacia* complex (Bcc) are a group of opportunistic pathogens that reside in the environment and are capable of infecting vulnerable individuals. Analysis of diverse Bcc isolates by multilocus sequence typing (MLST) has allowed us to characterise the population structure of infectious CF pathogens and has helped our investigations into tracing the source of continued infections.

By combining unambiguous MLST data with traditional polyphasic techniques in the identification and speciation of the Bcc, MLST has proved to be a very useful tool in aiding speciation decisions. As well as the inclusion of *B. ubonensis* to the Bcc a further five novel species have now been described (Vanleare *et al.*, In Press). These novel species have been named *B. latens* (formerly BCC1), *B. diffusa* (formerly BCC2), *B. arboris* (formerly BCC3), *B. seminalis* and *B. metallica*. In addition, a further two novel species have been proposed for BCC Group K (containing genome strain 383) and BCC Group AT (containing the Sargasso sea metagenome). In these cases a 3% cut-off in divergence of concatenated MLST loci represents a good standard for species demarcation. Most of these novel species and proposed species are very important clinically and their recent classification should now facilitate improved diagnostics for many previously unclassified Bcc members.

Models on the evolution and recombination of the Bcc are another new approach we can use to exploit MLST data to examine the recent emergence of these opportunistic populations. Preliminary data shows epidemic strains to be recently evolved from environmental strains and demonstrates how populations can diverge into different niches and form novel groups over time.

We have also correlated our MLST data with a large amount of information gathered at Cardiff University on the distribution of genomic islands and insertion sequences across the Bcc. We have found a significant correlation of mobile element distribution among closely related strains within MLST clonal complexes but not between more distantly related strains. This should facilitate further investigations into extent and impact of recombining elements and their role in phenotypic changes and on pathogenicity. By applying and combining the MLST framework with other techniques the complexities of problematic lineages such as ET-12 strains can be further unravelled.

Funding of this project was primarily by the UK Cystic Fibrosis Trust and more recently by the Medical Research Fund with the support of Micropathology Ltd for which we are grateful.
Burkholderia cepacia complex bacteria have both intrinsic and adaptive resistance to different classes of antibiotics. However, many of the putative resistance mechanisms encoded within the genome of B. cenocepacia have not been characterised. To define antibiotic resistance on a genomic scale, a set of microarray experiments was performed investigating the response of B. cenocepacia J2315 to a selection of antibiotics with different mechanisms of action. The antimicrobials examined included: (i) a carbapenem (meropenem), (ii) an aminoglycoside (amikacin), (iii) a folate-synthesis blocker (trimethoprim) and, additionally, the cationic drug chlorpromazine as an example of a non-antibiotic enhancer of microbial susceptibility. Cells were exposed to sub-inhibitory concentrations of each drug. In addition, gene expression from three spontaneous mutants of J2315 with elevated resistance to meropenem, trimethoprim and amikacin, respectively, was also determined using the microarray. Analysis of microarray data revealed that J2315 changed the expression of a variety of modifying enzymes and efflux systems when exposed to the antibiotics, however, in many cases the alteration in gene expression was minimal. The following eight examples show the upregulation of genes in different conditions: (i) In the presence of sub-inhibitory concentrations of amikacin, a phosphohydrolase and a putative acetyltransferase were upregulated 2- to 4-fold. Aminoglycoside deactivation by modification via acetyltransferases or phosphorylases are known resistance mechanisms, both the latter enzymes are possibly active in this way. (ii) The presence of meropenem in the growth medium strongly induced (50 to 100 fold) two beta-lactamases encoded on the second and third replicons, respectively. More than 25 putative efflux pump encoding genes are present in the B. cenocepacia J2315 genome and the microarray analysis linked upregulation of several efflux systems to specific antimicrobials. (iii) In the presence of chlorpromazine the most upregulated gene was an RND family efflux gene (15-fold change). (iv) A macrolide-linked efflux pump encoded on chromosome 2 was minimally induced after exposure to meropenem (1.6-fold) and chlorpromazine (2-fold). (v) Exposure to trimethoprim led to elevated expression of the aidA (10-fold) and also a putative ABC transporter gene (1.8-fold). (vi) The spontaneous mutant with adaptive resistance to amikacin showed constitutive overexpression of the acetyltransferase mentioned above (vii) and the mutant resistant to meropenem showed constitutive overexpression of degradative enzymes putatively involved in phenylacetic acid metabolism. (viii) The mutant with high level resistance to trimethoprim demonstrated massive constitutive expression (>100 fold) of the previously characterised ceeo RND family efflux pump system. Transcriptomic analysis of B. cenocepacia J2315 grown in the presence of clinically relevant antimicrobials has begun to define the resistance mechanisms it uses to evade eradication during CF infection. This research was supported by a grant from Cystic Fibrosis Foundation Therapeutics Inc.
INSIGHTS INTO THE MECHANISMS UNDERLYING THE ACQUISITION OF ANTIMICROBIAL RESISTANCE IN BURKHOLDERIA CENOCEPACIA DURING CHRONIC LUNG INFECTION OF A CYSTIC FIBROSIS PATIENT: AN EXPRESSION PROTEOMICS ANALYSIS

Pedro M. Santos, Ana Pinto-de-Oliveira, Mónica Cunha, Andreia Madeira and Isabel Sá-Correia

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The treatment of cystic fibrosis (CF) patients chronically infected with Burkholderia cepacia complex (Bcc) bacteria requires aggressive and prolonged antibiotic therapy, in addition to other stressing conditions to which they are exposed in the CF lung. The susceptibility patterns of sequential isolates of Bcc to clinically relevant antimicrobials obtained from Portuguese CF patients receiving care at the Hospital de Santa Maria, in Lisbon, in the course of chronic infection during a 5-year survey were recently compared in our group [1]. These isolates were previously genotyped and belong to the species B. cenocepacia (subgroups A and B), B. cepacia, B. multivorans, and B. stabilis [2, 3]. Phenotypic variants exhibiting different antimicrobial susceptibility patterns were obtained from sputum samples of clinically deteriorated CF patients during chronic lung infection. The isolation of resistant variants coincided with periods of pulmonary exacerbation and antibiotic therapy.

To get insights into the adaptive strategies employed by Bcc bacteria to cope with the stressing conditions to which they are exposed in the CF lung, in particular to antibiotic therapy, we have exploited expression proteomics, as we did before for another bacterial species/environmental stress [4, 5]. We have compared clonal serial isolates of B. cenocepacia (subgroup A) from a same CF patient evidencing different antibiotic resistance profiles. Two proteome reference maps, corresponding to the soluble and membrane protein fractions were constructed. Protein expression profiles were compared using difference gel electrophoresis (DIGE) technology. Results of this quantitative proteomic analysis will be presented and discussed.

ANTIBIOTIC RESISTANCE IN PLANKTONIC AND SESSILE BURKHOLDERIA CEPACIA COMPLEX ORGANISMS

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*Burkholderia cepacia* complex bacteria are opportunistic pathogens that can cause severe respiratory tract infections in cystic fibrosis (CF) patients. Treatment of infections caused by these organisms is particularly problematic due to the resistance to most commercially available antibiotics. Biofilm formation has been described for multiple *B. cepacia* complex strains and, compared to their planktonic counterparts, sessile (biofilm-grown) microorganisms are more resistant to antimicrobial agents. Consequently, the biofilm-forming capability of *B. cepacia* complex bacteria may contribute significantly to the problem of resistance. The goal of the present study was to determine the effects of several commonly used antibiotics on *B. cepacia* complex biofilms and to compare these with their effects on planktonic cells.

The action of six commonly used antibiotics (ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim-sulfamethoxazole) was evaluated on both planktonic and sessile cells of 33 strains belonging to the 15 described *B. cepacia* complex species. Conventional broth microdilution tests were performed to determine the minimal inhibitory concentration (MIC) for planktonic cultures. The effect of the antibiotics on the inhibition of biofilm growth (minimal biofilm inhibitory concentration; MBIC) was evaluated by using a resazurin-based viability staining after treating young biofilms (4 h adhesion) for 20 h.

The antibiotic susceptibilities of planktonic cells are highly divergent between the tested strains. In general, the MIC and MBIC of each antibiotic was highly similar within strains, indicating that the growth inhibitory effect of most antibiotics on planktonic cells is equal to that observed for young biofilms. Additional testing is under way to determine the bactericidal effect of these antibiotics on stationary phase planktonic and biofilm cultures.
OUTCOME POST-LUNG TRANSPLANT IN CYSTIC FIBROSIS PATIENTS INFECTED WITH NON-\textit{B. CENOCEPACIA} ORGANISMS. TORONTO EXPERIENCE.

Cecilia Chaparro$^{1,2}$, Elizabeth Tullis$^2$

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Introduction: The outcome of lung transplant of cystic fibrosis (CF) patients infected with \textit{B. cenocepacia} (genomavar III) still shows an increased rate of complications especially during the first six months post-op. Outcome with other \textit{Burkholderia} species is less well known as only a handful of cases have been reported. Some with a poor outcome related to infection complications and others with a more acceptable outcome.

Methods: We present here 5 cases of patients infected with non-\textit{cenocepacia} \textit{Burkholderia} and their outcome after lung transplant.

Results: Between March 1988 and December 2007 we have transplanted 212 CF patients. Of them, 57 were infected with \textit{B. cenocepacia} (genomavar III), and 5 with a non-\textit{B. cenocepacia} organisms. Four of them were infected with \textit{B. gladioli} and one with \textit{B. vietnamensis} (genomavar V). All of them had acquired the \textit{Burkholderia} prior to transplant.

Age of the patients at the time of the transplant was between 25 and 60. Survival post-transplant ranges from 1.1 to 7.5 years. Three of the patients have grown the \textit{Burkholderia} post transplant, 2 with \textit{B. gladioli} and the only patient with \textit{B. vietnamensis} (genomavar V). Most importantly, none of them has had any complication related to infection. Only one of the patients has died after a surgical complication without infection.

Conclusion: In our centre, CF lung transplant recipients infected with non-\textit{B. cenocepacia} show a good outcome compared to those infected with \textit{B. cenocepacia}. 
SURVEY OF BURKHOLDERIA MULTIVORANS SUSCEPTIBILITY IN A PEDIATRIC CLINIC POPULATION OVER 3 YEARS

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Burkholderia cepacia complex (BCC) infection in cystic fibrosis (CF) is most commonly associated with B. multivorans and B. cenocepacia. Some clinic populations have primarily B. cenocepacia, but the majority have both organisms. The Pediatric CF center in Seattle is somewhat unusual in that we have not identified any children with B. cenocepacia among our CF patients. In the past 3 years a total of 16 patients followed in our center have had respiratory cultures positive for B. cepacia complex. All of these patients had B. multivorans. In 15 patients, it was the sole BCC organism; in one transiently infected patient, B. multivorans was replaced by B. cepacia (genomovar I), which was also transient. Three patients were culture positive for B. multivorans for all three years of the survey, 7 were transiently positive, 1 died, and 1 left the pediatric CF center. The remaining 4 patients acquired the organism during the survey period. Antibiotic susceptibility to 6 agents was consistently monitored. Ceftazidime (Ctz), meropenem (Mer), ticarcillin/ clavulanate (Tim), and trimethoprim/sulfamethoxazole (T/S) were evaluated for all 3 years. Quinolones were represented by ciprofloxacin in 2005 and the first part of 2006, and by levofloxacin in the second half of 2006 and all of 2007. Tetracyclines were represented by doxycycline in the first part of 2005 and by minocycline in the remainder of the study. Susceptibility testing was by broth microdilution in 2005 and by Etest in 2006 and 2007. Organisms from individual patients with multiple positive cultures did not appear to acquire resistance over time. In addition, for the majority of drugs there was not more than a single dilution change in median MIC (MIC₅₀) over the three year period. The one exception to this was Tim for which the MIC₅₀ increased from 32 in 2005 to >256mcg/µL in subsequent years. The 3 agents that appeared most active against B. multivorans isolates based on in vitro testing were: Ctz (90.1%, 58.3% and 100% susceptible in 2005, 2006, and 2007, respectively), T/S (72.7%, 83.3%, and 88.9%), and Mer (63.6%, 75%, and 88.9%). The least active agents were Tim (18%, 8.3%, and 22.2%) and quinolones (9%, 25%, and 66.7%); however, the numbers are small. In this pediatric CF center population B. multivorans was the predominant BCC isolated. Among the 16 culture positive patients, some were transiently colonized and few had severe disease. Importantly, there were many active antimicrobial agents available for treatment.
IDENTIFICATION AND CHARACTERISATION OF A NOVEL ORPHAN LUXR HOMOLOGUE IN BURKHOLDERIA CENOCEPACIA

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Burkholderia cenocepacia is an opportunistic pathogen which can cause chronic and fatal infections in immunocompromised individuals. Quorum Sensing (QS) is a mechanism employed by diverse bacteria to coordinately regulate gene expression. In B. cenocepacia, two well-characterised QS systems, CepIR and CciIR, are part of a complex regulatory network that both positively and negatively affect gene expression including that of specific virulence determinants. We identified a novel regulator, homologous to LuxR, encoded by BCAM0188 and determined that it is a component of the QS network in B. cenocepacia. BCAM0188 does not have an adjacent AHL synthase and is conserved in B. cenocepacia strains, but absent from other species of the B. cenocepacia complex. A transcriptional profiling approach was employed in order to identify genes regulated by BCAM0188. Microarray analysis revealed differential expression of 275 genes (125 increased, 150 decreased) in the BCAM0188 mutant compared to its parental strain, K56-2. A number of genes which were subject to high levels of negative regulation by BCAM0188 are predicted to encode proteins of unknown function or proteins involved in lectin biosynthesis, transcriptional regulation and efflux pumps. Semi-quantitative RT-PCR confirmed negative regulation by BCAM0188 for 4 targets of interest. Microarray analysis also indicated that the expression of the zinc metalloprotease-encoding genes, zmpA and zmpB, was increased and decreased, respectively, in the BCAM0188 mutant compared to K56-2. However, independent analysis using lux-based reporter assays showed increased expression of both zmpA and zmpB in the BCAM0188 mutant compared to K56-2. Furthermore, increased protease production was confirmed phenotypically using skim milk agar plates. Ongoing work is aimed at further characterisation of this novel regulator and its role in the complex interplay between CepIR and CciIR QS systems in B. cenocepacia.
PRODUCTION OF THE ANTIFUNGAL COMPOUND PYRROLNITRIN IS QUORUM SENSING-REGULATED IN MEMBERS OF THE BURKHOLDERIA CEPACIA COMPLEX

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Members of Burkholderia cepacia complex (Bcc) are recognized as phytopathogens and opportunistic human pathogens, but are also known for their capacity to suppress soil borne fungal pathogens. The strong biocontrol activity observed with some Bcc strains is, at least in part, a result of the production of a large variety of compounds with antifungal activity, including cepacin, altericidins, xylocandins, pyrrolnitrin, siderophores, and a nonribosomally synthesized lipopeptide. The aim of our study was to investigate whether expression of antifungal traits by Bcc strains is dependent on AHL-dependent quorum sensing (QS) systems that operate in these organisms.

Using a quorum quenching approach, i.e. by heterologous expression of the Bacillus sp. AiiA lactonase in various Burkholderia sp. hosts, we show that antifungal activity in the large majority of investigated strains is dependent on a functional QS system. We next wished to identify the QS-regulated factors responsible for the observed antifungal activities. These investigations revealed that synthesis of pyrrolnitrin (prn), a very common broad-spectrum antibiotic produced by many Pseudomonas and Serratia species, is QS-regulated in several of the investigated Bcc strains.

To study the underlying molecular mechanisms of prn biosynthesis in better detail we constructed mutants in Burkholderia sp. 383 that are defective in the CepIR QS system. We demonstrate that inactivation of the CepIR system abolishes antifungal activity against Rhizoctonia solani and prn production was no longer detectable by GC-MS. Furthermore using fusions of lacZ and gfp to the promoter of the prn operon we demonstrate that expression of pyrrolnitrin is positively regulated by CepR at the transcriptional level.
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A new group of nitrogen fixing plant associated _Burkholderia_ species has emerged in the last few years, having plant association and nitrogen fixation as common traits. The purpose of this study was to determine if these species possess an _N_-acyl homoserine lactone (AHL) quorum sensing (QS) cell-cell signaling system, and whether it was important for nitrogen fixation and other important phenotypic traits. It was determined that _B. kururiensis_, and other members of this _Burkholderia_ species cluster, contain at least one highly conserved system, designated _BraI/R_, which produces and responds to _N_-dodecanoyl-3-oxo-homoserine lactone (C12-3-oxo-AHL). It was determined that the _BraI/R_ AHL QS system is not involved in the regulation of nitrogen fixation and in several other important phenotypes indicating that it might not be a global regulatory system. The _BraI/R_ system is similar to the _LasI/R_ of _Pseudomonas aeruginosa_ and the _braI/R_ genes have, just like _lasI/R_, in between the repressor gene called _rsaL_. _B. kururiensis_ synthesizes very low levels of C12-3-oxo-AHL with the situation dramatically changing when _rsaL_ is missing since the _rsaL_ mutant produces over 2000-fold more AHLs compared to the parent strain. This unique stringent regulation indicates that _RSA_ could be an on/off switch for AHL QS in _B. kururiensis_ and the ability to produce very high levels of AHL production also questions the role of this molecule in the novel group of _Burkholderia_. The presence of a well conserved and distinct AHL QS system among all the diazotrophic _Burkholderia_ is a further indication that they are closely related, and that this system might play an important and conserved role in the life style of this novel group of bacterial species.
STUDIES ON THE ROLE OF QUORUM SENSING IN BURKHOLDERIA PLANTARII AND BURKHOLDERIA GLUMAE VIRULENCE

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Burkholderia plantarii and Burkholderia glumae are pathogens of rice and very close relatives to the members of the Burkholderia cepacia complex (BCC). We have previously shown that in both pathogens N-acyl homoserine lactone quorum sensing (QS) is important for virulence and that a QS-regulated lipase was a virulence factor in B. glumae. We have now also studied B. plantarii and determined that pathogenic strains produce large amounts of anthranilic acid which is present up to 6 mM in the spent supernatants. The production of anthranilic acid by B. plantarii is connected to QS and our experiments have also shown that it is phytotoxic to rice. Interestingly non-pathogenic/less pathogenic B. plantarii isolates do not produce such high amounts of anthranilic acid indicating that the ability to produce large amounts is an adaptive response of rice pathogens. We did not detect anthranilic acid in several B. glumae strains thus this ability was associated only with B. plantarii. Importantly, anthranilic acid is a precursor for the synthesis of many compounds one of which is the QS signal molecule 3-hydroxy-4-quinolone known as PQS which is produced by Pseudomonas aeruginosa and Burkholderia pseudomallei. It has been established that pathogenic strains of B. glumae produce PQS whereas pathogenic B. plantarii isolates do not indicating a possible link with anthranilic acid production. Finally, both B. plantarii and B. glumae are pathogenic to Caenorhabditis elegans and to the larvae of the wax moth Galleria mellonella and thus these host can serve as alternative infection models for the study of virulence mechanisms of these important Burkholderia species.
Session 4: Polysaccharides

(4A)
CONDITIONAL MUTATIONS IN THE AMINOARABINOSE BIOSYNTHESIS AND TRANSFER (ARN) GENE CLUSTER OF B. CENOCEPACIA KS6-2 INDUCE CHANGES IN PHOSPHOLIPIDS AND LIPID A-CORE OLIGOSACCHARIDE

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_Burkholderia cenocepacia_ is a member of the _B. cepacia_ complex, a group of environmental Gram negative bacteria that are highly resistant to the majority of clinically useful antimicrobials and also resistant to antimicrobial peptides (APs). LPS is the major component of the outer membrane of Gram negative bacteria and consists of the O antigen polysaccharide, core oligosaccharide and Lipid A. APs bind to Gram negative bacteria through electrostatic interactions with negatively charged phosphate groups covalently attached to residues on the lipid A and core oligosaccharide. Bacteria can resist the action of APs by modifying their lipid A with the positively charged sugar 4-amino-4-deoxy-L-aminoarabinose (ara4N). In _E. coli_ and _Salmonella_ sp. this modification is regulated by environmental signals and dispensable for growth. _B. cenocepacia_ LPS contains ara4N as a modification of the Lipid A, as well as part of the core oligosaccharide. We have demonstrated that the _arn_ cluster of genes putatively involved in the synthesis of ara4N and its transfer to the LPS is essential for _B. cenocepacia_ viability. The loss of viability of conditional mutants in the _arn_ cluster is accompanied by increased membrane permeability, sensitivity to SDS, and accumulation of membranous material suggesting that the lack of ara4N causes a general cell envelope defect. We hypothesize that these defects are associated with the lack of ara4N in the lipid A-core moiety of the LPS molecule. To better study the compositional and structural changes in the lipid A-core and also other lipid components of the bacterial cell envelope in the _arn_ conditional mutants, we recreated these mutants in the heptoseless _B. cenocepacia_ K56-2 strain RSF34. In this manner, we can investigate the changes in both lipid A and a short inner core disaccharide composed of Kdo and Ko residues. We labeled the cells with $^{32}$P, extracted the glycerophospholipids of the different strains grown under non-permissive conditions and separated them by TLC. The lack of expression of the _arn_ cluster correlates with changes in the pattern of phosphorylated lipids extracted from _B. cenocepacia_. Efforts to identify the lipid species produced by _B. cenocepacia_ in the absence of ara4N using a variety of chemical and physical approaches are currently in progress.
PLANT PATHOGEN *BURKHOLDERIA* LIPOPOLYSACCHARIDES

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The *Burkholderia* genus consists of several species of closely related and extremely versatile Gram-negative bacteria found naturally in soil, water, and the rhizosphere of plants. Strains of *Burkholderia* have been used in biological control of plant diseases and bioremediation, while some strains are plant pathogens or opportunistic pathogens of humans with cystic fibrosis. The ecological versatility of these bacteria is likely due to their unusually large genomes, which are often comprised of several (typically two or three) large replicons, as well as their ability to use a large array of compounds as sole carbon sources.

As ubiquitous and vital components of the cell surface of Gram-negative bacteria, lipopolysaccharides (LPSs) have multiple roles in these various plant-microbe interactions. LPS contributes to the reduced permeability of the Gram-negative outer membrane. This barrier function allows growth of bacteria in unfavorable conditions that may be encountered in niches on or within plant hosts. For example, the exclusion of preformed or induced antimicrobial substances of plant origin probably contributes to the ability of pathogenic bacteria to parasitize plants. LPS-defective mutants show increased *in vitro* sensitivity to antibiotics and antimicrobial peptides and upon introduction into susceptible plants, the numbers of viable bacteria often decline very rapidly. LPS may also promote bacterial attachment to plant surfaces. Conversely, perception of LPS by plant cells can lead to the direct triggering of defense responses or to the priming of the plant to respond more rapidly and/or to a greater degree to subsequent pathogen challenge. These induced responses presumably act to eliminate or contain potential pathogens 1,2.

The ability of plants to recognize LPS and the consequences of this recognition for plant-microbe interactions are subjects of considerable current interest. A first step toward the comprehension of these complex biological mechanisms is the elucidation of the full structural detail of *Burkholderia* LPSs.

From the chemical point of view: lipopolysaccharide (S-LPS) structure is constituted by three chemically and biogenetically distinct regions: a glycolipid moiety, the Lipid A; an oligosaccharide region, the core region; a polysaccharide, the O-specific chain (O-polysaccharide, O-chain). LPS not containing O-chain are termed Rough (R) LPS or lipoooligosaccharide (LOS). LOSs may occur in both wild and laboratory strains possessing mutations in the genes encoding the O-specific polysaccharide biosynthesis or transfer.

Within this frame the structure of LPS from plant pathogen *Burkholderia* is comprehensively described.

DIFFERENTIAL MUCOID EXOPOLYSACCHARIDE PRODUCTION IN 
BURKHOLDERIA CEPACIA COMPLEX

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Burkholderia cepacia complex members, the other main CF pathogen Pseudomonas aeruginosa are capable of elaborating a mucoid exopolysaccharide (EPS). EPS production is not clearly visible on routine laboratory media; however media such as Yeast Extract medium (YEM) permit identification of isolates capable of producing EPS. We conducted a survey of the B. cepacia complex strain panel; a collection of environmental isolates; and an extensive collection of clinical isolates from the Vancouver CF clinics. These results demonstrated EPS production is common and that all species of the B. cepacia complex can elaborate EPS on YEM. It was notable, however, that the proportion of non-mucoid isolates was much higher in the more virulent B. cenocepacia in both clinical and environmental isolates. Furthermore, analysis of clinical isolates by case demonstrated that phenotypic transition during infections sometimes occurred, however rather than the typical nonmucoid to mucoid transition seen in P. aeruginosa; we observed a number of mucoid to nonmucoid transitions. We are also investigating the basis of the biochemical differences between mucoid and nonmucoid variants of B. cenocepacia.
FUNCTIONAL CHARACTERIZATION OF BCEC, AN UDP-GLUCOSE DEHYDROGENASE FROM BURKHOLDERIA CEPACIA REQUIRED FOR CEPACIAN POLYSACCHARIDE SYNTHESIS

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Bacteria belonging to the Burkholderia cepacia complex (Bcc) have emerged as opportunistic pathogens in cystic fibrosis (CF) patients. This microbes express often a mucoid phenotype, associated with the production of abundant amounts of exopolysaccharides (EPS), suggesting a possible role of EPS in Bcc virulence and persistence. Cepacian is the main EPS produced by Bcc isolates and is composed of a branched acetylated heptasaccharide repeat-unit with D-glucose, D-rhamnose, D-mannose, D-galactose and D-glucuronic acid in the ratio 1:1:1:3:1, respectively [1]. The cepacian biosynthesis is a multi-step process directed by genes belonging to the cepacian gene cluster bce [2]. This study focused on the functional analysis of the cepacian gene bceC which encodes an UDP-glucose dehydrogenase from the CF isolate Burkholderia cepacia IST408, a high-producer of cepacian. UDP-glucose dehydrogenase (EC 1.1.1.22) is a key enzyme required for the synthesis of the cepacian polysaccharide precursor UDP-glucuronic acid. Database searches and phylogenetic analysis revealed that BceC orthologs can be found in the Burkholderia genomes (2 to 3 per genome) and are encoded by syntenic clusters linked to lipopolysaccharide and polysaccharide biosynthesis. The bceC gene was recombinantly expressed in Escherichia coli and purified for biochemical characterization. The enzyme has an optimum temperature and pH of 45ºC and 8.5, respectively. The estimated apparent $K_m$ values for UDP-glucose and NAD$^+$ were 0.23 and 0.53 mM, respectively. A bceC-deficient mutant showed a significant reduced ability to produce the EPS cepacian and was phenotypically nonmucoid, demonstrating that the enzymatic activity of BceC is required for cepacian production. Additionally, the bceC mutant is also defective for biofilm formation. Because of its central role in cepacian synthesis and biofilm formation, BceC is a potential target for therapeutic inhibitors to combat Burkholderia infections in CF patients.

EXTENSION OF THE bce CLUSTER OF GENES INVOLVED IN THE BIOSYNTHESIS OF CEPACIAN BY THE GENUS BURKHOLDERIA

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Exopolysaccharides (EPS) are important virulence determinants in several pathogenic bacteria and their role in persistence and virulence has been discussed by several authors (1, 2). Cepacian is one of the four known exopolysaccharides produced by Burkholderia cepacia complex (Bcc) and it was found in the majority of the clinical isolates (1, 3). It is composed of branched acetylated heptasaccharide repeat-units with D-glucose, D-rhamnose, D-mannose, D-galactose and D-glucuronic acid in the ratio 1:1:1:3:1, respectively (4). In Bcc, B. pseudomallei, B. thailandensis and B. oklahomensis, the genes responsible for cepacian biosynthesis locate within two different clusters 150 kb apart from each other, while in B. phymatum, B. phytofirmans and B. xenovarans these two regions are contiguous and present similar structural arrangement. The first region was previously characterized and includes eleven genes, from bceA to bceK. It includes two genes which products are involved in nucleotide sugar biosynthesis, five glycosyltransferases and four other genes encoding proteins for the polymerization and export of cepacian (5). Proteins such as BceA, BceD and BceF, among others have been characterized (6, 7). The second region includes eight additional genes putatively encoding three proteins involved in nucleotide sugar biosynthesis, a bifunctional glycosyltransferase, a repeat-unit transporter and three other proteins of unknown function. Quantification of the polysaccharide produced by several Bcc and non Bcc strains was carried out. We propose this second region as an extension of bce gene cluster and confirmed that cepacian production is a common feature in both Bcc and non Bcc strains whether they are isolated from clinical or environmental sources.

1) Zlosnik JE et al., J Clin Microbiol (in press)
BURKHOLDERIA CENOCEPACIA pgaC, A GENE INVOLVED IN THE SYNTHESIS OF THE POLYSACCHARIDE POLY-N-ACETYL-GLUCOSAMINE (PNAG), IS REQUIRED FOR VIRULENCE IN C. ELEGANS

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Burkholderia cenocepacia is an important opportunistic pathogen causing deadly lung infections in cystic fibrosis patients. This species has also been shown to be virulent in other models of infection. In particular, strain K56-2 can infect and kill the nematode, Caenorhabditis elegans. In Staphylococcus spp., one recognized virulence factor in the C. elegans infection model is the polysaccharide, poly-N-acetyl-glucosamine (PNAG), which is responsible for intestinal tract colonization and virulence. In this study, we examined whether PNAG contributes to B. cenocepacia K56-2 virulence in C. elegans.

Using the Escherichia coli K-12 PNAG biosynthetic locus (pga) for homology searches to the B. cenocepacia J2315 genome, we recognized genes potentially responsible for the synthesis of this polysaccharide. The predicted proteins encoded by the J2315 genes BCAM1228, BCAM1227, and BCAM1226 are homologs of E. coli PgaA, PgaB, and PgaC, respectively, and the protein encoded by BCAM1225 has a similar predicted mass, pl, and membrane topology as PgaD. The pgaC gene is predicted to encode a putative N-acetyl-glucosaminyltransferase, which is thought to be the first committed step in the synthesis of PNAG. Therefore we generated a K56-2 pgaC mutant by allelic replacement and confirmed it by PCR.

To determine the effect of this mutation on PNAG production, strains were grown in typticase soy broth + 1% glucose as biofilms on polystyrene plates for 48 hours at 37°C. Using these in an ELISA with antibody to Staphylococcus PNAG, we observed that the pgaC mutant produced significantly less PNAG than the wild-type strain.

Next we assessed the effect of this mutation on the ability to infect C. elegans. L4 stage nematodes were placed on bacterial lawns of either wild-type K56-2 or K56-2 pgaC grown on NGM agar and animal health was followed for 8 days. The pgaC mutant was highly attenuated in nematode killing compared to K56-2. Microscopic examination of nematodes exposed to either K56-2 or the pgaC mutant showed no sign of bacterial attachment to the cuticle; however, animals exposed to the pgaC mutant appeared to have fewer intestinal tract bacteria than those exposed to K56-2. Quantification of digestive tract bacteria, performed by manual disruption, serial dilution, and plating, confirmed that animals exposed to the pgaC mutant had significantly fewer intraluminal colony forming units than K56-exposed animals. Taken together, these results indicate that PNAG facilitates intestinal tract colonization and disease in bacterivorous nematodes by diverse bacteria, including B. cenocepacia.
INTERACTION OF BCC EXOPOLYSACCHARIDES WITH COMPONENT OF THE INNATE IMMUNE SYSTEM: BACTERIAL ESCAPE FROM HOST DEFENCE MECHANISMS

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Infecting bacteria use a variety of tools to promote an effective defence against host antimicrobial agents both of the innate and adaptive immune system. Among others, bacterial exopolysaccharides (EPS) have been also recognised as important factors in infection set up and maintenance. As a matter of fact, soon after infection establishment P. aeruginosa exhibits a mucoid phenotype and clinical isolates revert to a non-mucoid phenotype when cultivated in vitro. Regarding BCC, it was reported that B. cenocepacia strain C9343 showed a mucoid appearance and interfered with clearance in an animal model [1]. A recent publication [2] demonstrated that EPS produced by the same B. cenocepacia strain inhibited neutrophil chemotaxis and scavenged reactive oxygen specie (ROS), thus impairing some of the host defence mechanism. In addition, it was demonstrated that EPS are able to decrease antimicrobial peptides (AP) activity [3].

In order to characterise at a molecular level the mechanism with which EPS scavenge ROS, alginate and cepacian were treated with sodium hypochlorite. Capillary viscosity measurements indicated that both alginate and cepacian molecular masses were largely decreased by hypochlorite ions action. The depolymerisation was rather fast, since the capillary flow rate of EPS solution reached that of the solvent in only two hours. Size exclusion chromatography and determination of reducing-end concentration confirmed the results of capillary viscometry measurements. The structure of the oligomeric species produced by ROS will be analysed by mass spectrometry and NMR spectroscopy.

Minimum inhibiting concentration assays carried out on a reference strain of E. coli showed that alginate is very efficient in lowering the antimicrobial activity of AP from primates, while cepacian had very low or no effect at all. However, an EPS mixture produced by B. cenocepacia strain C9343 [1] and composed of cepacian, PS-I and dextran, formed a complex with AP followed by its precipitation. This behaviour can explain the mechanism of action of AP deactivation by EPS and, at the same time points at a synergistic effect of different EPS towards host AP. Moreover, it offers an hypothesis on the advantage of synthesizing EPS mixtures, as many isolates of BCC do.

(4H)

VIRULENCE FACTORS OF THE BURKHOLDERIA CEPACIA COMPLEX; STUDIES OF THE EPS AND LPS SACCHARIDES.


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The Burkholderia cepacia complex (Bcc) was first identified in 1950 as the causative agent of soft rot of onions, and has emerged over the past two decades as a major pulmonary pathogen in cystic fibrosis (CF) patients. Worst-case scenario is the onset of the “cepacia syndrome” – a fatal necrotizing pneumonia with bacteraemia that occurs in 20% of all infected CF patients. The main problems with Bcc infection are the massive inflammatory response and the inherent resistance to antimicrobial peptides and antibiotics. The members of the Bcc are thought to produce a range of putative virulence factors, two of which are exopolysaccharide (EPS) and lipopolysaccharide (LPS, endotoxin). Both of these saccharides are thought to be involved in inflammation and persistence in host, as well as antibiotic-resistance mechanisms which are observed in the clinic.

We returned to the “natural” host, and found that agar containing an onion extract caused EPS production (the mucoid phenotype) in isolates of the Bcc that had been previously considered non-mucoid. Chemical analysis suggested that the onion components responsible for EPS induction were primarily the carbohydrates sucrose, fructose and fructans. Additional sugars were investigated, and all alcohol sugars tested were able to induce EPS production, in particular mannitol and glucitol. Representatives of the B. cenocepacia ET12 lineage did not produce EPS under any growth conditions investigated. This finding correlated with the presence of an 11 bp deletion in the bceB gene which encodes a glycosyltransferase responsible for the catalysis of the first step of the assembly of the EPS repeat unit. Knock-outs of this bceB gene were also non-mucoid. These novel and surprising insights into EPS biosynthesis highlight the diverse metabolic profile of the Bcc and show that a potential virulence factor may not be detected by routine laboratory culture. Our results also highlight a potential hazard in the use of inhaled mannitol as an osmolyte to improve mucociliary clearance in individuals with cystic fibrosis.

The LPS/endotoxin from members of the BCC is unique – it is highly inflammatory and cannot be neutralised by polymyxin. To probe this further we have investigated the inflammatory potential of LPS isolated from B. cenocepacia ET-12 isolates K56-2, J2315 and a mutant SAL-1, presenting smooth, rough and deep-rough LPS respectively. The LPSs were used to stimulate human monocyte-derived macrophages to elicit a cytokine response (TNF-α and a range of interleukins) alone, and in the presence of cationic antimicrobial peptides (cAMPs). Our results provide further evidence that the LPSs from the BCC have unique chemical and biological properties.
Session 5: Pathogenesis

(5A)
CHARACTERIZATION OF MgtC, AN INNER MEMBRANE PROTEIN REQUIRED FOR INTRACELLULAR SURVIVAL OF *BURKHOLDERIA CENOCEPACIA*

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Our laboratory has identified *mgtC* as a gene necessary for the survival of *Burkholderia cenocepacia* *in vivo* (Hunt *et al.*, 2004). In both *Salmonella typhimurium* and *Mycobacterium tuberculosis*, *mgtC* plays a role in adaptation to low magnesium, low pH environments and is highly expressed under magnesium-deplete conditions. We have shown that *mgtC* is also needed for growth of *B. cenocepacia* in magnesium-depleted medium and for bacterial survival within murine macrophages (Maloney and Valvano, 2006). Recent studies demonstrated that the *B. cenocepacia* containing vacuole has a neutral pH (Lamothe *et al.*, 2007), and a similar environment in *Salmonella*-infected macrophages is not Mg²⁺ depleted (Martin-Orozco *et al.*, 2006). Therefore, the role of MgtC in intracellular survival may not be related to low Mg²⁺ but rather to other macrophage-killing mechanisms. We compared the growth *in vitro* of wild type and *mgtC*-deficient *B. cenocepacia* in the presence of compounds thought to mimic *in vivo* conditions. In addition, experiments using our macrophage infection model cells were performed using inhibitors of specific macrophage responses. We have demonstrated that the reduced intracellular survival of the *mgtC* mutant in macrophages is not due to increased sensitivity to low pH, oxidative stress, nitrosative stress, potassium concentration, oxygen tension or cationic peptides, nor does it appear to be the consequence of a general membrane defect since *mgtC* mutants can resist detergents and antimicrobial peptides. To elucidate how MgtC may be involved in protecting *B. cenocepacia* from the intracellular environment of the host cells we are currently investigating the regulation of *mgtC* and whether MgtC interacts with other proteins.
INVASION OF BURKOLDERIA CEPACIA COMPLEX INTO LUNG EPITHELIAL CELLS INVOLVES GLYCOSPHINGOLIPID RECEPTORS

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The invasion of Burkholderia cepacia complex (Bcc) into lung epithelial cells is well-established. B. cenocepacia and B. multivorans, the most virulent and clinically relevant species, invade most readily. However, the mechanisms of invasion are poorly understood. Prior to invasion, these bacteria need to bind to the lung epithelial cells. Previously, it has been shown that Bcc strains bound to many glycolipid receptors, including asialoGM1, asialoGM2 and globosides, on lung epithelia. We have now demonstrated that these glycolipid receptors are essential for invasion. Invasion was examined in two different lung epithelial cell lines, 16HBE14o- cells which express functional cystic fibrosis transmembrane regulator (CFTR) protein and CFBE41o- cells which are homozygous negative for the ΔF508 mutation. Enzyme treatment to remove galactose moieties from the lung cell surface dramatically inhibited invasion, while removal of sialic acid groups did not. In addition, treatment of lung epithelial cells with inhibitors of the sphingolipid biosynthetic pathways at non-cytotoxic levels also significantly reduced invasion by Bcc strains. In contrast, results obtained following treatment with the mucolytic agent, N-acetylcysteine, indicated that mucus adhesion did not play a major role in the invasion process. Binding of biotinylated strains to purified glycolipids separated by TLC was examined and different patterns of binding were observed. In conclusion, it is apparent that invasion of lung epithelial cells is mediated via binding to glycosphingolipid receptors. We are currently evaluating glycoconjugate derivatives to examine if these can be used to prevent attachment of Bcc to epithelial cells, which may represent a potential therapy in the future in the prevention of colonisation.

This project has been funded under the Programme for Research in Third Level Institutions (PRTLI) administered by HEA.
STRUCTURE AND PRO-INFLAMMATORY ACTIVITY OF ENDOTOXIN FROM THE BURKHOLDERIA CEPACIA COMPLEX

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Members of genus Burkholderia comprise opportunistic pathogens responsible for important infections in immunocompromised and in cystic fibrosis (CF) patients. The B. cepacia complex (Bcc) \(^{[1-3]}\) is a group of microorganisms composed of at least ten closely related genomovars. All genomovars have been shown to cause infections although B. cenocepacia and B. multivorans (genomovars III and II, respectively) are the most frequently isolated genomovars from CF patients. Moreover, pre-operative infections with Bcc are often associated with a poor prognosis in CF lung transplant recipients.

The hallmark of Bcc infection is the inflammation to which contributes the biological activity of lipopolysaccharides (LPSs), which are the main components of the outer membrane of Gram-negative bacteria and are heat-stable complex amphiphilic macromolecules indispensable for the bacterial growth and survival. The lipopolysaccharides (LPS) \(^{[4-7]}\), also termed endotoxins, are potent immunostimulator factors and are involved in pathways controlling inflammation and host defenses; moreover, their structural characteristic confers decreased permeability to the Bcc outer membrane and resistance to classes of antibiotic as β-lactams as well as cationic antimicrobial compounds. It has been demonstrated that LPSs undergo significant structural changes to allow persistence of bacterial infections during the interaction with the CF microenvironment. Changing of these bacterial structures may influence host reactions and improve bacterial survival in infected tissues as those of patients with CF. The comprehension of the bacterial modifications of virulence factors that allow resistance, survival and persistence of infections will help in better understanding the molecular mechanisms of adaptation to new physiological conditions and will be useful in the design of new antimicrobial compounds and therapies.

Lipopolysaccharides are constituted by three chemically and biogenetically distinct regions: a glycolipid moiety, the Lipid A; an oligosaccharide region, the core region; a polysaccharide, the O-specific chain (O-polysaccharide, O-chain). LPS not containing O-chain are termed Rough (R) LPS or lipooligosaccharide (LOS). LOSs may occur in both wild and laboratory strains possessing mutations in the genes encoding the O-specific polysaccharide biosynthesis or transfer.

The structure and immunostimulatory activity of LPSs from Bcc clinical isolates is here described, to establish how LPS structural changes influence the immunostimulatory properties (and thus the immune response).

EFFECTS OF LACTOFERRIN IRON SATURATION ON THE ABILITY OF BURKHOLDERIA CEPACIA COMPLEX (BCC) TO INVADE LUNG EPITHELIAL CELLS AND FORM BIOFILMS

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The objectives of this study were to investigate the effects of recombinant human lactoferrin (rHL) on the ability of Burkholderia cepacia complex (Bcc) strains to form biofilms and invade lung epithelial cells.

We examined whether the iron saturation level of rHL, at physiological levels (0.9mg/ml) observed in sputum samples of CF patients, influenced the biofilm forming ability of various strains of Bcc. The study found that rHL has anti-biofilm activity which is specific to some Bcc strains only (LMG13010, C5393, C1962 and JTC) and this activity is strongly dependent on lactoferrin iron saturation. In the presence of apo-rHL biofilm formation was 50% less than without lactoferrin, however complete biofilm inhibition was not seen. This finding is consistent with a previous study, which demonstrated that apolactoferrin-induced iron deficiency resulted in inhibition of biofilm formation and development in Pseudomonas aeruginosa. Furthermore, we investigated the ability of rHL, with different iron saturation levels, to influence the invasion of Bcc strains in A549 monolayers. We found that the ability of Bcc strains to invade cultured A549 cells was significantly enhanced in the presence of apolactoferrin. However, this activity was also dependent on the lactoferrin iron saturation level and was specific to each strain tested. In particular, the invasive ability of LMG 13010 and LMG 18941 was 25-30 times greater in the presence of apo- rHL. These findings strongly suggest that in Bcc strains the process of invasion is iron dependent and is inversely proportional to the iron availability.

The findings presented here demonstrate a significant relationship between the iron saturation level of the lactoferrin and both the invasion efficiency of members of Bcc and their ability to form biofilms in vitro.
BURKHOLDERIA CENOCEPACIA ZMPA AND ZMPB METALLOPROTEASES DEGRADE HOST CYTOKINES AND ANTIMICROBIAL PEPTIDES

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Strains from all members of the *Burkholderia cepacia* complex (Bcc) have been isolated from cystic fibrosis (CF) patients; the most prevalent in Canada being *B. cenocepacia*. The direct role of extracellular proteases in *B. cenocepacia* pathogenesis is not known; however, several studies have suggested that two secreted zinc metalloproteases, ZmpA and ZmpB may be required for maximal virulence of *B. cenocepacia in vivo*. Both the *zmpA* and *zmpB* genes are present in strains from *B. cepacia*, *B. cenocepacia*, *B. stabilis*, *B. ambifiaria*, and *B. pyrrocinia* but absent from *B. multivorans*, *B. vietnamiensis*, *B. dolosa* and *B. anthina*. ZmpB has previously been found to possess broader substrate specificity than ZmpA. In the present study, we investigated whether ZmpA and/or ZmpB could degrade host cytokines which potentially could alter the balance of host cytokines. Both proteases were found to digest both pro- and anti-inflammatory cytokines. Both ZmpA and ZmpB digested interferon-γ, IL1-beta, IL-6, IL-10, and TGF-1beta. Interestingly, under the conditions employed, only ZmpA digested TNF and only ZmpB digested IL-8. We also examined whether ZmpA/ZmpB could potentially impair the host defense by degrading antimicrobial peptides. Antimicrobial peptides are a structurally diverse group of peptides involved in innate immunity. Two groups of defensins have been identified in humans, α- and β- defensins. β-defensin-1 was degraded by ZmpB but not ZmpA. Neither ZmpA nor ZmpB cleaved the α-defensin, HNP-1. Another antimicrobial peptide group, the cathelicidins, has only one known representative in humans (hCAP-18). hCAP-18 is proteolytically processed resulting in LL-37. In digestion studies, ZmpA but not ZmpB cleaved LL-37. Preliminary liquid killing assays indicate that K56-2zmpB and K56-2zmpAzmpB mutants are more sensitive to the antimicrobial peptide, protamine. These results suggest that ZmpA and ZmpB could interfere with host defense mechanisms against *B. cenocepacia*.
(5F)
THE ACYL CARRIER PROTEIN, INVOLVED IN THE VIRULENCE OF BURKHOLDERIA CENOCEPACIA J2315, IS CONSERVED WITHIN THE BURKHOLDERIA GENUS

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Aiming at the identification of virulence determinants of bacteria of the Burkholderia cepacia complex that might be exploited to develop new therapeutic approaches and/or to develop new methods for early detection of respiratory infections, a collection of mutants derived from B. cenocepacia strains J2315 was prepared by random plasposon mutagenesis using previously established techniques [1,2]. This collection has been screened for mutants exhibiting reduced virulence to the nematode model of infection Caenorhabditis elegans. One of the mutants exhibited reduced virulence to the nematodes using the fast-killing assay. Cloning and sequence analysis of the interrupted gene revealed that it encodes an acyl carrier protein (ACP). Bacterial ACPs are the donors of the acyl moiety involved in type II fatty acid biosynthesis (FAS II), being also required for the synthesis of mature haemolysin, synthesis of lipid A of LPS, and acylhomoserine lactones [3]. The mutant was found to exhibit an increased ability to form biofilms in vitro, a more hydrophobic cell surface and reduced ability to kill the nematode C. elegans using slow-killing assays.

Remarkably, the ACP amino acid sequence was found to be 100 % conserved within the genomes of 52 Burkholderia strains sequenced so far. ACP and other proteins involved in FAS II are being studied as targets for antimicrobials development [3]. Altogether, these results highlight its potential as a target to develop antibacterial agents to combat infections caused not only by Bcc species, but also by other Burkholderia species, especially B. pseudomallei and B. mallei.

CHARACTERIZATION OF A NOVEL 1-CYS PEROXIREDOXIN OF BURKHOLDERIA CENOCEPACIA

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Peroxiredoxins are ubiquitous proteins that catalyze the reduction of hydroperoxides, thus conferring resistance to oxidative stress. We recently reported that a putative peroxiredoxin of Burkholderia cenocepacia is co-expressed with the arn locus. The arn locus is associated with aminoarabinose modification of lipopolysaccharide, resulting in resistance to antimicrobial peptides. Thus the linkage of a peroxiredoxin to the arn locus combines putative resistance mechanisms to both oxidative and non-oxidative killing by phagocytic cells. In the present study we have characterized the peroxiredoxin, a homologue of the previously described bacterioferritin comigratory protein (BCP). We report that the linkage of bcp to the arn locus is unique to organisms of the Burkholderia genus and Ralstonia pickettii group (i.e. those beta-proteobacteria that exhibit innate resistance to antimicrobial peptides). The Burkholderia bcp homologue is constitutively expressed throughout the growth cycle. However, presumably due to the shifting repertoire of anti-oxidant proteins throughout the growth cycle, the anti-oxidant activity of BCP is most pronounced at the lag phase and early log phase of growth. We have confirmed that the Burkholderia BCP homologue possesses thiol-dependent peroxidase activity. Additionally, we have used a combination of site-directed mutagenesis and high-resolution mass spectrometry to interrogate the catalytic mechanism of the Burkholderia BCP homologue, and compare it to the previously described BCP of Escherichia coli. We report that the E. coli BCP harbours a resolving cysteine and functions as an atypical 2-Cys peroxiredoxin. However, the Burkholderia BCP lacks the resolving cysteine and consequently functions via the distinct 1-Cys mechanism. Sequence analysis of BCP homologues indicates that this 1-Cys peroxiredoxin of Burkholderia is abundant in diverse bacterial genera, and represents a novel member of the thiol-dependent peroxidases.
CHARACTERIZATION OF A LYSOGENIC BURKHOLDERIA CEPACIA COMPLEX PHAGE WITH POTENTIAL FOR USE IN PHAGE THERAPY.

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Because members of the Burkholderia cepacia complex (BCC) are normally resistant to high levels of antibiotics, alternative treatment therapies are needed. One potentially useful form of alternative treatment is phage therapy, in which bacteriophages rather than chemical antibiotics are used in order to eliminate the pathogenic bacteria. Several phages specific to the BCC have been isolated and characterized in an effort to identify phages that will be most efficacious in a treatment regimen. Although lytic phages pose the fewest problems associated with their use, many BCC strains have been found to harbour lysogenic phages that may prove useful as phage therapy agents. One such phage is KS10, a prophage we originally identified in *B. cenocepacia* K56-2. A survey of 21 strains and 20 clinical isolates of the BCC revealed that KS10 is able to form plaques on lawns of *B. ambifaria* LMG 19467 and *B. cenocepacia* PC184. The genome of KS10 encodes 49 potential proteins, including capsid proteins similar to Mu and BcepMu, and tail proteins similar to Mu, though it is lacking the invertible G tail genes region. The organization of the KS10 genome differs from most phages in that its capsid genes are not aligned into one module but rather separated by approximately 11 kb, giving evidence of one or more prior genetic rearrangements. There were no potential virulence factors identified in KS10, though many hypothetical proteins were identified with no known function. Time course experiments suggest that KS10 prophage induction to lysis is variable depending upon environmental conditions and the genetic background of the bacterial host.
ANTIBIOTIC SUSCEPTIBILITY, VIRULENCE AND TAXONOMIC STATUS OF BURKHOLDERIA CEPACIA-COMPLEX MULTIRESISTANT STRAINS ISOLATED FROM ITALIAN CYSTIC FIBROSIS PATIENTS

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*B. cepacia*-complex (Bcc) strains are often multidrug resistant due to innate and acquired mechanisms of resistance, but few data are available about the antibiotic susceptibility patterns of these bacteria in cystic fibrosis (CF) patients. We report the *in vitro* activity of commonly used antimicrobial agents against 72 multiresistant Bcc strains selected over a 4-year period from 1000 CF patients attending 2 Italian centers. The minimum inhibitory concentrations (MICs) were determined by E test; the genomovar status was determined by *recA*-based PCR assays and the presence of genetic elements characteristic of epidemic lineages such as *BCESM* and *IS 1363* was evaluated by specific tests.

Temocillin, meropenem and ceftazidime were the most active agents inhibiting 90%, 56% and 35% of strains respectively. The majority of Bcc isolates belonged to *B. cenocepacia* (76%), the III-A (26%) and III-B lineages being the most represented (50%). The remaining isolates belonged to *B. cepacia gvr* I (16%), *B. multivorans* (2.6%), *B. stabilis* (2.6%), and *B. vietnamiensis* (2.6%). All the isolates belonging to the III-A lineage were *BCESM* positive and one *IS1363* positive. Only 5 strains belonging to the III-B lineage were positive for the *BCESM* and one for *IS1363*. Some differences were demonstrated among susceptibility patterns of the different groups; strains identified as *B. cenocepacia* lineage III-A showed the highest resistance level to the tested antibiotic agents while *B. cepacia gvr* I members showed a lower degree of resistance.

Temocillin, which is not frequently used, represents the most active drug against Bcc multiresistant strains. Bcc strains belonging to genomovars considered less clinically relevant for CF patients, such as *gvr* I, can show an high level of antibiotic resistance.
ZEBRA FISH AS A NOVEL VIRULENCE MODEL FOR BURKHOLDERIA CENOCEPACIA

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The molecular mechanisms which allow B. cenocepacia to colonize the lung, subvert host defenses, and then invade and destroy deeper lung tissue are still poorly understood. A limited number of infection models are available to study B. cenocepacia virulence. Especially cell invasion assays have proven difficult to conclusively show and study intracellular replication, due to the persistence of extracellular bacteria in the presence of high doses of antibiotics. Good models are essential to analyze in detail the molecular mechanisms that allow this intracellular pathogen to resist host killing, and allow the bacteria to survive and multiply inside immune and other host cells. Currently we are developing and validating a new model for B. cenocepacia using zebra fish that will allow us to study the immunopathology and physiopathology of the infection in more detail. Zebra fish have recently become recognized as a promising model to study human pathogens. In the young embryos, an innate immune system that resembles that of humans, is already developed, and the translucence of the zebra fish embryos allow easy real time analysis of infections. Promising preliminary data using real time analysis show that the bacterium is able to invade, survive, and replicate intracellularly, followed by invasion of other cells, resulting in rapid death of the embryo, often preceded by septicemia. We have observed a difference in pathogenicity between several clinical isolates. We are now studying in more detail the pathogenicity of different Bcc strains and mutants, as well as the bacterial strategy to survive intracellularly. Here we will discuss our recent progress, and discuss the value of this promising model.
INVESTIGATIONS ON THE PATHOGENICITY OF \textit{BURKHOLDERIA CEPACIA} COMPLEX STRAINS IN TWO DIFFERENT VIRULENCE MODELS

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Over the past few decades, strains of the \textit{Burkholderia cepacia} complex (Bcc) have emerged as important pathogens for patients suffering from cystic fibrosis (CF). Identification of virulence factors and assessment of the pathogenic potential of \textit{Burkholderia} strains have increased the need for appropriate infection models.

We have previously used the nematode \textit{Caenorhabditis elegans} to identify virulence factors in \textit{B. cenocepacia}. However, some questions cannot be addressed with this infection model, especially the effect of temperature on pathogenicity, as \textit{C. elegans} cannot be maintained above 20 °C. Another disadvantage of this model is that the infection dose cannot be varied as bacteria are ingested by the nematodes.

In this study we used the larvae of the greater wax moth \textit{Galleria mellonella} as a host organism for assessing the virulence of Bcc strains. Its innate immune system is similar to the one of mammals and includes phagocytosis, nodulization and encapsulation. The size of the animal also allows to inject defined cell numbers and the larvae can be maintained at 30°C to 37°C.

Our study aimed at comparing the \textit{G. mellonella} with the \textit{C. elegans} infection model, to investigate if certain virulence factors are host specific and what the contribution of individual factors to virulence in the different pathogenesis models is. In addition to several Bcc wild type strains, we tested selected mutants of \textit{B. cenocepacia} H111, a CF isolate that effectively kills \textit{C. elegans} as well as \textit{G. mellonella}. While some virulence factors were important for pathogenicity in both model systems others were host specific. Most interestingly, AidA, a protein of unknown function that is essential for nematode killing of \textit{B. cenocepacia} H111, is not required for virulence of the strain in the \textit{G. mellonella} infection model.

This suggests that in addition to a set of virulence factor required for pathogenicity in all hosts there are also factors that are specific for particular hosts.
PATHOGENICITY OF ENVIRONMENTAL *BURKHOLDERIA CENOCEPACIA* STRAINS

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The widespread presence in the rhizosphere of *Burkholderia cenocepacia* poses the question of whether rhizosphere strains are pathogenic for CF patients or not. At this aim, we explored the virulence of eight environmental *B. cenocepacia* strains, recovered mainly from maize rhizosphere, and ten clinical strains isolated from CF patients, typed by using multi locus sequence typing (MLST). We investigated if rhizosphere and CF *B. cenocepacia* strains differed in their capacity to form biofilm in vitro and to induce systemic infection or chronic persistence in a murine model of airway infection. Interestingly, environmental and clinical strains formed similar amounts of biofilm in microtitre plate wells (\(P=0.89\)), with OD\(_{595nm}\) mean values of 0.9927 and 1.059, respectively. Although strain-to-strain differences were observed in the in vivo infection model, statistic analysis performed on all data together (chronic infection and mortality) showed that *B. cenocepacia* strains of environmental origin were as virulent as those from CF patients and were able to persist in the lungs of infected mice, with no significant differences in bacterial loads and localization 14 days after challenge. Lung histology of mice infected with clinical strain LMG16656\(^T\) and rhizosphere strain MEX1 revealed an extensive inflammatory cell infiltrate in the thickened alveolar septa, where bacterial cells were visualized as microcolonies by indirect immunofluorescence. These data suggest that both clinical and rhizosphere *B. cenocepacia* strains have a similar capacity to maintain a chronic respiratory infection which may be due to production of similar virulent factors not depending on their origin. Furthermore, to assess the host influence on *B. cenocepacia* pathogenicity of environmental strains, we evaluated the changes in virulence of the rhizosphere strain MEX1 after serial passages in mice. Results obtained revealed that this strain becomes able to increase its capacity to establish chronic infection after serial passages in mice, suggesting adaptation of *B. cenocepacia* to murine lung tissues.

This work was supported by the Italian Cystic Fibrosis Research Foundation (grants FFC#11/2004 and FFC#7/2006) with the contribution of “Associazione Laziale e Associazione Lombarda per la lotta alla fibrosi cistica e Delegazione FFC di Latina”.

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\((6E)\)
Abstract not suitable for oral presentation

BATCH MICROBIAL DEGRADATION OF PHENOLS BY INDIGENOUS PSEUDOMONAS FLUORESCENCE

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The potential of various organisms to metabolize organic compounds has been observed to be a potentially effective means in disposing of hazardous and toxic wastes. Phenolic compounds have long been recognized as one of the most recalcitrant and persistent organic chemicals in the environment. The bioremediation potential of an indigenous *Pseudomonas fluorescens* was studied in batch culture using synthetic phenol in water in the concentration range of (100–500) mg/L as a model limiting substrate. The effect of initial phenol concentration on the degradation process was investigated. Phenol was completely degraded at different cultivation times for the different initial phenol concentrations. Increasing the initial phenol concentration from 100mg/L to 500mg/L increased the lag phase from 0 to 66hrs and correspondingly prolonged the degradation process from 84hrs to 354hrs. There was decrease in biodegradation rate as initial phenol concentration increased. The Monod model has been used to interpret the free cell data on phenol biodegradation. The kinetic parameters have been estimated up to initial phenol concentration of 500mg/L. The *r_{max}* decreased and *K_s* increased with higher concentration of phenol. The *r_{max}* has been found to be a strong function of initial phenol concentration.
**PRELIMINARY PARTICIPANTS LIST**

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