18th Annual Meeting
April 9-12, 2014
Nimes, France
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wednesday, April 9, 2014</strong></td>
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<tr>
<td>19.00-21.00</td>
<td>Welcome Reception/ Registration - Atria Novotel Nimes</td>
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<tr>
<td><strong>Thursday, April 10, 2014</strong></td>
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<tr>
<td>7.30-8.15</td>
<td>Breakfast and registration</td>
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<tr>
<td>8.15-8.30</td>
<td>Welcome - Introductory remarks</td>
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<tr>
<td>8.30-9.20</td>
<td><strong>KEYNOTE lecture</strong></td>
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<td>Dr. Sophie de Bentzmann</td>
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<td></td>
<td>The shady side of <em>Pseudomonas aeruginosa</em></td>
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<tr>
<td>9.20-10.00</td>
<td>I Epidemiology: identification and detection</td>
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<td>Moderator: John LiPuma</td>
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<tr>
<td>9.20-9.40</td>
<td>1. PCR detection of <em>B. multivorans</em> in environmental samples</td>
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<td>C. Peeters and P. Vandamme</td>
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<td>9.40-10.00</td>
<td>2. Epidemiology of <em>Bcc</em> and related organisms in French patients with cystic fibrosis</td>
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<td></td>
<td>Christine Segonds, Michelle Thouverez, Marie Sponga, Lydie Lemonnier</td>
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<td>and the Observatoire cepacia Study Group</td>
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<td>10.00-10.30</td>
<td>Coffee break</td>
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<td><strong>Session 2</strong></td>
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<tr>
<td>10.30-12.30</td>
<td>II Interactions with the host: Evolution of <em>Bcc</em> during chronic infection</td>
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<td>Moderators: Amal Amer, Pavel Drevinek</td>
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<td>10.30-10.50</td>
<td>3. Within-Cystic Fibrosis patient genotypic variation in <em>Burkholderia multivorans</em> sequential isolates</td>
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<td>Inês N. Silva, Pedro M. Santos, Jörg D. Becker, and Leonilde M. Moreira</td>
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<td>10.50-11.10</td>
<td>4. <em>Burkholderia cenocepacia</em> adapts by increasing attachment to host lung epithelial cells over time of infection</td>
</tr>
</tbody>
</table>
11.10-11.30  5. Changes in expression of virulence factors over the course of infection with Burkholderia cenocepacia ST32
Louise Cullen, Kirsten Schaffer, Máire Callaghan and Siobhán McClean

11.30-12.50  III Interactions with the host: Phagocytes and immune response
Moderators: David Speert, David O'Callaghan

11.30-11.50  6. Human macrophages provide a rich replication niche that allows Burkholderia cenocepacia to escape neutrophil killing and enhances proliferation
Allison McDonald and David P. Speert

11.50-12.10  7. Identification of TecA, the Burkholderia cenocepacia type 6 secretion system effector protein affecting eukaryotic cytoskeleton architecture
Daniel F. Aubert, Sherry Hu and Miguel A. Valvano

12.10-12.30  8. MicroRNA, autophagy and Burkholderia cenocepacia. Is there a link?
Mia F. Tazi, Duaa Dakhllallah, Benjamin Kopp, Kyle Caution, Anwari Akhter, Youssra Saqr, Melissa Piper, Clay Marsh, Amal Amer

12.30-12.50  9. Modeling the innate immune response during inflammatory and persistent Burkholderia cepacia complex infections in zebrafish embryos
Jennifer Mesureur, Julien Rougeot, Annemarie H. Meijer, David O'Callaghan, Annette Vergunst

12.50-14.10  Lunch

Session 3

14.10-15.30  IV Molecular Microbiology
Moderators: Arsénio Fialho, Gabriella Pessi

14.10-14.30  10. Unraveling the functions of BCAM0224, a trimeric autotransporter adhesin from Burkholderia cenocepacia
Dalila Mil-Homens, Maria Inês Leça, Sofiane El-Kirat-Chatel, Audrey Beaussart, Yves F. Dufrêne and Arsénio M. Fialho

14.30-14.50  11. Response to nitrogen limitation in B. cenocepacia H111
Gabriella Pessi, Martina Lardi, Nadine Schmid, Claudio Aguilar and Leo Eberl

14.50-15.10  12. Characterisation of putative small RNAs of Burkholderia cenocepacia J2315 identified by differential RNA sequencing
Andrea M Sass, Sanne Kiekens, Heleen Van Acker, Tom Coenye

15.10-15.30  13. A Burkholderia cenocepacia MurJ (MviN) homolog is essential for cell wall peptidoglycan synthesis and bacterial viability
Yasmine Fathy Mohamed and Miguel A. Valvano

15.30-16.00  Coffee break
**Session 4**

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
</tr>
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<tbody>
<tr>
<td>16.00-16.40</td>
<td>IV Molecular Microbiology - continued</td>
<td>Moderators: Arsenio Fialho, Leo Eberl</td>
</tr>
<tr>
<td>16.00-16.20</td>
<td>14. Redundancy and specificity of iron transport systems in the genus Burkholderia</td>
<td>Anugraha Mathew, Aurelien Carlier and Leo Eberl</td>
</tr>
<tr>
<td>16.40-17.40</td>
<td>V Genomics and metabolomics</td>
<td>Moderators: Peter Vandamme, Jose Degrossi</td>
</tr>
<tr>
<td>16.40-17.00</td>
<td>16. Causes of heterogeneity in evolutionary rate in divided bacterial genomes</td>
<td>Marcus M. Dillon and Vaughn S. Cooper</td>
</tr>
<tr>
<td>17.20-17.40</td>
<td>18. Development of genetic tools and genome sequencing of Burkholderia contaminans isolates</td>
<td>Ma. Agustina López De Volder, Ruhi Bloodworth, Carrie Selin, José Degrossi and Silvia T. Cardona</td>
</tr>
</tbody>
</table>

**Free time/ dinner on your own**
Friday April 11, 2014

7.30-8.30 Breakfast

Session 5

8.30-10.10 VI Antimicrobial resistance, novel drugs and disinfectants
Moderators: Tom Coenye, Miguel Valvano

8.30-8.50 19. New drugs and new targets to fight Burkholderia cenocepacia
Silvia Buroni, Viola C. Scoffone, Francesca Spadaro, Vadim Makarov, and Giovanna Riccardi

8.50-9.10 20. Phenotypic changes of Burkholderia contaminans associated to biocides resistance
Agustina López de Volder, Daniela Figoli, Verónica Pioli, Fabricio Rugnone, Sergio Teves and José Degrossi

9.10-9.30 21. Nonmevalonate pathway for isoprenoid biosynthesis as novel target for antibacterial therapy against the BCC
Annelien Everaert, René Chofo, Serge Van Calenbergh, Tom Coenye

9.30-9.50 22. Mining the Burkholderia genomes for novel antibiotics
Eshwar Mahenthiralingam, Lijang Song, Simon Harris, Paul Coupland, Matthew Dunn, Cerith Jones, Matthew Moore, Julian Parkhill and Gregory L. Challis

9.50-10.10 23. Use of essential oils from medicinal plants to fight Burkholderia cepacia complex strains

10.10-10.30 24. Fosmidomycin potentiates the effects of colistin on Burkholderia multivorans clinical isolates
Rebecca J. Malott, Chia-Hung Wu, James E. A. Zlosnik, Dianne K. Newman, and David P. Speert

10.30-10.50 Coffee break

Session 6

10.40-11.40 VI Antimicrobial resistance, novel drugs and disinfectants - continued
Moderators: Silvia Cardona, Siobhan McClean

Yasmine Sonmez, Camille Bechetoille, Sandrine Perrotto, Alban Payet-
11.00-11.20 26. Developing a high throughput chemogenomic approach for profiling bioactives against Burkholderia cenocepacia
April S. Gislason, Ruhi A. M. Bloodworth, Wubin Qu, Xuan Li, Chenggang Zhang and Silvia T. Cardona

11.20-11.40 27. Characterization of growth inhibitors of Burkholderia cenocepacia K56-2 and generation of an HDTM library
Carrie Selin and Silvia T. Cardona

11.40-12.40 VII Plant-associated Burkholderia species
Moderators: Vittorio Venturi, Eshwar Mahenthiralingam

11.40-12.00 28. Functional analysis of the candidatus Burkholderia kirkii genome: insights into the nature of an obligate leaf nodule symbiosis
A. Carlier, M. Pinto, S. Sieber, U. Omasits, C. Ahrens, K. Gademann and L. Eberl

12.00-12.20 29. Plant-induced gene expression in an endophyte belonging to the group of plant beneficial Burkholderia
Bruna G. Coutinho and Vittorio Venturi

12.20-12.40 30. Adaptation of Burkholderia toward symbiosis with Mimoseae
Agnieszka Klonowska, Caroline Burnaud, Rémy Melkonian, Gilles Béna, Damien Mornico, Pierre Tisseyre, Clémence Chaintreuil, Karine Heulin, Gisèle Laguérre and Lionel Moulin

12.40-14.00 Lunch

14.30-18.30 Guided Tour Ancient Nimes

20.30- Conference diner: Le Lisita, boulevard des Arenes
Saturday, April 12, 2014

Session 7

<table>
<thead>
<tr>
<th>Time</th>
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</thead>
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| 8.45-10.25 | VIII Biofilms and quorum sensing
Moderators: Alan Brown, Paola Cescutti |
| 8.45-9.05  | 31. Exopolysaccharides in biofilms of Burkholderia cenocepacia and Burkholderia multivorans
Ambra Delneri, Claudia Buriola, Mustafa Fazli, Tim Tolker-Nielsen, Roberto Rizzo and Paola Cescutti |
| 9.05-9.25  | 32. Biofilm formation by cystic fibrosis-pathogenic Burkholderia cepacia complex (BCC) bacteria allows them to evade neutrophil antimicrobial activities
Mark Murphy, Maire Callaghan and Emma Caraher |
| 9.25-9.45  | 33. Development of a biosensor for investigating the BDSF quorum sensing system
Angela Suppiger, Claudio Aguilar and Leo Eberl |
| 9.45-10.05 | 34. Involvement of toxin anti-toxin modules in Burkholderia cenocepacia biofilm persistence
Heleen Van Acker, Andrea Sass, Hans J. Nelis & Tom Coenye |
| 10.05-10.25| 35. The attenuated virulence of a Burkholderia cenocepacia paaABCDE mutant is due to inhibition of quorum sensing by release of phenylacetic acid
Tanya Pribytkova, Steve P. Bernier, John L. Sorensen, Michael G. Surette, and Silvia T. Cardona |

10.25-11.00 Coffee break

11.00-12.00 Closing remarks- discussion about the next meeting

12.30 Lunch on your own

We gratefully acknowledge the generous sponsorship of

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Belgian Science Policy Office
Abstracts
Keynote Lecture: THE SHADY SIDE OF PSEUDOMONAS AERUGINOSA

Sophie de Bentzmann

UMR7255-Laboratoire d’Ingénierie des Systèmes Macromoléculaires, CNRS - Aix Marseille Université, Marseille, France

Contact : bentzman@imm.cnrs.fr

Pseudomonas aeruginosa is a Gram-negative environmental species and an opportunistic microorganism, establishing itself in vulnerable patients, such as those with cystic fibrosis (CF) or hospitalized in intensive care units (ICU). It has become a major cause of nosocomial infections worldwide and a serious threat to Public Health because of overuse and misuse of antibiotics that have selected highly resistant strains against which very few therapeutic options exist. Its genome is highly plastic and evolves very fast under environmental constraints as it will be illustrated in a series of isolates that we recently sequenced and compared. This bacterium is also a fantastic model for studying new molecular determinants (Type I and IVb conjugative and non conjugative pili, T1SS, lectins,...) involved in several process such as adhesion, uptake in host cells, sedentary community lifestyle (biofilm), conjugation, secretion and related virulence. Several examples will illustrate each aspect of the research we recently performed in this field.

P. aeruginosa has also a unique ability to survive in specific habitats through coordination of appropriate gene expression in response to encountered environmental changes. The complexity of its regulatory networks which integrate environmental signals through adequate and specific signaling pathways and the number of regulatory genes in its genome are incredibly high and involve two-component and ECF signaling pathways, quorum sensing and other molecules including c-di-GMP, as well as a number of transcriptional regulators. Two TCS (an unorthodox GacS/GacA and a classical PprA/PprB) signalling pathways will be illustrated with recent findings we obtained in particular on the multicomponent signal transduction system made of the LadS hybrid histidine kinase and the TCS GacS/GacA, on sRNA Rsm Y and Z the targets controlled by GacS/GacA, on the PprA/PprB regulon and on a new transcriptional regulator Cpr.
1. PCR DETECTION OF *B. MULTIVORANS* IN ENVIRONMENTAL SAMPLES

C. Peeters\(^1\) and P. Vandamme\(^1\)

\(^1\)Laboratory of Microbiology Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Contact: Charlotte Peeters, Charlotte.Peeters@UGent.be

Recent epidemiological surveys reveal that *B. multivorans* is the most prevalent Bcc cystic fibrosis pathogen in many countries (e.g. Belgium and France). The concept that environmental pressures can select for traits that confer virulence leads to the possibility that soil or other environments could be regular sources of new pathogens. The continued emergence of unique *B. multivorans* strains in CF patients suggests acquisition from nonhuman sources, such as the natural environment. Yet, there are so few environmental *B. multivorans* isolates that its true environmental niche is considered unknown.

To gain more insight in the environmental niche of *B. multivorans*, samples were taken from river and recreational waters and soil in Belgium. After total DNA-extraction of the environmental samples, *B. multivorans* was detected using a *B. multivorans* specific recA PCR assay. Using this culture-independent technique, *B. multivorans* was detected in both water (12/112) and soil (24/28) samples. To obtain pure cultures for MLST analysis, PCR positive samples were plated using semi-selective isolation conditions and obtained isolates were identified using matrix-assisted laser desorption ionization time-of-flight spectroscopy (MALDI-TOF MS) and/or 16S rRNA and recA sequence analysis. A wide diversity of *Burkholderia* strains, representing at least a dozen novel *Burkholderia* species, was recovered, but not *B. multivorans*.

In conclusion, the culture-independent approach demonstrated that *B. multivorans* is indeed present in the environment. However, isolation of *B. multivorans* from the environment is not as straightforward as compared to other *Burkholderia* species.

This research was supported by the Special Research Council of Ghent University. The *Burkholderia cepacia* complex National Reference Center is supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System.
2. EPIDEMIOLOGY OF Bcc AND RELATED ORGANISMS IN FRENCH PATIENTS WITH CYSTIC FIBROSIS

Christine Segonds1, Michelle Thouerez2, Marie Sponga3, Lydie Lemonnier3 and the Observatoire cepacia Study Group

1Observatoire cepacia, Laboratoire de Bactériologie-Hygïène, Hôpital Purpan, CHU Toulouse, France
2Service de Bactériologie et Hygiène Hospitalière, CHU Besançon, France
3Vaincre la Mucoviscidose, Paris, France
Contact: Christine Segonds, segonds.c@chu-toulouse.fr

Biological and clinical data concerning colonisations with Bcc and related organisms in French patients with CF are collected and analysed by the Observatoire cepacia and the French CF Registry. Clinical isolates are identified by means of a combination of ARDRA, RecA based species-specific PCR or RecA sequencing. Genotyping is performed using PCR-ribotyping and PFGE or MLST. In 2011, 116 patients out of 5993 were colonised with Bcc organisms (prevalence, 1.9% ; incidence 0.5%). Bcc prevalence was of 2.4% in male patients versus 1.6% in female patients and the mean age at primary colonization was 16.6 y. The distribution of species was the following: *B. multivorans*, 63 (54.3%); *B. cenocepacia* 35 (30.2%); other species 18 (15.5%). The five epidemic lineages which emerged in the 1990s, i.e ST 15/16 and ST 419/180 (*B. multivorans*); ST 32 (*B. cenocepacia* IIIA), ST 122 and 279 (*B. cenocepacia* III B) were still involved in 41 of the 116 patients (35%), but only in 4 new cases. A sixth epidemic clone (ST751) emerged in the late 2000s and was involved in 4 patients and 2 new cases. *Burkholderia gladioli* was identified in 14 patients (prevalence, 0.23%; incidence 0.03%) and other colistin-resistant species in 19 patients: *Burkholderia thailandensis* (1 patient); *Burkholderia fungorum* (1 patient); *Inquilinus limosus* (7 patients) and *Pandoraea* species (10 patients). Thirty-five patients with Bcc and two patients with *B. gladioli* underwent lung transplantation between 2003 and 2011. Bloodstream infections (BSI) occurred in 11 patients colonized with Bcc, and in the 2 patients colonized with *B. gladioli*. Fourteen of the 37 patients (38%) died within 6 months post-transplantation, 8 (22%) died later after transplantation, and 15 (40%) are still alive to-date. In conclusion, the implementation of infection control measures allowed a dramatic decrease of the spread of epidemic lineages in France, but vigilance is still needed. As previously reported, Bcc as well as *B. gladioli* colonised patients are at risk of post-transplantation BSI.
3. WITHIN- CYSTIC FIBROSIS PATIENT GENOTYPIC VARIATION IN BURKHOLDERIA MULTIVORANS SEQUENTIAL ISOLATES

Inês N. Silva¹, Pedro M. Santos², Jörg D. Becker³, and Leonilde M. Moreira¹,⁴

¹IBB- Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, IST, Lisbon, Portugal
²Center of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal
³Instituto Gulbenkian de Ciência, Rua da Quinta Grande nº6, Oeiras, Portugal
⁴Department of Bioengineering, Instituto Superior Técnico (IST), Av. Rovisco Pais, 1049-001 Lisbon, Portugal

Contact: Inês Silva, ines.silva@tecnico.ulisboa.pt

Bacteria from Burkholderia cepacia complex (Bcc) are characterized by large size genomes comprising a high number of protein-encoding genes that confer bacteria the ability to rapidly adapt and colonize new environmental niches such as airways of cystic fibrosis patients. In order to get insights on the evolution of the genome content during long-term colonization of cystic fibrosis airways with the emerging opportunistic pathogen Burkholderia multivorans, we have sequenced and assembled the genomes of 12 B. multivorans clinical isolates recovered from a CF patient within a 13 year period. Genomic DNA of B. multivorans D2095 was sequenced by Illumina HiSeq2000 system and paired-end and mate-pair sequence reads were de novo assembled, originating 9 scaffolds and estimating a genome size of 6,73 Mbp. A draft annotation was performed using Prokka (Prokaryotic Genome Annotation System) which predicted a total of 6342 coding sequences for B. multivorans D2095 genome. The genomes of the other 11 clinical isolates were sequenced with paired-end MiSeq sequencing and the resulting reads were mapped to B. multivorans D2095 scaffolds. Comparative genomics allowed the identification of major rearrangements conducting to genome reduction in the last clinical isolate and duplication of a 37-kb prophage genomic region in some of the isolates. A total of 144 variants, from which 119 (13 indels, 2 tandem repeats and 104 SNPs) were located within CDS and estimating a SNP rate per year of 2.4 (R = 0.8) was estimated. 88 out of the 104 SNPs identified were non-synonymous and there were 10 genes with more than one mutated site. A prediction on the effect of each non-synonymous mutation on protein function suggests that 16 out of 88 are likely to affect protein function. The results obtained confirm genome reduction during chronic infection as observed for other CF pathogens, suggest the existence of different clones of B. multivorans coexisting at the same time and evidence the exceptionally high genomic plasticity.

II. Interactions with the host: Evolution of BCC during chronic infection
4. *BURKHOLDERIA CENOCEPACIA* ADAPTS BY INCREASING ATTACHMENT TO HOST LUNG EPITHELIAL CELLS OVER TIME OF INFECTION.

Louise Cullen¹, Kirsten Schaffer², Máire Callaghan¹ and Siobhán McClean¹*

1: Centre of Microbial Host Interactions (CMHI) Institute of Technology Tallaght, Dublin 24, Ireland  
2: St Vincent’s University Hospital (SVUH), Elm Park, Dublin 4, Ireland

Contact: Siobhán McClean (siobhan.mcclean@ittdublin.ie)

*B. cenocepacia* chronically colonises the lungs of cystic fibrosis (CF) patients. Although it is generally confined to the lung, bacteraemia can develop in a subgroup of patients contributing to a sharp decline in health. The mechanisms by which *B. cenocepacia* chronically colonises or causes bacteraemia have not been elucidated. Bacteria can change phenotype during infection, adapting to the host environment. We aim to identify the alterations in *B. cenocepacia* phenotype from initial colonisation to chronic infection. In addition, we aim to determine the alterations that may result in *B. cenocepacia* escaping the lung and moving into the blood circulation. We have investigated a series of genetically identical, sequential *B. cenocepacia* isolates from two siblings with CF, one with chronic infection confined to the lung (Pt1), the other with bacteraemia (Pt2). All isolates were relatively avirulent *in vivo* in the *Galleria mellonella* virulence model. Interestingly, the blood isolates from Pt2 were considerably more virulent in this model than the sputum isolate from that patient (P=0.014). Mucoid production by *B. cenocepacia* has been associated with better patient outcomes; however, all but one isolate were non-mucoid when grown on YEM. Attachment of both sputum and blood isolates to CF lung epithelial cells (CFBE41o-⁻) increased with time of patient colonisation (p<0.005 and 0.001 respectively), indicating an increased potential for host epithelial interactions over time. This key adaptation was confirmed by confocal microscopy. We are currently comparing the proteomes of all isolates to identify alterations in protein expression that may be involved in enhanced host cell attachment. Overall, it is clear that *B. cenocepacia* adapts and increases in virulence during colonisation.
Microarray data comparing a transcriptome of sputum and blood isolates of *B. cenocepacia* ST32 indicated different expression in genes for flagella, type 3 secretion system (T3SS), bacterial capsule and quorum sensing among others, despite the fact that the isogenic isolates were cultivated in identical growth conditions.

The aim of this study was to confirm microarray findings by real time PCR as well as by functional tests for motility, and to check the gene expression on a wider collection of ST32 isolates (sequential isolates from 7 patients who succumbed to cepacia syndrome).

For swimming activity, we have tested 29 sputum and 7 blood isolates (a range of 3 to 7 isolates per patient; spanning the length of the infection from 2 up to 16 years). In addition, 10 of the sputum isolates and all 7 blood isolates were tested by real time PCR to check their expression of following genes: 2 for capsule (BCAM0859 and BCAM0860), 2 for T3SS (BCAM2048 and BCAM2050) and 2 for quorum sensing (BCAM1870 and BCAM1871) which were all up-regulated in blood isolates vs. sputum isolates; and 2 for flagella (BCAL0114 and BCAL0142) which were found to be down-regulated in previous microarray experiments. The isolates were cultivated 4 hours in control medium (BSM) after which their RNA was extracted and subjected to RQ-PCR assays.

The motility of isolates was observed to be decreasing with the time of infection: while in general early isolates showed swimming activity, the latter isolates demonstrated a loss of this activity. RQ-PCR analysis for the 8 genes provided results consistent with the microarray data and swimming activity for all but 2 isolates: the blood isolates showed on average 32-fold increase in expression for capsule genes, 24-fold up-regulation of genes for quorum sensing, 12-fold increase in expression of genes for T3SS and similar or down-regulated expression of genes for flagella.

Results from microarray analysis were confirmed by real time PCR on a larger panel of clinical isolates. With the worsening clinical condition there is a trend towards the loss of bacterial motility. We are testing whether this phenomenon could be utilized as a negative prognostic marker in the chronic *B. cenocepacia* infection.

Supported by IGA MZ NT12405-5, MSMT LD11029, GAUK 2120176.
6. HUMAN MACROPHAGES PROVIDE A RICH REPLICATION NICHE THAT ALLOWS *BURKHOLDERIA CENOCEPACIA* TO ESCAPE NEUTROPHIL KILLING AND ENHANCES PROLIFERATION

Allison McDonald\textsuperscript{1,2} and David P. Speert\textsuperscript{1,2,3}

\textsuperscript{1}Centre for Understanding and Preventing Infection in Children, Vancouver, Canada  
\textsuperscript{2}Department of Microbiology and Immunology, University of British Columbia  
\textsuperscript{3}Department of Pediatrics, Faculty of Medicine, University of British Columbia

Contact: David Speert, dspeert@cfri.ubc.ca

Pulmonary innate immunity protects against inhaled pathogens through a combination of defenses that include tissue resident macrophages and recruited neutrophils. *Burkholderia cenocepacia* causes severe respiratory infections in immunocompromised individuals that are characterized by neutrophil infiltration and excessive inflammation. The ability of *B. cenocepacia* to survive and replicate intracellularly may contribute to its ability to evade the host response. We aimed to investigate the collaborative effects of macrophages and neutrophils to control *B. cenocepacia* survival. While neutrophils were faster to phagocytose *B. cenocepacia*, the bacteria were able to quickly replicate within primary monocyte-derived macrophages, which offered a large survival advantage compared to bacterial growth in media alone in the absence of leukocytes. Proliferation required entry into macrophages and neither macrophage-released factors nor macrophage lysates were capable of enhancing bacterial growth. Only when neutrophils were co-cultured in large excess to macrophages were they able to control the growth of *B. cenocepacia* and the release of inflammatory mediators from macrophages. This study suggests that macrophages have a dominant effect over neutrophils in the ability to affect *B. cenocepacia* survival and growth and that an excess of neutrophils is required to abrogate the enhanced growth provided by the intramacrophage replication niche. This phenomenon is consistent with the massive infiltration of neutrophils during acute respiratory infection with *B. cenocepacia*. 
7. IDENTIFICATION OF TECA, THE BURKHOLDERIA CENOCEPACIA TYPE 6 SECRETION SYSTEM EFFECUTOR PROTEIN AFFECTING EUKARYOTIC CYTOSKELETON ARCHITECTURE

Daniel F. Aubert, Sherry Hu and Miguel A. Valvano

¹ Department of Microbiology, University of Western Ontario, London, Ontario N6A 5C1, Canada; ² Centre for Infection and Immunity, Queen's University Belfast, BT9 5GZ, Belfast, United Kingdom

Contact: Miguel Valvano; m.valvano@qub.ac.uk

The Type 6 Secretion System (T6SS) is a versatile weapon widespread among Gram-negative pathogens and symbionts. Some T6SS deliver toxins to kill or inhibit the growth of susceptible bacteria, while others have evolved to target eukaryotic cells. Deletion of atsR, a negative regulator of virulence factors in B. cenocepacia K56-2, increases T6SS activity. Also, macrophages infected with K56-2 ΔatsR display dramatic alterations in their cytoskeleton architecture with the formation of unusual “beads on a string-like” structures. This phenotype relies on the T6SS to affect the activation of multiple Rho family GTPases by an unknown mechanism. Systematic deletion of each gene within the T6SS cluster and vgrGs in K56-2 ΔatsR identified critical core components and accessory proteins of the T6S machinery but failed to uncover an effector protein affecting the host cytoskeleton. Screening of a K56-2 ΔatsR transposon library in our macrophage infection model identified a mutant with an insertion in a gene that we have named tecA (for T6SS effector protein affecting the cytoskeleton architecture), which was unable to elicit cytoskeletal rearrangements. tecA encodes a small protein of unknown function and is unique to B. cenocepacia species. Burkholderia multivorans ATCC17616 ΔatsRbm has an active T6SS but lacks a TecA homolog. While it was initially unable to induce cytoskeletal rearrangements upon macrophage infection, expression of TecA was sufficient to make ATCC17616 ΔatsRbm induce the formation of “beads on a string-like” structures. Based on these findings we propose that TecA is the T6SS effector protein affecting the cytoskeleton architecture in macrophages infected by B. cenocepacia.
8. MICRORNA, AUTOPHAGY AND BURKHOLDERIA CENOCEPACIA. IS THERE A LINK?

Mia F. Tazi¹,²,⁵, Duaa Dakhallah²,³,⁵, Benjamin Kopp⁴, Kyle Caution¹,²,⁵, Anwari Akhter¹,²,⁵, Youssra Saqr¹,⁵, Melissa Piper²,³,⁵, Clay Marsh²,³,⁵, Amal Amer¹,²,⁵

¹Department of Microbial Infection and Immunity, Center for Microbial Interface Biology, ²Department of Internal Medicine, ³Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, ⁴Center for Microbial Pathogenesis at Nationwide Children’s Hospital, ⁵The Dorothy M. Davis Heart and Lung Research Institute and The Ohio State University, Columbus, Ohio, 43210

Each year 1,000 children and adults are diagnosed with Cystic Fibrosis (CF), a lethal, inherited disease that most critically affects the lungs. The disease is directly caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene rendering its protein dysfunctional. Autophagy, a highly regulated biological process, normally clears these defective proteins and invading microbes. However, this process is deficient in CF patients and CF mice. Consequently, specific pathogens are able to replicate within CF macrophages thus exacerbating inflammation.

Burkholderia cenocepacia (B.c) is a significant CF pathogen as it harbors innate antibacterial resistance to the majority of treatments, may cause fatal sepsis, and is transmitted by direct patient contact. CF patients infected with B.c are not considered for lung transplant due to poor survival rates. MicroRNAs (miRs) non-coding RNAs that post-transcriptionally regulate targeted mRNA expression, have become significant innovative therapies. The majority of miRs comprising the miR-17~92a cluster are predicted to target several autophagy mRNAs. This redundancy suggests its relevance in the regulation of autophagy. The underlying mechanism for alterations in CF immune responses to B.c remains unknown. We hypothesize that CF macrophages express elevated miR-17~92a expression that downregulates autophagy mRNA targets contributing to autophagy dysfunction, consequently promoting B.c infection and inflammation. Our data demonstrate that miR-17~92a expression is upregulated in CF macrophages. B.c infection further upregulates miR-17~92a cluster expression. High levels of miR-17~92a expression is accompanied by downregulation autophagy mRNAs contributing to the further deterioration of autophagy activity. Using specific miR-17 antagomirs to downregulate miR-17, we found that B.c burden and inflammation are controlled in a CF model of infection. Thus, the expression of members of the miR-17~92a cluster correlates with autophagy activity which modulates the pathophysiology of CF.
Chronic respiratory infection in cystic fibrosis patients is characterized by a high level of pro-inflammatory cytokines, leukocyte infiltration, and inflammation in the lungs due to colonization by pathogenic bacteria. Chronic infections caused by bacteria belonging to the *Burkholderia cepacia* complex (Bcc) can be symptom free, but often cause pulmonary exacerbation with progressive worsening of lung function, sometimes resulting in acute fatal necrotizing pneumonia and sepsis. The reasons for these unpredictable, sudden transitions are not understood. Using zebrafish embryos, which have an innate immune system very similar to that of humans, we previously found that *B. cenocepacia* K56-2, belonging to the epidemic ET12 lineage, is highly virulent for zebrafish embryos; it causes a rapidly fatal (2 days) systemic inflammatory infection. In contrast, embryos can control infection with strains such as *B. stabilis* LMG14294, which cause a persistent infection. Intravenously injected bacteria are rapidly phagocytosed by macrophages, and we have shown that an intracellular stage is important for infection. Using qRT-PCR, we observed that intracellular *B. cenocepacia* K56-2 causes a very rapid (2-3 hours) strong increase in pro-inflammatory (IL-1β, IL-8, TNFα) gene expression that is maintained during later stages of infection. In contrast, similar numbers of intracellular *B. stabilis* only induces moderate changes in pro-inflammatory gene expression during early stages. In an attempt to better understand the molecular basis for these different infection outcomes, we performed a global host transcriptome analysis during different stages of both infection types. RNA-seq analysis revealed interesting infection responsive host gene expression patterns. Whereas many host genes were differentially regulated during early (3 hours) as well as later (24 hours) stages of infection caused by *B. cenocepacia* K56-2, only few genes showed changes in expression level upon persistent infection with *B. stabilis* LMG14294. In particular, the Toll-like receptor (TLR) and apoptosis pathways were strongly activated during acute infection. The “silent” intracellular persistence of *B. stabilis* coincided with increased expression of genes encoding complement proteins. We will discuss how we are using the zebrafish model to further study the role of the TLR pathway, including the central adaptor protein MyD88, bacterial ligands and intracellular stages in the induction of the highly excessive innate inflammatory response.
10. UNRAVELING THE FUNCTIONS OF BCAM0224, A TRIMERIC AUTOTRANSPORTER ADHESIN FROM BURKHOLDERIA CENOCEPACIA

Dalila Mil-Homens¹, Maria Inês Leça¹, Sofiane El-Kirat-Chatel², Audrey Beaussart², Yves F. Dufrêne² and Arsénio M. Fialho¹

¹IBB-Institute for Biotechnology and Bioengineering, Instituto Superior Técnico, Lisbon, Portugal; ²Université Catholique de Louvain, Institute of Life Sciences, Louvain-la-Neuve, Belgium

Contact: Arsénio M. Fialho, afialho@ist.utl.pt

B. cenocepacia is able to attach to receptors on the lung epithelial cells and either invade and persist via intracellular vacuoles or translocate through the epithelium. However the mechanisms of interaction between this pathogen and the host lung epithelium remain poorly understood. Trimeric autotransporter adhesins (TAAs) are surface-exposed proteins known to be involved in a wide range of host interaction traits on pathogenic gram-negative bacteria [1]. We have previously identified in the genome of the epidemic clinical isolate B. cenocepacia J2315, a novel cluster of genes putatively encoding three TAAs (BCAM0219, BCAM0223 and BCAM0224) [2,3]. In this study, we characterized the BCAM0224 protein aiming to establish its role in B. cenocepacia pathogenicity. We show that BCAM0224 occurs on the bacterial surface and adopts a trimeric conformation. Further, we demonstrated that BCAM0224 is needed for the earlier stages of biofilm formation and is required for swarming motility. In addition, BCAM0224 plays an important role in evasion of the human innate immune system, providing resistance against the bactericidal activity of serum via the complement classical pathway. Moreover, we used the killing of Galleria mellonella as a model host to state the role of BCAM0224 in B. cenocepacia virulence [2]. We also used human bronchial epithelial cell lines (16HBE14o- and CFBE41o-), which have a non-CF and a CF phenotypes, respectively, to show that this TAA mediates bacterial adhesion to and invasion of host cells. Finally, we used single molecule atomic force spectroscopy to show that BCAM0224 mediates low-affinity homophilic and heterophilic (collagen, pneumocytes) interactions involved in bacterial aggregation and host cell adhesion, and that the adhesin behaves has a stiff nanospring when subjected to external force [4]. Collectively, our results show that BCAM0224 is a multifunctional virulence factor of B. cenocepacia, playing different roles in host-cells interaction mechanisms and harbouring remarkable binding properties (i.e. large binding strength, low affinity, broad specificity and spring elasticity).

11. RESPONSE TO NITROGEN LIMITATION IN B. CENOCEPACIA H111

Gabriella Pessi1, Martina Lardi1, Nadine Schmid1, Claudio Aguilar1 and Leo Eberl1

1Institute of Plant Biology, Department of Microbiology, UZH Zurich, Switzerland

Contact: Gabriella Pessi, gabriella.pessi@botinst.uzh.ch

Nitrogen is one of the most important and limiting elements in the environment. When bacterial growth is limited by the nitrogen source, a complex regulatory response activates the assimilatory metabolic pathways of nitrogen metabolism. These regulatory mechanisms can vary with the nitrogen source and the phylogenetic affiliation of the bacterium. To date there are no reports on how B. cenocepacia adapts to changes in nitrogen availability in the environment. Inspection of the B. cenocepacia H111 genome sequence revealed the presence of two genes encoding a set of nitrogen sensor proteins called PII and genes coding for the two-component regulatory system NtrBC, which is involved in nitrogen signalling in many bacteria. Using global approaches including RNA-Seq and proteomics, we investigated the molecular mechanisms underlying the control of nitrogen metabolism and found that the two PII proteins, the alternative sigma factor RpoN (σ54) and the putative transcriptional activator NtrC are up-regulated during nitrogen starvation. We constructed rpoN and ntrC mutant strains and show that they were impaired in growth in minimal medium containing ammonium or urea as sole nitrogen source, suggesting an important role of these proteins in nitrogen metabolism. Among the functions activated by nitrogen-limiting conditions we found the production of the exopolysaccharide cepacian as well as synthesis of a potential PHB granule-associated phasin. We are currently investigating how their biosynthesis is regulated at the molecular level.

12. CHARACTERISATION OF PUTATIVE SMALL RNAS OF BURKHOLDERIA CENOCEPACIA J2315 IDENTIFIED BY DIFFERENTIAL RNA SEQUENCING

Andrea M Sass, Sanne Kiekens, Heleen Van Acker, Tom Coenye

1Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Gent, Belgium

Contact: Andrea Sass, andrea.sass@ugent.be

Small RNAs in bacteria, as well as in other organisms, play an important role in gene regulation. They play a central role in response to environmental growth conditions by posttranscriptional gene regulation, altering gene expression by interfering with translation, or by influencing mRNA degradation rates or protein activity.

In Burkholderia sp., small RNAs are up to date largely uncharacterised. To identify small RNAs expressed in B. cenocepacia biofilms, RNA from biofilm grown cells was sequenced using differential RNA sequencing, a method which allows precise mapping of transcription start sites. Transcription start sites were then screened for putative small RNAs, based on their position relative to annotated genes, relative expression, secondary structure formation and conservation within genomes of sequenced Burkholderia sp. Small RNAs in bacteria tend to be very abundant and most are located within intergenic regions. Therefore, a subset of putative small RNAs with relatively strong expression and located within intergenic regions, sufficiently distant to annotated genes, was selected for further characterisation. Differential expression of putative small RNAs in biofilms compared with planktonically grown cultures was analysed by qPCR and interactions of small RNAs with potential target genes were analysed by in silico methods. The relevance of selected putative small RNAs for growth and biofilm formation was investigated by expressing antisense RNA on a rhamnose-inducible vector.
13. A *BURKHOLDERIA CENOCEPACIA* MURJ (MVIN) HOMOLOG IS ESSENTIAL FOR CELL WALL PEPTIDOGLYCAN SYNTHESIS AND BACTERIAL VIABILITY

Yasmine Fathy Mohamed\(^1\)\(^2\) and Miguel A. Valvano\(^1\)

\(^1\) Centre for Infection and Immunity, Queen's University Belfast, Belfast, BT9 7AE, United Kingdom; \(^2\) Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Alexandria University, Egypt

**Contact:** Miguel Valvano; m.valvano@qub.ac.uk

The cell wall peptidoglycan (PG) of *Burkholderia cenocepacia*, an opportunistic pathogen, has not been characterized. However, the *B. cenocepacia* genome contains homologues of genes encoding PG biosynthetic functions in other bacteria. PG biosynthesis involves the formation of the undecaprenyl-pyrophosphate-linked N-acetyl glucosamine-N-acetyl muramic acid-MurNAc-pentapeptide, known as lipid II, which is built on the cytosolic face of the cell membrane. Lipid II is then translocated across the membrane and its glycopeptide moiety becomes incorporated into the growing cell wall mesh; this translocation step is critical for PG synthesis. We have investigated candidate flippase homologs of the MurJ family in *B. cenocepacia*. Our results show that BCAL2764, herein referred to as *murJ\(_{Bc}\)*, is indispensable for viability. Viable *B. cenocepacia* could only be obtained through a conditional mutagenesis strategy by placing *murJ\(_{Bc}\)* under the control of a rhamnose-inducible promoter. Under rhamnose depletion the conditional strain stopped growing and individual cells displayed morphological abnormalities consistent with a defect in PG synthesis. Bacterial cells unable to express MurJ\(_{Bc}\) underwent cell lysis, while partial MurJ\(_{Bc}\) depletion sensitized the mutant to the action of β-lactam antibiotics. Depletion of MurJ\(_{Bc}\) caused accumulation of PG precursors consistent with the notion that this protein plays a role in lipid II flipping to the periplasmic compartment. Reciprocal complementation experiments of conditional *murJ* mutants in *B. cenocepacia* and *Escherichia coli* with plasmids expressing MurJ from each strain indicated that MurJ\(_{Bc}\) and MurJ\(_{Ec}\) are functional homologs. Together, our results are consistent with the notion that MurJ\(_{Bc}\) is a PG lipid II flippase in *B. cenocepacia*.

14. REDUNDANCY AND SPECIFICITY OF IRON TRANSPORT SYSTEMS IN THE GENUS *BURKHOLDERIA*

Anugraha Mathew, Aurelien Carlier and Leo Eberl

Institute of Plant Biology, University of Zurich, Switzerland

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

Like many other bacteria, *Burkholderia* sp. takes up iron in its ferric form via siderophore-dependent transporters. We identified a novel iron uptake locus, *ftr\(_{Bc}\)ABCD*, in the genome of *B. cenocepacia* H111, which is also conserved in other members of the genus *Burkholderia*. Mutants deficient in both siderophore-dependent and Ftr\(_{Bc}\)ABCD systems failed to grow under iron-limited conditions and radiolabeled iron transport assays showed that these mutants were impaired in iron uptake. This finding indicates that *Burkholderia* species employ an alternative, siderophore-independent, Ftr\(_{Bc}\)ABCD-dependent iron uptake system. Although siderophore and Ftr systems appear to be functionally redundant in vitro, they seem to have varying contributions under iron limited conditions in different habitats. Most importantly, we show that Ftr\(_{Bc}\)ABCD system is dispensable for the virulence of *B. cenocepacia* H111 while siderophores are critical for the pathogenicity. However, Ftr\(_{Bc}\)ABCD is highly conserved within the genus *Burkholderia* while the production of siderophores seems to be facultative, especially among environmental isolates. Our preliminary result suggests that the Ftr system contributes significantly to the growth of *Burkholderia* strains in soil. We are currently investigating the importance of Ftr system in *Burkholderia* legume symbionts which do not synthesize siderophores but have a functional Ftr\(_{Bc}\)ABCD system. Furthermore, we are introducing the siderophore biosynthesis cluster into these strains to analyze whether siderophores enhance nodulation efficiency or the plants would select for siderophore-deficient mutants.
15. THE CAUSES & CONSEQUENCES OF EXOPOLYSACCHARIDE PRODUCTION IN BURKHOLDERIA SPECIES: NUTRITIONAL CUES, TRANSCRIPTIONAL NETWORKS & VIRULENCE

Carmen C. Denman, Matthew T. Robinson, Jennifer E. Marin, Andrea M. Sass, Eshwar Mahenthiralingam, Alan R. Brown

1Biosciences, College of Life & Environmental Sciences, University of Exeter, Exeter. UK; 2Organisms & Environment Division, Cardiff School of Biosciences, Cardiff University, Cardiff, UK.

1Present address: London School of Hygiene & Tropical Medicine, London, UK. 2Present address: Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Gent, Belgium

Contact: Alan Brown, a.r.brown@exeter.ac.uk

Whilst exopolysaccharide (EPS) is recognised as a putative virulence determinant in species of the Burkholderia cepacia complex (BCC), the transcriptional networks and nutritional cues that promote its biosynthesis remain poorly defined. Mannitol is one of several sugars and sugar alcohols that can induce the overproduction of EPS, most commonly studied using mannitol-rich yeast extract medium (MYEM). However, our studies highlight that this mannitol-induced EPS biosynthesis is also dependent on other (as yet undefined) parameters of the culture medium. RNA-seq and microarray analysis has been performed to investigate the specific transcriptional response of Burkholderia multivorans isolates C1576 and ATCC17616 to growth on various media that either support or do not support EPS biosynthesis, revealing remarkable strain-to-strain variation in the transcriptional response that underlies EPS induction. In addition, microarray datasets from Burkholderia cenocepacia J2315 and B. multivorans ATCC17616 have been mined for evidence of induction of EPS-related genes in response to diverse environmental stressors and culture conditions. Whilst J2315 is incapable of producing EPS (due to a previously documented deletion with the bceB gene), the bce-I and bce-II gene clusters of J2315 are upregulated in stationary phase glycerol minimal media and also in response to heat stress, suggesting that the transcriptional network(s) controlling EPS biosynthesis remain intact within this strain. Preliminary studies on the role of EPS in the rice pathogen Burkholderia glumae will also be presented, in which we show a similar pattern of EPS induction to that observed in BCC species and that the bceB gene is also pivotal for EPS production in B. glumae.
16. CAUSES OF HETEROGENEITY IN EVOLUTIONARY RATE IN DIVIDED BACTERIAL GENOMES

Marcus M. Dillon and Vaughn S. Cooper

Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH, 03824, USA.

Contact: Vaughn Cooper, vaughn.cooper@unh.edu

Many bacterial genomes like *Burkholderia* are composed of multiple chromosomes, in which one is primary, larger and harbors more essential genes, and others are secondary, smaller, and contain more variable genes. Studies of multi-chromosome bacteria have revealed that secondary chromosomes are replicated later in the cell cycle, are expressed less, and evolve more rapidly. However, the extent to which this rapid evolution results from reduced purifying selection on less essential, less expressed genes or as an inherent feature of the mutational process affecting secondary chromosomes has yet to be addressed experimentally. We conducted a mutation accumulation experiment using daily single-colony bottlenecks to collect and enumerate mutations in the near absence of natural selection using three bacteria with multiple chromosomes: *Burkholderia cenocepacia*, *Vibrio cholerae*, and *Vibrio fischeri*. After more than 5,000 generations of evolution, genome sequencing of 50 lines from each species enabled us to calculate and compare mutation rates between chromosomes and between regions within the same chromosomes. In *Vibrio*, mutation rates on secondary chromosomes were higher than those on primary chromosomes, which suggests that delayed replication timing has the important consequence of increasing the mutation rate. However, in *Burkholderia* the mutation rate was greater on chromosome 1, and more surprisingly, mutations were biased towards G+C bases, which has never been reported. Thus, selection appears to actively shape *Burkholderia* genomes to purify the primary chromosome of variation and enrich G+C content even beyond the mutation bias, and yet tolerate more variation in late-replicated, weakly expressed genome locations. This dynamic affects genes near the terminus of primary chromosomes and especially on secondary chromosomes, and contributes to these regions becoming evolutionary test beds enriched for variation within and among species. More broadly, the methods developed for this project enable low-cost, high-throughput, and accurate genome-wide genotyping portable for a wide range of microevolutionary studies.
17. FROM GENOME TO PHENOME AND BACK: UNDERSTANDING THE HIGH METABOLIC VERSATILITY OF BURKHOLDERIA CEPIACIA COMPLEX


1Department of Biology, University of Florence, Italy
2Laboratory of Microbiology, Dept. Biochemistry and Microbiology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium
3Department of Sciences for Agriculture and Food Production and Environment, University of Florence, Italy
4Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy
5Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy
6ENEA, Casaccia Res. Ctr., Via Anguillarese 301, 00123 Roma, Italy

Contact: elena.perrin@unifi.it, renato.fani@unifi.it

Strains of the Burkholderia cepacia complex (Bcc) are able to colonize many different environments; they can have a free-living lifestyle, but they may also colonize multicellular eukaryotes intracellularly and, although they are considered highly beneficial in the environment, they can also cause life-threatening infections in immuno-compromised and Cystic Fibrosis (CF) patients. This heterogeneous lifestyle and the consequent high metabolic versatility is accompanied by unusually large genomes (7.5-8.5 Mb, with a GC content of approximately 67% and divided in multiple replicons), suggesting that particular genome structures and genetic content may support and explain in evolutionary terms such high metabolic diversity.

Then, the purpose of this work was to provide a model framework of relationships between genomes and phenotypic diversity in the 18 Bcc type strains, through a multi-level, systems biology approach. The genome sequences of these 18 strains were obtained and their assembly revealed that sizes vary between 6.23 and 9.72 Mb. In addition a Pulse Field Gel Electrophoresis analysis was performed, confirming the presence of multiple replicons in each strain. Further analysis on the sequences obtained allowed the identification of peculiar patterns as concerning, on one hand, genes involved in pathogenesis, virulence and antibiotics resistance and on the other hand genes involved in plants growth promotion, nitrogen fixation and degradation of toxic agents.

Large scale phenotypic characterization was also performed on these strains, adopting the Phenotype MicroArray (PM) technique. The ability of these strains to grow using different sources of carbon, nitrogen, sulfur and phosphorus and also to grow in the presence of different pH, osmolytes and toxic compounds was tested. In addition the M.I.C. of different classes of antibiotics were determined. All those data were then used to perform an analysis of relationships between genome data and phenome results with the software suite DuctApe, to provide a first model of genome-metabolic description and differentiation of Bcc strains.
18. DEVELOPMENT OF GENETIC TOOLS AND GENOME SEQUENCING OF BURKHOLDERIA CONTAMINANS ISOLATES

Ma. Agustina López De Volder¹, Ruhi Bloodworth², Carrie Selin², José Degrossi¹ and Silvia T. Cardona²,³

¹Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
²Department of Microbiology, University of Manitoba, Winnipeg, Canada
³Department of Medical Microbiology & Infectious Disease, University of Manitoba, Winnipeg, Canada

Contact: Silvia.Cardona@umanitoba.ca

Burkholderia contaminans is a new species of the B. cepacia complex (Bcc). It can be found in natural environments, as contaminant of industrial products and as opportunistic pathogen infecting the lung of cystic fibrosis (CF) patients. Although B. contaminans is the prevalent species in CF patients in Argentina, knowledge about its mechanism of pathogenesis is very limited when compared with other Bcc members like B. cenocepacia or B. multivorans, which are common in North America and Europe.

In this work, we describe phenotypic characteristics of Argentinian isolates, determine the feasibility of applying genetic tools developed for B. cenocepacia and sequenced the genomes of four B. contaminans isolates.

While most of B. contaminans isolates presented filament morphology, similar to B. anthina, some isolates exhibited short rod morphology, similar to B. cenocepacia. Filament morphology correlated with the presence of yellow-green pigment and 50% of the strains of B. contaminans produced β-Haemolisis. Standard transposon mutagenesis techniques were successful using the antibiotic marker trimethoprim, and gentamicin or ampicillin to select against donor and helper strains.

The genomes of the type strain LMG23361T and three clinical isolates were sequenced with an Illumina MiSeq sequencer. Read assembly was performed with Velvet and the contigs were preliminarily annotated with the Rast server (http://rast.nmpdr.org/). Seven genes used in the Burkholderia cepacia complex Multilocus Sequence Typing (MLST) database (http://pubmlst.org/bcc/) were retrieved from the Rast server and compared against those deposited in the MLST database. This analysis confirmed that the sequenced genomes indeed corresponded to B. contaminans. However, all Argentinian isolates formed a new sequence type. Further analysis of the draft genomes indicated no evidence of the genomic island BcenGI11, which is related to virulence in B. cenocepacia J2315. To increase contigs length and produce a new assembly, more genome sequencing is performed with PacBio technology.

In summary, we are developing the research tools that will allow a better understanding of this emerging opportunistic pathogen prevalent among Argentinian cystic fibrosis patients.
19. NEW DRUGS AND NEW TARGETS TO FIGHT BURKHOLDERIA CENOCEPACIA

Silvia Buroni¹, Viola C. Scoffone¹, Francesca Spadaro¹, Vadim Makarov², and Giovanna Riccardi¹

¹Dipartimento di Biologia e Biotecnologie, Università degli Studi di Pavia, Pavia, Italy; ²Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia.

Contact: Silvia Buroni, silvia.buroni@unipv.it

Due to prolonged antibiotic therapies resistance has emerged among Burkholderia cenocepacia clinical isolates and the current therapy is aimed at reducing the bacterial load in cystic fibrosis (CF) patients rather than eradicate it. Consequently, finding new drugs, as well as novel targets, is fundamental for both improvement of CF patient life expectancy and fighting infections caused by drug-resistant strains. In this perspective, two different strategies can be pursued:

A) FROM DRUG TO TARGET

The 2-thiopyridine derivative 1-oxido-5-(trifluoromethyl)pyridin-2-yl thiocyanate (11026103) has been synthesized and shown to be active against B. cenocepacia. Preliminary in vivo results suggest that the former is not toxic. We identified a mechanism of resistance, which relies on the extrusion of 11026103 drug by RND-4 efflux transporter. As our results indicated that RND-4 made a significant contribution to the antibiotic resistance of B. cenocepacia, we are also performing heterologous expression trials of this transporter for structural studies and drug design.

We plan to evaluate the in vivo efficacy of these molecules, as well as to synthesize new and more active compounds.

B) FROM TARGET TO DRUG

Quorum sensing (QS) is an intercellular communication process, based on the synthesis and secretion of signal molecules, which control the expression of target genes involved in virulence. Presently, QS components are considered one of the most promising drug targets. We expressed and purified B. cenocepacia QS synthases: CepI, CciI, and BCAM0581 and we are setting up an enzymatic assay for the screening of new QS inhibitors to be used as new antivirulence compounds.

Our studies aim at addressing the lack of therapeutic solutions through drug discovery, determining mechanisms of action, and proposing new targets.
20. PHENOTYPIC CHANGES OF BURKHOLDERIA CONTAMINANS ASSOCIATED TO BIOCIDES RESISTANCE

Agustina López de Volder¹, Daniela Figoli¹, Verónica Pioli¹, Fabricio Rugnone², Sergio Teves¹,² and José Degrossi¹.

¹ Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Buenos Aires, Argentina.  
² Laboratorio Proanalisis S.A. Buenos Aires, Argentina.

Contact: José Degrossi, jdegross@ffyb.uba.ar

The innate resistance to biocides is one of the key factors in the role of the Burkholderia cepacia complex (Bcc) as problematic contaminants of hospital settings, industrial processes and mass consumption products. In addition to the risk for Public Health that it poses, adaptive tolerance to preservatives linked to changes in antibiotics resistance and other virulence factors was recently described in some Bcc species (1, 2).

In this work we studied the influence of growth conditions on biocides resistance and phenotypical changes associated to adaptive tolerance of Burkholderia contaminans, the most frequent Bcc species isolated in Argentina.

Resistance profiles to different biocides were compared by diffusion agar test performed on triptic soy agar and minimal medium, in aerobic and microaerophilic conditions.

Three B. contaminans strains (LMG 23361T, FFI6 isolated from purified water and FFi18 recovered from a pharmaceutical product) were used to study adaptive tolerance towards chlorhexidine, benzalconium chloride and a commercial mixture of methylisothiazolinone/chlormethylisothiazolinone (Kathon CG®).

All strains developed resistance to the biocides used in this study. Adapted strains increased four to ten folds the tolerance to biocides, being FFI6 the one with highest tolerance levels.

Influence of growth conditions and phenotypic changes associated to adaptive tolerance were similar in all tested strains. However important variations among biocides were observed. For instance, growth on minimal medium increased the resistance to benzalconium chloride of all strains and only chlorhexidine adapted strains didn’t produce the green yellow pigment observed in the parental and other adapted strains. Virulence in Drosophila melanogaster model was higher in most adapted strains than the observed with wild-type strains, and antibiotics resistance profiles were modified in all adapted cells.

This study confirms the resistance and the ability to develop tolerance to biocides of B. contaminans, a frequent contaminant but little studied Bcc member. Results suggest that mechanisms involved in the resistance to biocides are influenced by growth conditions. The increases in virulence of adapted strains reveal the potential clinical impact that could be associated to the biocide resistance.

References


In contrast to the more common mevalonate pathway, some bacteria use an alternative pathway for isoprenoid biosynthesis, the DOXP or nonmevalonate pathway. Since this pathway is not present in human cells, it is possibly a novel target for the development of antibacterial chemotherapy. The rate-limiting step in this pathway is the conversion of DOXP to MEP, catalyzed by the enzyme DXR. Previous studies confirmed that DXR inhibitors such as fosmidomycin and FR900098 can be used for the treatment of malaria parasite *Plasmodium falciparum*, but there is a paucity of research investigating the nonmevalonate pathway in bacteria. The first purpose of this research is to evaluate the biological efficacy of new components, mainly fosmidomycin derivatives, in state-of-the-art *in vitro* bacterial model systems. These susceptibility tests are performed on planktonic cells of several Gram-negative and Gram-positive bacteria including *Burkholderia cenocepacia*, *Burkholderia cepacia* and *Burkholderia multivorans*. All fosmidomycin derivatives tested show MIC values \( \geq 250 \, \mu M \) on planktonic cells of each strain, in each examined condition. Further experiments will be carried out later to investigate the importance of efflux, cell penetration, intracellular activation, etc. on the *in vitro* and *in vivo* activity of fosmidomycin derivatives. The second purpose is to perform strategic basic research on the nonmevalonate pathway in *Burkholderia cenocepacia*. Therefore, the expression kinetics and the essentiality of the different DOXP genes are determined *in vitro* and *in vivo*, both in planktonic and in sessile cells. The third purpose is to identify different resistance mechanisms against inhibitors of the nonmevalonate pathway and to examine how to circumvent these mechanisms. Results for the second and third part of this investigation are not obtained yet and will be described later.
22. MINING BURKHOLDERIA GENOMES FOR NOVEL ANTIBIOTICS

Eshwar Mahenthiralingam,1* Lijang Song,2 Simon Harris,3 Paul Coupland,3 Matthew Dunn,3 Cerith Jones,1 Matthew Moore,1 Julian Parkhill,3 and Gregory L. Challis2

1 Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Main Building, Park Place, Cardiff, UK
Wales CF10 3AT, UK
2 Department of Chemistry, University of Warwick, Coventry, West Midlands, England CV4 7AL, UK
3 The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Cambridge CB10 1SA, UK

*Corresponding author: MahenthiralingamE@cardiff.ac.uk

Burkholderia bacteria have large (6-9 Mb), high GC (>67%), multireplicon genomes that encode a massive variety of metabolic and catabolic functions. As biotechnological agents they have been used for bioremediation, biological control and the commercial production of enzymes such as lipases. Secretion of a range of antimicrobials plays a major role in the ability of Burkholderia to kill other soil microorganisms and protect plants from attack by a range of pathogens. Although multiple Burkholderia antibiotics have been chemically characterized, their full potential as a novel source of antimicrobials has not been systematically evaluated. Next generation sequencing technologies has provided an unprecedented ability to acquire genome-scale DNA sequencing data. As a result, genome-mining microorganisms for biosynthetic pathways likely to be involved in natural product biosynthesis has become a rapid and cost-effective means to identify novel antimicrobials. AntiSMASH is a bioinformatic pipeline for the identification of gene clusters encoding secondary metabolites of multiple chemical classes. An in silico survey of secondary metabolite gene clusters in 30 Burkholderia genomes (29 from www.burkholderia.com and 1 novel genome), 26 Pseudomonas genomes (from www.pseudomonas.com) and 14 Streptomyces genomes (from GenBank) was performed. Streptomyces devoted 14.1% of their genomic capacity to secondary metabolite production, correlating with their existing use as one of the major sources of clinically relevant antibiotics. Burkholderia were just below Streptomyces, with 10.2% of their genome encoding secondary metabolite biosynthesis genes, while only 5% of the Pseudomonas genome was associated with these functions. The mean number of secondary metabolite biosynthetic gene clusters ranked as follows: Streptomyces = 32 > Burkholderia = 21 > Pseudomonas = 7. Moreover, Burkholderia contained several large gene clusters (50 to 100+ Kb) encoding multiple modular polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) assembly lines, equivalent to those found in Streptomyces. As part of this antiSMASH survey, we included a novel draft Burkholderia gladioli genome for a cystic fibrosis isolate, BCC0238, which had been determined using Pacific Biosciences (PacBio) Single Molecule Real-Time sequencing. Assembly of the long and short reads from a single PacBio run produced an 8.6 Mb, 10 contig, draft genome; both of the B. gladioli BCC0238 large chromosomal replicons (> 4Mb each) were intact within this sequence. In summary, Burkholderia encode a wealth of antimicrobial secondary metabolite gene clusters and their correlation to known and novel antibiotics will be described.
The discovery of antibiotics has witnessed dramatic control of bacterial and microbial pathogens. However, resistant bacterial strains have emerged and have spread throughout the world because of the remarkable genetic plasticity of the microorganisms and the mobility of the world population. The situation is complicated by the fact that in the recent years no new class of antibiotics was discovered. In this context it is worth of noticing that medicinal plants, spice and Essential Oils (EOs) bearing plants possess different pharmacological activities. In particular, the EOs inhibition of a wide range of microorganisms has been described and has been screened worldwide as potential sources of novel antimicrobial compounds. Additionally, EOs are a combinatorial drug, consisting of a complex blend of active molecules, which target many cellular components simultaneously, reducing the probability to develop resistance with respect to common single-molecule antibiotics. Particularly interesting, from this viewpoint, is the possibility to treat infections of Cystic Fibrosis (CF) patients. Among the CF pathogens the *Burkholderia cepacia* complex (Bcc) members cause serious respiratory infections difficult to eradicate due to their intrinsic resistance to most clinically available antibiotics. In this work we have checked the ability of EOs extracted from six different medicinal plants (*Eugenia caryophyllata*, *Origanum vulgare*, *Rosmarinus officinalis*, *Lavandula officinalis*, *Melaleuca alternifolia* and *Thymus vulgaris*) to inhibit the growth of 80 strains belonging to the 18 known Bcc species either of clinical or environmental origin. Data obtained with the disk diffusion tests showed that all the Bcc strains exhibited, although at a different extent, a high degree of sensitivity to each of the six EOs tested. The most effective EOs were those extracted from *Origanum vulgare*, *Thymus vulgaris* and *Eugenia caryophyllata*. Very interestingly, these three EOs gave an inhibitory halo much larger than that produced by ciprofloxacin (one of the antibiotics used in CF infections therapy). The determination of Minimum Inhibitory Concentration revealed that the three above mentioned EOs had a bactericidal activity also when diluted up to 0.125% (vol/vol) on almost all strains tested. Moreover, no mutant strain Bcc resistant to the six EOs was detected in our large-scale screening.

In conclusion, we show that EOs from medicinal plants deserve a special attention as a source for new antibacterial formulations to be used against CF bacterial pathogens.
24. FOSMIDOMYCIN POTENTIATES THE EFFECTS OF COLISTIN ON BURKHOLDERIA MULTIVORANS CLINICAL ISOLATES

Rebecca J. Malott¹, Chia-Hung Wu², James E. A. Zlosnik¹, Dianne K. Newman², and David P. Speert¹

¹Centre for Understanding and Preventing Infection in Children, Department of Pediatrics, University of British Columbia, Vancouver, Canada
²Division of Biology, California Institute of Technology, Pasadena, USA

Contact: Rebecca Malott, rebecca.malott@gmail.com

Burkholderia cepacia complex (Bcc) pulmonary infections in people living with cystic fibrosis (CF) are difficult to treat due to the extreme intrinsic resistance of most isolates to a broad range of antimicrobials. Fosmidomycin is an antibacterial and antiparasitic agent that disrupts the isoprenoid biosynthesis pathway, a precursor to hopanoid biosynthesis. Membrane hopanoids are involved in membrane stability and contribute to polymyxin resistance in Bcc bacteria. Although Bcc species are highly resistant to monotherapy with either fosmidomycin or polymyxins, we explored the hypothesis that inhibition of isoprenoid biosynthesis by fosmidomycin would potentiate the inhibitory effects of polymyxins on \textit{B. multivorans} growth. Checkerboard minimum inhibitory concentration (MIC) assays determined that isolates of our chosen Bcc model species, \textit{B. multivorans}, were highly resistant to treatment with fosmidomycin or colistin (polymyxin E) alone, but antimicrobial synergy was observed in certain clinical isolates when the agents were used in combination. MICs for colistin of RAPD type-matched early and late \textit{B. multivorans} respiratory isolates from ten CF patients were evaluated by broth microdilution. Two of the late isolates had colistin MICs of 64 µg/ml, whereas the remainder of the isolates had colistin MICs of >512 µg/ml. Treatment with 256 µg/ml of fosmidomycin further decreased the colistin MIC of the two more susceptible isolates to as low as 8 µg/ml, a concentration achievable with colistin inhalation therapy. A different late clinical isolate with a colistin MIC of >512 µg/ml was rendered at least 64-fold more susceptible to colistin by the addition of 256 µg/ml of fosmidomycin, indicating that even isolates with extreme colistin resistance can become more susceptible to colistin with the addition of fosmidomycin. Efforts are ongoing to determine the molecular mechanism of this exciting synergy.

VI. Antimicrobial resistance, novel drugs and disinfectants
Identification of *Burkholderia* spp. in the sputum of Cystic Fibrosis (CF) patients is associated with a rapid decline of lung function and poor outcome. The most severe cases concern patients that succumbed rapidly to a pneumonic illness with high temperatures and respiratory failure: the Cepacia Syndrome. One of the major problems associated with *Burkholderia* spp. infections is their intrinsic resistance to most of the clinically available antibiotics, including aminoglycosides, quinolones, polymyxins, and β-lactams. Identification and characterization of new products having the potential to kill *Burkholderia* spp. and other multi-resistant bacteria that colonizes the lung of CF patients are needed. Alaxia aims to address this unmet medical need by developing a first-in-class hypothiocyanite (OSCN⁻) and bovine lactoferrin (bLF) combination therapy.

ALX-009 is a fixed combination of OSCN⁻ and bLF for the management of lung infections, including *Burkholderia* spp., in CF patients. OSCN⁻ is a highly reactive chemical species with a proven broad microbiological action that attacks the free thiols of bacterial proteins. In contrast, bLF is generally considered as a bacteriostatic compound mainly because of its iron chelating properties, leading to deprivation of the iron ions necessary for bacterial growth. MIC values were obtained according to the CLSI guidelines with slight modifications. Our results corroborate the bactericidal capability of OSCN⁻ and the isolate-specific bactericide activity of bLF. We also prove the interest to combine both compounds. Indeed, the MIC ranges for OSCN are of 25-150µg/ml with no naturally resistant strain identified so far. bLF alone shows bactericidal activity only for 12% of the tested clinical isolates and MIC values ranged for 0.25 to 32mg/ml. Additionally, preliminary data analysis demonstrated that the interaction between these two compounds have FIC index varying from ≥ 0.1 to ≤ 1.0 thus indicating a synergistic/additive effect. Several combinations having the potential to kill over 96% of the clinical isolates tested were identified. In conclusion, the data obtained by Alaxia over ca. 120 clinical isolates of Burkholderia cepacia complex and other *Burkholderia* spp. demonstrates the high bactericidal potential of the OSCN⁻/bLF combination. Today selected combinations are being tested in other *in vitro* experiments to identify the best associations being transferred to the clinic.
26. DEVELOPING A HIGH THROUGHPUT CHEMOGENOMIC APPROACH FOR PROFILING BIOACTIVES AGAINST *BURKHOLDERIA CENOCEPACIA*

April S. Gislason¹, Ruhi A. M. Bloodworth¹, Wubin Qu², Xuan Li³, Chenggang Zhang² and Silvia T. Cardona¹

¹University of Manitoba Department of Microbiology, Winnipeg, MB R3T 2N2
²Beijing Institute of Radiation Medicine, State Key Laboratory of Proteomics, Cognitive and Mental Health Research Center, Beijing 100850, China
³University of Minnesota Duluth, Department of Mathematics and Statistics, 140 Solon Campus Center, 1117 University Drive, Duluth MN 55812-3000

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

Development of novel antibiotics against multi-drug resistant Gram-negative bacteria, like *Burkholderia cenocepacia*, is crucial in order to contend with the pervasive evolution of antibiotic resistance. Whole cell screens have identified small molecules which inhibit the growth of *B. cenocepacia*, but the absence of understanding their mechanism of action prevents their further development. Previously, we developed a chemogenomic approach to match antibiotics to their respective targets using a library of *B. cenocepacia* K56-2 conditional growth (CG) mutants. In this enhanced sensitivity assay (ESA), depleting the expression of an essential protein in a CG mutant by manipulating a rhamnose inducible promoter specifically sensitizes the CG mutant to antibiotics that target the depleted essential product, resulting in a severe growth defect. However, the ESA is time consuming, labour intensive and impractical to use as screen for finding antibiotic targets.

We are developing the ESA into a high throughput screen where the growth defect of the CG mutants will be quantified using next generation sequencing. The ESA will be advanced into a high throughput screen by pooling and coculturing strains, followed by detection through multiplexed sequencing. To achieve this, primers were designed to amplify the transposon-genome interface of CG mutants in one multiplex reaction. Indices were then added to the amplicons in a second PCR so each pool could be analyzed *en masse* in one sequencing run.

We have been able to produce balanced amplification for 25 of the 56 CG mutants originally tested and detected a synthetic ten-fold depletion of each mutant, demonstrating that the multiplexed sequencing is able to detect and quantify the relative abundances of these CG mutant strains. Preliminary results confirm that using these 25 CG mutants, the high throughput ESA is able to detect the enhanced sensitivity of the gyrB mutant (>10-fold depletion compared to the no antibiotic control) in response to treatment with sublethal concentrations of novobiocin, an antibiotic which specifically targets GyrB.

The high throughput ESA is being further developed to profile antibiotics against the CG mutants and for the identification of novel gene target-bioactive matches in *B. cenocepacia* K56-2.
27. CHARACTERIZATION OF GROWTH INHIBITORS OF BURKHOLDERIA CENOCEPACIA K56-2 AND GENERATION OF AN HDTM LIBRARY.

Carrie Selin¹ and Silvia T. Cardona¹,²

¹Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada
²Department of Medical Microbiology and Infectious Disease, University of Manitoba, Winnipeg, Manitoba, Canada

Contact: Silvia T. Cardona, Email: Silvia.Cardona@umanitoba.ca

Infections with Burkholderia cepacia complex (Bcc) bacteria are difficult to eradicate in cystic fibrosis patients due to the intrinsic resistance of Bcc to most of the available antibiotics. Since the products of essential genes are targets of the most effective antibiotics, identification of novel essential genes holds promise for novel antibiotic therapies. Deep sequencing of high-density transposon mutant (HDTM) libraries can be effective at identifying the essential genome of a given microorganism. The chemogenomic approach can take it one step further, where the HDTM is utilized to determine the fitness contribution of each gene in the presence of an antibacterial drug or bioactive molecule. The relative abundance of each mutant in the population can then be quantified by NGS. With this in mind, we decided to identify potential new bioactive compounds that inhibit the growth of B. cenocepacia K56-2 by utilizing a high throughput screening (HTS) of 30,259 compounds from the Canadian Chemical Biology Network Compound Collection (CCC). The whole-cell based HTS revealed 87 Bce bioactives, which were then prioritized according to potency, chemical properties and structure clustering. This prioritization highlighted 41 Bce bioactives of interest. We chose 11 compounds from this prioritized group that clustered independently from one another. These compounds were subject to an MIC analysis (CLSI guidelines), a toxicity screen and an in vivo antibacterial activity assay using C. elegans as the host model. Our pipeline of in vitro and in vivo analysis selected two compounds, MAC-151023 and MAC-0036650, both of low toxicity and in vitro and in vivo antibacterial activities. MAC-151023 belongs to the Maybridge Library of synthetic small molecules and is a quinolino-benzothiazine. A search by structure against the PubChem database returned the compound CID 659101. Based on the Bioactivity database, this compound was active in 155 screens out of more than 600 screens performed, suggesting that MAC-151023 is a very promiscuous drug. MAC-0036650, a novel phenyl-thiazol-butenamide, from the Maybridge Library was one of the 20 most potent compounds in the primary screen. A search of MAC-0036650 by structure in the PubChem database returned the compound CID 5605429 and the bioactivity database shows that this compound was active in 6 screens out of 61.

The small Bce bioactives identified in the HTS can be used in combination with the HDTM libraries to find mutants that may show sensitivity to these molecules, which will allow us to determine the gene target of this molecule. To this end, I am generating a high-density transposon mutant library of 2-million Tn-mutants in B. cenocepacia K56-2. The sequencing-ready library will be sequenced on an Illumina flow cell and the sequence reads will be first selected based on 100% identity with the transposon end, and then mapped to the genome of K56-2 using MAQ software. The precise insertion sites will be identified and the number and frequency of insertions per position will be counted and normalized by dividing the number of insertion sites by gene length. Statistical analysis of essentiality will be performed according to a bimodal distribution.

In summary our research will address gene essentiality in B. cenocepacia K56-2 at the genomic level and characterize Bce bioactives and gene targets as a starting point in mode of action determination, as well as the contribution of each gene to intrinsic mechanisms of resistance.
The leaves of several members of the genera *Psychotria*, *Pavetta* and *Sericanthe* display small structures called leaf galls or nodules that house *Burkholderia* spp. bacteria. The bacteria are transmitted hereditarily through the seeds, and cannot be acquired from the environment. Very little is known about the biology of the symbiosis, except that the bacteria are essential for plant development: Bacteria-free seeds germinate, but the resulting aposymbiotic plants appear severely stunted and eventually wither and die. Leaf nodule symbiosis has often been described as obligate, since the bacteria cannot be cultured and aposymbiotic plants do not reproduce. Exactly what the bacteria do for the plant is unknown.

We have recently studied the genome of *Candidatus* Burkholderia kirkii, the leaf symbiont of *Psychotria kirkii*. Most functions encoded by the small genome of *B. kirkii* are also found in endophytic *Burkholderia* species that are not typically associated with *Rubiaceae*. Genes unique to *B. kirkii*, presumably specific to that species' lifestyle, are found on a plasmid and possibly code for the synthesis of secondary metabolites of the C7N aminocyclitol family. High throughput expression analysis confirmed that a large share of the metabolism of *B. kirkii* is directed towards the synthesis of secondary metabolites, with enzymes of the secondary metabolism among the most abundant proteins in *P. kirkii* leaf nodules. We were able to recover and chemically characterize C7N aminocyclitol molecules from the leaves of *P. kirkii*. Finally, we could show that the putative genes for C7N aminocyclitol biosynthesis are highly conserved among 8 species of *Psychotria* and *Pavetta* symbionts.

We propose that secondary metabolism plays a central role in the leaf nodule symbiosis, and that the *Psychotria/Burkholderia* symbiosis may be the first described example of a vertically-transmitted protective symbiosis in plants.
29. PLANT-INDUCED GENE EXPRESSION IN AN ENDOPHYTE BELONGING TO THE GROUP OF PLANT BENEFICIAL BURKHOLDERIA

Bruna G. Coutinho and Vittorio Venturi

Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, AREA Science Park, Padriciano 99, 34149, Trieste, Italy.

Contact: Bruna G. Coutinho, coutinho@icgeb.org

The genus *Burkholderia* is composed of functionally diverse species, and it can be divided into several clusters. One of these, designated the plant-beneficial-environmental (PBE) *Burkholderia* cluster, is formed by non-pathogenic species, which in most cases have been found to be associated with plants. Recently we sequenced the genome of *Burkholderia kururiensis* M130, which is one of the few characterized rice endophytes showing strong growth-promoting effects and increasing rice yields. The genome sequencing allowed us to identify several genes related to plant growth promotion, including the *accD* gene encoding 1-aminocyclopropane-1-carboxylate deaminase, genes for the production of indole-3-acetic acid and the *nif* gene cluster. However, very little is known on how endophytes enter and colonize plants. For this reason, we analysed the changes in gene expression occurring during the interaction with the plant by identifying genes of *B. kururiensis* M130 that are differentially regulated in the presence of rice plant extract. This was achieved via a more traditional screen of a transposon mutant genome bank and also by the more recent wide-genome scale RNAseq approach. The results indicate that the endophyte undergoes a dramatic change in its gene expression profile in the presence of rice plant extract with the up- or downregulation of 31.1% of its protein coding genes by more than 2-fold. Interestingly, a great number of differentially expressed genes encode membrane transporters and secretion systems, which indicates that the exchange of molecules is a major factor in this environment. Genes related to mobility and chemotaxis were also over-represented which suggests recognition and an intimate interaction between bacteria and plant. This work stresses the close signalling taking place between plants and bacteria and helps us understand the great adaptation an endophyte undergoes to survive and live inside plants.
30. ADAPTATION OF BURKHOLDERIA TOWARD SYMBIOSIS WITH MIMOSEAE

Agnieszka Klonowska, Caroline Burnaud, Rémy Melkonian, Gilles Béna, Damien Mornico, Pierre Tisseyre, Clémence Chaintreuil, Karine Heulin, Gisèle Laguerre and Lionel Moulin
Laboratoire des Symbioses Tropicales et Méditerranéennes, IRD, UMR 113, Montpellier, France

Contact : Agnieszka Klonowska, agnieszka.klonowska@ird.fr

Rhizobia represent a polyphyletic group of bacteria able to develop a nitrogen fixing symbiosis with legume plants. Since the discovery of symbiotic species among Burkholderia in 2001 (Moulin et al., 2001), β-rhizobia (rhizobia from the beta-subclass of Proteobacteria) have attracted particular interest as uncommon rhizobia spread in all tropical regions mainly associated with Mimosa, a large genus whose major center of diversification is in Brazil. For last 5 years our team devoted its attention to the study of the β-rhizobia adaptation toward nitrogen fixing symbiosis using phylogenetic, physiologic, genetic and transcriptomic approaches. Our study of the diversity of Burkholderia associated with Mimosa pudica in several tropical countries showed that symbionts distribution can be linked to geographic localisation and soil properties (Mishra et al., 2012; Klonowska et al., 2012). It can also be linked to the variations of Burkholderia competitiveness toward different M. pudica ecotypes as suggested the study of symbiotic traits of β-rhizobia (Melkonian et al., 2013). In order to describe the symbiotic process developed by Burkholderia we initiated the transcriptomic study of early symbiotic stage and active symbiosis of B. phyramid. This study showed complex response of B. phyramid specific response to the plant root exudates showing the up-regulation of many genes related to plant rhizosphere (motility, degradation of plant components) and to plant host (molecular dialog, synthesis of phytotoxins, secretion systems). Finally, we investigated if legume genera phylogenetically close to Mimosa (i.e. the “Piptadenia group” within the Mimoseae tribe) could be also associated with β-rhizobia. Results showed that Burkholderia are deep rooted within the Mimoseae tribe, being found in nodules of numerous Piptadenia and Anadenantera species (Bournaud et al., 2013).

Reference
31. EXOPOLYSACCHARIDES IN BIOFILMS OF *BURKHOLDERIA CENOCEPACIA* AND *BURKHOLDERIA MULTIVORANS*

Ambra Delneri¹, Claudia Buriola¹, Mustafa Fazli², Tim Tolker-Nielsen², Roberto Rizzo¹ and Paola Cescutti¹

¹Dipartimento di Scienze della Vita, Università degli Studi di Trieste, via L. Giorgieri 1, Ed. C11, 34127 Trieste
²Costerton Biofilm Center, Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Contact : Paola Cescutti, pcescutti@units.it

*B. cenocepacia* and *B. multivorans* are two of the species constituting the *Burkholderia cepacia* Complex (BCC), a group of phenotypically very similar micro-organisms capable of infecting cystic fibrosis (CF) patients, sometimes with lethal outcome. All eighteen species that currently make up the complex can cause CF infection, with *B. cenocepacia* being the most predominant (50-80%), followed by *B. multivorans* (9-37%). The remaining species generally cause less than 10% of infection cases [1]. Mucoid as well as non-mucoid strains are isolated from CF patients and the relationship between mucoidicity and virulence still needs to be clarified. Although the presence of biofilm in the lungs has been established only for *P. aeruginosa* [2], most likely also species of the BCC forms biofilms in the host. Exopolysaccharides (EPOLs) are considered important components of the biofilm matrix, but little is known on the type of EPOLs produced in biofilms; most of the literature data derives either from genetic studies or from polysaccharides produced in non-biofilm mode.

In our laboratory we are determining the types of EPOLs produced by *B. cenocepacia* strains BTS2 and H111 and *B. multivorans* C1576 in biofilms. For *B. cenocepacia* BTS2 and *B. multivorans* C1576 Yeast Extract Mannitol (YEM) and Müller Hinton (MH) media were used; the former stimulates EPOLs biosynthesis and the latter is generally used in microbiological tests. In order to obtain enough matrix for structural analysis, biofilms were formed on cellulose membranes deposited on agar plates and/or on glass slides. Structural data on EPOLs were then obtained mainly by NMR spectroscopy and were compared with those of known polysaccharides.

In the case of *B. cenocepacia* H111, the interest was directed towards the identification of EPOLs produced by a gene cluster described as essential for biofilm formation [3]. Such gene cluster is transcriptionally regulated by the protein Bcam1349 which is also c-di-GMP responsive [4]. We compared biofilms formed by H111 WT with those of a strain overexpressing the bcam1349 gene from the multicopy number plasmid pBcam1349 in WT (WT+pBcam1349) and of the mutant MF255 (bcal1389 mutant strain (no cellulose) overexpressing pBcam1349). For matrix isolation and purification we resorted to the production of pellicles. The WT strain produced a small amount of pellicle compared to the other two strains. NMR spectroscopy showed a complex anomic region suggesting the presence of more than one EPOL as well as signals belonging to proteins and other non saccharidic material. Sugar composition analysis revealed rhamnose and mannose as main monosaccharides, accompanied by smaller quantities of glucose.

BIOFILM FORMATION BY CYSTIC FIBROSIS-PATHOGENIC BURKHOLDERIA CEPACIA COMPLEX (BCC) BACTERIA ALLOWS THEM TO EVADE NEUTROPHIL ANTI-MICROBIAL ACTIVITIES

Mark Murphy¹,², Maire Callaghan¹,² and Emma Caraher¹,²

¹Centre for Microbial-Host Interactions and ²Centre of Applied Science for Health, Institute of Technology Tallaght, Dublin, Ireland

Contact: Mark Murphy, mark.murphy@postgrad.ittdublin.ie

Colonisation of the airway of people with cystic fibrosis (CF) by Burkholderia cepacia complex bacteria occurs despite the continuous recruitment of neutrophils. As such, we have investigated whether residence in biofilms contributes to the inability of neutrophils to eradicate Bcc bacteria from the airway.

Using the neutrophil-like, differentiated cell line, dHL60, we have shown that Bcc biofilm biomass increased in the presence of these cells, likely the result of incorporating dHL60 cellular debris such as DNA. Using flow cytometry, we have shown that mature biofilms (72 hr) induced necrosis in the cells which would in turn facilitate this increase. We have demonstrated through confocal microscopy that mature biofilms can act as a barrier to the migration of the cells, which would limit their anti-microbial efficacy. Additionally, dHL60 cells displayed a reduced ability to recognise biofilm-dwelling Bcc bacteria; the cells displayed significantly less migration toward biofilms than toward planktonic bacteria in transwell migration assays (P<0.05) and secreted significantly less IL-8 when cultured in the presence of biofilms, with respect to planktonic bacteria (P<0.001). Reduced expression of IL-8 mRNA under the same conditions was confirmed by qRT-PCR.

Hence, biofilm formation may contribute to the persistence of Bcc bacteria in the CF airway, by reducing recognition of the bacteria and acting as a barrier, while the presence of neutrophils reinforces that biofilm. These findings suggest that disrupting or preventing biofilm formation can improve the efficacy of the neutrophil-mediated anti-microbial response to Bcc bacteria.

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33. DEVELOPMENT OF A BIOSENSOR FOR INVESTIGATING THE BDSF QUORUM SENSING SYSTEM

Angela Suppiger¹, Claudio Aguilar¹ and Leo Eberl¹

¹Department of Microbiology, University of Zurich, Zurich, Switzerland

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

*Burkholderia cenocepacia* is able to use two different chemical languages for inducing gene expression in a cell density-dependent manner: *N*-acyl-homoserine lactones (AHLs) and *cis*-2-dodecenoic acid (BDSF). Recent work has shown that changes in the intracellular c-di-GMP level are controlled by BDSF and the phosphodiesterase activity of RpfR (BDSF receptor), which in turn leads to differential expression of target genes. In addition it was found that the *B. cenocepacia* BDSF- and AHL-dependent quorum sensing (QS) systems regulate specific as well as overlapping sets of genes. However, how the changes in c-di-GMP levels are translated into altered gene expression is currently unknown. Interestingly, a bioinformatic analysis revealed that homologs of *rpfF*<sub>Bc</sub> (BDSF synthase) and *rpfR* are conserved in a wide range of bacterial species, including *Burkholderia*, *Achromobacter*, *Yersinia*, *Serratia*, *Enterobacter* and *Cronobacter*. Here, we describe a biosensor that can be used to monitor the production of BDSF signal molecules. To this end, a sensor plasmid was engineered in which the promoter region of a BDSF-regulated gene was placed in front of a promoterless reporter gene. This construct was introduced into a *B. cenocepacia* *rpfF* mutant background. The response of the biosensor was characterized for a range of BDSF concentrations. Our results show that the biosensor is highly sensitive, responding to concentrations as low as 10 nM of synthetic BDSF. We also tested several bacterial strains for BDSF production using either spent supernatants or cross-streak experiments and showed that production of BDSF is particularly common among members of the Bcc. We anticipate that this biosensor can be of interest for researchers working on BDSF signaling.
34 INVOLVEMENT OF TOXIN ANTITOXIN-MODULES IN *BURKHOLDERIA CENOCEPACIA* BIOFILM PERSISTENCE

Heleen Van Acker, Andrea Sass, Hans J. Nelis & Tom Coenye

Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

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

Biofilms are involved in the recalcitrance of infections due to the presence of persister cells. Although the molecular basis of persistence is still largely unknown, type II toxin antitoxin (TA)-modules are thought to play a role. Those TA-modules consist of a toxin, which can inhibit an important cellular target and an antitoxin which can form a complex with the toxin thereby inactivating it. In the present study, we investigated whether TA-modules contribute to persistence in *B. cenocepacia* J2315.

Using bioinformatics tools, 17 pairs of genes were identified as possible TA-modules. Overexpression of the different toxins had various effects on growth, persistence and biofilm formation. Toxins of which the overexpression resulted in growth inhibition, had a positive influence on the number of surviving cells, while toxins of which the overexpression did not have influence on growth, had no or a negative influence on the persister fraction. Furthermore, the expression of the TA-modules was compared between treated and untreated sessile and planktonic WT cultures. For 10 toxin encoding genes, the expression was higher in untreated sessile cells than in untreated planktonic cells. Nine toxin encoding genes were upregulated after treatment with tobramycin, but none after treatment with ciprofloxacin. These results indicate that most, but not all TA-modules contribute to persistence in *B. cenocepacia* J2315 and that this contribution depends on the mode of growth and the antibiotic used.

Interestingly, one of these TA-modules is located in the *Burkholderia cepacia* epidemic strain marker region. This toxin was identified as a global regulator influencing quorum sensing and activating cellular pathways involved in persistence, iron acquisition, virulence and biofilm formation. These results may help explain why infections with strains of the *B. cenocepacia* ET12 lineage are so difficult to treat.
35. THE ATTENUATED VIRULENCE OF A BURKHOLDERIA CENOCEPACIA PaaABCDE MUTANT IS DUE TO INHIBITION OF QUORUM SENSING BY RELEASE OF PHENYLACETIC ACID

Tanya Pribytkova¹, Steve P. Bernier⁴, John L. Sorensen³, Michael G. Surette⁴,⁵, and Silvia T. Cardona¹,².

¹Department of Microbiology, University of Manitoba, Winnipeg, Canada
²Department of Medical Microbiology & Infectious Disease, University of Manitoba, Winnipeg, Canada
³Department of Chemistry, University of Manitoba, Winnipeg, Canada
⁴Department of Medicine, Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada
⁵Department of Biochemistry and Biological Sciences, McMaster University, Hamilton, Ontario, Canada

Contact: Silvia.Cardona@umanitoba.ca

Although metabolically versatile Burkholderia cepacia complex (Bcc) are well known pathogens of cystic fibrosis patients, how Bcc metabolism affect interactions with other bacteria and the host is poorly understood. The phenylacetic acid (PAA) degradation pathway of Burkholderia cenocepacia is active in cystic fibrosis-like conditions and mutants of the PAA degradation pathway are attenuated for virulence in rat and nematode models of infection. Recent findings that exogenously added or endogenously produced PAA inhibits quorum sensing (QS) related pathogenicity in Pseudomonas aeruginosa, prompted us to investigate if the mechanism of attenuation in B. cenocepacia PAA degradation mutants was related to QS inhibition.

We first demonstrate that in conditions that allow infection in Caenorhabditis elegans, a B. cenocepacia mutant of the phenylacetyl-CoA monooxygenase complex (PaaABCDE) releases PAA. Addition of PAA further decreased the pathogenicity of the paaABCDE mutant, which cannot metabolize PAA, but did not affect the one of the wild type (WT), due to PAA consumption. Mutant spent medium or exogenously added PAA to a biosensor system, led to a reduced detection of acyl-homoserine lactone (AHL). According to PAA-related QS inhibition, the reduced protease activity of the paaABCDE mutant and the pathogenicity in C. elegans, increased with the addition of AHL.

Taken together, we demonstrate that the attenuated phenotype of B. cenocepacia paaABCDE mutant is due to QS inhibition. Further, we provide a biological explanation for the activation of the PAA degradation pathway of B. cenocepacia during cystic fibrosis conditions and propose that the PAA metabolism of Bcc bacteria can stimulate P. aeruginosa virulence during Burkholderia-Pseudomonas interactions.
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<thead>
<tr>
<th>Surname</th>
<th>First Name</th>
<th>Affiliation</th>
<th>City</th>
<th>Country</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amer</td>
<td>Amal</td>
<td>Ohio State University</td>
<td>Columbus, Ohio</td>
<td>USA</td>
<td><a href="mailto:Amal.amer@osumc.edu">Amal.amer@osumc.edu</a></td>
</tr>
<tr>
<td>Bechetoille</td>
<td>Camille</td>
<td>Stragene France-Alaxia</td>
<td>Lyon</td>
<td>France</td>
<td><a href="mailto:camille.bechetoille@bioalaxia.eu">camille.bechetoille@bioalaxia.eu</a></td>
</tr>
<tr>
<td>Brown</td>
<td>Alan</td>
<td>Biosciences, University of Exeter</td>
<td>Exeter</td>
<td>United Kingdom</td>
<td><a href="mailto:a.r.brown@exeter.ac.uk">a.r.brown@exeter.ac.uk</a></td>
</tr>
<tr>
<td>Burns</td>
<td>Jane</td>
<td>Seattle Children’s Research Institute</td>
<td>Seattle</td>
<td>USA</td>
<td><a href="mailto:jane.burns@seattlechildrens.org">jane.burns@seattlechildrens.org</a></td>
</tr>
<tr>
<td>Buroni</td>
<td>Silvia</td>
<td>Dip. Biologia e Biotecnologie-University of Pavia</td>
<td>Pavia</td>
<td>Italy</td>
<td><a href="mailto:silvia.buroni@unipv.it">silvia.buroni@unipv.it</a></td>
</tr>
<tr>
<td>Cardona</td>
<td>Silvia</td>
<td>University of Manitoba</td>
<td>Winnipeg</td>
<td>Canada</td>
<td><a href="mailto:Silvia.Cardona@umanitoba.ca">Silvia.Cardona@umanitoba.ca</a></td>
</tr>
<tr>
<td>Carlier</td>
<td>Aurélien</td>
<td>Institute of Plant Biology, University of Zurich</td>
<td>Zurich</td>
<td>Switzerland</td>
<td><a href="mailto:aurelien.carlier@access.uzh.ch">aurelien.carlier@access.uzh.ch</a></td>
</tr>
<tr>
<td>Cescutti</td>
<td>Paola</td>
<td>Dept. Life Sciences, Univ. of Trieste</td>
<td>Trieste</td>
<td>Italy</td>
<td><a href="mailto:rizzor@units.it">rizzor@units.it</a></td>
</tr>
<tr>
<td>Coenye</td>
<td>Tom</td>
<td>Ghent University</td>
<td>Gent</td>
<td>Belgium</td>
<td><a href="mailto:tom.coenye@ugent.be">tom.coenye@ugent.be</a></td>
</tr>
<tr>
<td>Cooper</td>
<td>Vaughn</td>
<td>University of New Hampshire</td>
<td>Durham, NH</td>
<td>USA</td>
<td><a href="mailto:vaughn.cooper@unh.edu">vaughn.cooper@unh.edu</a></td>
</tr>
<tr>
<td>de Bentzmann</td>
<td>Sophie</td>
<td>UMR7255- CNRS - Aix Marseille Université</td>
<td>Marseille</td>
<td>France</td>
<td><a href="mailto:bentzman@imm.cnrs.fr">bentzman@imm.cnrs.fr</a></td>
</tr>
<tr>
<td>Degrossi</td>
<td>José</td>
<td>Universidad de Buenos Aires</td>
<td>Buenos Aires</td>
<td>Argentina</td>
<td><a href="mailto:jdegross@ffyb.uba.ar">jdegross@ffyb.uba.ar</a></td>
</tr>
<tr>
<td>Drevinek</td>
<td>Pavel</td>
<td>2nd Faculty of Medicine, Charles University</td>
<td>Prague</td>
<td>Czech Republic</td>
<td><a href="mailto:paveldrevinek@Lfmotol.cuni.cz">paveldrevinek@Lfmotol.cuni.cz</a></td>
</tr>
<tr>
<td>Eberl</td>
<td>Leo</td>
<td>Institute of Plant Biology, University of Zurich</td>
<td>Zurich</td>
<td>Switzerland</td>
<td><a href="mailto:leberl@botinst.uzh.ch">leberl@botinst.uzh.ch</a></td>
</tr>
<tr>
<td>Everaert</td>
<td>Annelien</td>
<td>Ghent University</td>
<td>Ghent</td>
<td>Belgium</td>
<td><a href="mailto:annelien.everaert@ugent.be">annelien.everaert@ugent.be</a></td>
</tr>
<tr>
<td>Fialho</td>
<td>Arsenio</td>
<td>Institute for Biotechnology and Bioengineering, Instituto Superior Técnico</td>
<td>Lisbon</td>
<td>Portugal</td>
<td><a href="mailto:dalilamil-homens@ist.utl.pt">dalilamil-homens@ist.utl.pt</a></td>
</tr>
<tr>
<td>Gislason</td>
<td>April</td>
<td>University of Manitoba</td>
<td>Winnipeg</td>
<td>Canada</td>
<td><a href="mailto:gislasonapril@gmail.com">gislasonapril@gmail.com</a></td>
</tr>
<tr>
<td>Gomes</td>
<td>Margarida</td>
<td>INSERM U1047, University of Montpellier 1</td>
<td>Nimes</td>
<td>France</td>
<td><a href="mailto:margarida.gomes@ist.utl.pt">margarida.gomes@ist.utl.pt</a></td>
</tr>
<tr>
<td>Gonçalves</td>
<td>Bruna</td>
<td>ICGB</td>
<td>Trieste</td>
<td>Italy</td>
<td><a href="mailto:coutinho@icgeb.org">coutinho@icgeb.org</a></td>
</tr>
<tr>
<td>Jones</td>
<td>Cerith</td>
<td>Cardiff University</td>
<td>Cardiff</td>
<td>UK</td>
<td><a href="mailto:JonesC146@cardiff.ac.uk">JonesC146@cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Juarez Perez</td>
<td>Victor</td>
<td>Stragene France - Alaxia</td>
<td>Lyon</td>
<td>France</td>
<td><a href="mailto:v.juarez@stragene.fr">v.juarez@stragene.fr</a></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
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<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalferstová</td>
<td>Lucie</td>
<td>Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University</td>
<td>Prague</td>
<td>Czech Republic</td>
<td><a href="mailto:lucie.kalferstova@lfmotol.cuni.cz">lucie.kalferstova@lfmotol.cuni.cz</a></td>
</tr>
<tr>
<td>Klonowska</td>
<td>Agnieszka</td>
<td>IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes</td>
<td>Montpellier</td>
<td>France</td>
<td><a href="mailto:agnieszka.klonowska@ird.fr">agnieszka.klonowska@ird.fr</a></td>
</tr>
<tr>
<td>LiPuma</td>
<td>John</td>
<td>University of Michigan</td>
<td>Ann Arbor, MI</td>
<td>USA</td>
<td><a href="mailto:jlipuma@umich.edu">jlipuma@umich.edu</a></td>
</tr>
<tr>
<td>Mahenthiralingam</td>
<td>Eshwar</td>
<td>Cardiff University</td>
<td>Cardiff</td>
<td>United Kingdom</td>
<td><a href="mailto:MahenthiralingamE@cardiff.ac.uk">MahenthiralingamE@cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Malott</td>
<td>Rebecca</td>
<td>CUPIC, University of British Columbia</td>
<td>Toronto</td>
<td>Canada</td>
<td><a href="mailto:rebecca.malott@gmail.com">rebecca.malott@gmail.com</a></td>
</tr>
<tr>
<td>Mathew</td>
<td>Anugraha</td>
<td>Institute of Plant Biology, University of Zurich</td>
<td>Zurich</td>
<td>Switzerland</td>
<td><a href="mailto:a.mathew@access.uzh.ch">a.mathew@access.uzh.ch</a></td>
</tr>
<tr>
<td>McClean</td>
<td>Siobhán</td>
<td>Centre of Microbial Host Interactions, Institute of Technology Dublin</td>
<td>Dublin</td>
<td>Ireland</td>
<td><a href="mailto:siobhan.mcclean@ittdublin.ie">siobhan.mcclean@ittdublin.ie</a></td>
</tr>
<tr>
<td>McDonald</td>
<td>Allison</td>
<td>University of British Columbia</td>
<td>Vancouver</td>
<td>Canada</td>
<td><a href="mailto:allimcdo@gmail.com">allimcdo@gmail.com</a></td>
</tr>
<tr>
<td>Mesureur</td>
<td>Jennifer</td>
<td>INSERM U1047, University of Montpellier 1</td>
<td>Nimes</td>
<td>France</td>
<td><a href="mailto:jennifer.mesureur@univ-montp1.fr">jennifer.mesureur@univ-montp1.fr</a></td>
</tr>
<tr>
<td>Mil-Homens</td>
<td>Dalila</td>
<td>Institute for Biotechnology and Bioengineering</td>
<td>Lisbon</td>
<td>Portugal</td>
<td><a href="mailto:dalilamil-homens@ist.utl.pt">dalilamil-homens@ist.utl.pt</a></td>
</tr>
<tr>
<td>Moreira</td>
<td>Leonilde</td>
<td>Instituto Superior Técnico-Department of Bioengineering</td>
<td>Lisbon</td>
<td>Portugal</td>
<td><a href="mailto:lmoreira@tecnico.ulisboa.pt">lmoreira@tecnico.ulisboa.pt</a></td>
</tr>
<tr>
<td>Murphy</td>
<td>Mark</td>
<td>Institute of Technology Tallaght</td>
<td>Dublin</td>
<td>Ireland</td>
<td><a href="mailto:mark.murphy@postgrad.ittdublin.ie">mark.murphy@postgrad.ittdublin.ie</a></td>
</tr>
<tr>
<td>O’Callaghan</td>
<td>David</td>
<td>INSERM U1074, University of Montpellier 1</td>
<td>Nimes</td>
<td>France</td>
<td><a href="mailto:david.ocallaghan@univ-montp1.fr">david.ocallaghan@univ-montp1.fr</a></td>
</tr>
<tr>
<td>Peeters</td>
<td>Charlotte</td>
<td>LM-UGent, Ghent University</td>
<td>Gent</td>
<td>Belgium</td>
<td><a href="mailto:charlotte.peeters@ugent.be">charlotte.peeters@ugent.be</a></td>
</tr>
<tr>
<td>Perrin</td>
<td>Elena</td>
<td>Department of Biology, University of Florence (FI)</td>
<td>Sesto fiorentino</td>
<td>Italy</td>
<td><a href="mailto:elena.perrin@unifi.it">elena.perrin@unifi.it</a></td>
</tr>
<tr>
<td>Pessi</td>
<td>Gabriella</td>
<td>Institute of Plant Biology, University of Zurich</td>
<td>Zurich</td>
<td>Switzerland</td>
<td><a href="mailto:gabriella.pessi@botinst.uzh.ch">gabriella.pessi@botinst.uzh.ch</a></td>
</tr>
<tr>
<td>Rizzo</td>
<td>Roberto</td>
<td>Dept. Life Sciences, Univ. of Trieste</td>
<td>Trieste</td>
<td>Italy</td>
<td><a href="mailto:rizzor@units.it">rizzor@units.it</a></td>
</tr>
<tr>
<td>Rodrigues Feliciano</td>
<td>Joana Rita</td>
<td>INSERM U1047/Instituto Superior Técnico</td>
<td>Nîmes, Lisbon</td>
<td>France, Portugal</td>
<td><a href="mailto:joana.feliciano@ist.utl.pt">joana.feliciano@ist.utl.pt</a></td>
</tr>
<tr>
<td>Sass</td>
<td>Andrea</td>
<td>Ghent University</td>
<td>Gent</td>
<td>Belgium</td>
<td><a href="mailto:andrea.sass@ugent.be">andrea.sass@ugent.be</a></td>
</tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Segonds</td>
<td>Christine</td>
<td>Observatoire Cepacia, laboratoire de Bactériologie</td>
<td>Toulouse</td>
<td>France</td>
<td><a href="mailto:segonds.c@chu-toulouse.fr">segonds.c@chu-toulouse.fr</a></td>
</tr>
<tr>
<td>Selin</td>
<td>Carrie</td>
<td>University of Manitoba</td>
<td>Winnipeg</td>
<td>Canada</td>
<td><a href="mailto:umselinc@cc.umanitoba.ca">umselinc@cc.umanitoba.ca</a></td>
</tr>
<tr>
<td>Silva</td>
<td>Inês</td>
<td>Institute for Biotechnology and Bioengineering</td>
<td>Lisbon</td>
<td>Portugal</td>
<td><a href="mailto:ines.silva@tecnico.ulisboa.pt">ines.silva@tecnico.ulisboa.pt</a></td>
</tr>
<tr>
<td>Sonmez</td>
<td>Yasmine</td>
<td>Alaxia</td>
<td>Lyon</td>
<td>France</td>
<td><a href="mailto:yasmine.sonmez@bioalaxia.eu">yasmine.sonmez@bioalaxia.eu</a></td>
</tr>
<tr>
<td>Speert</td>
<td>David</td>
<td>UBC</td>
<td>Vancouver</td>
<td>Canada</td>
<td><a href="mailto:dspeert@cfri.ubc.ca">dspeert@cfri.ubc.ca</a></td>
</tr>
<tr>
<td>Spiewak</td>
<td>Helena</td>
<td>The University of Sheffield</td>
<td>Sheffield</td>
<td>United Kingdom</td>
<td><a href="mailto:h.spiewak@sheffield.ac.uk">h.spiewak@sheffield.ac.uk</a></td>
</tr>
<tr>
<td>Suppiger</td>
<td>Angela</td>
<td>Institute of Plant Biology, University of Zurich</td>
<td>Zurich</td>
<td>Switzerland</td>
<td><a href="mailto:a.suppiger@access.uzh.ch">a.suppiger@access.uzh.ch</a></td>
</tr>
<tr>
<td>Thomas</td>
<td>Mark</td>
<td>University of Sheffield</td>
<td>Sheffield</td>
<td>United Kingdom</td>
<td><a href="mailto:m.s.thomas@shef.ac.uk">m.s.thomas@shef.ac.uk</a></td>
</tr>
<tr>
<td>Valvano</td>
<td>Miguel</td>
<td>Queen's University Belfast</td>
<td>Belfast</td>
<td>United Kingdom</td>
<td><a href="mailto:m.valvano@qub.ac.uk">m.valvano@qub.ac.uk</a></td>
</tr>
<tr>
<td>Vandamme</td>
<td>Peter</td>
<td>LM-UGent, Ghent University</td>
<td>Gent</td>
<td>Belgium</td>
<td><a href="mailto:peter.vandamme@ugent.be">peter.vandamme@ugent.be</a></td>
</tr>
<tr>
<td>Venturi</td>
<td>Vittorio</td>
<td>ICGEB</td>
<td>Trieste</td>
<td>Italy</td>
<td><a href="mailto:venturi@icgeb.org">venturi@icgeb.org</a></td>
</tr>
<tr>
<td>Vergunst</td>
<td>Annette</td>
<td>INSERM U1047, University of Montpellier 1</td>
<td>Nîmes</td>
<td>France</td>
<td><a href="mailto:annette.vergunst@univ-montp1.fr">annette.vergunst@univ-montp1.fr</a></td>
</tr>
<tr>
<td>Zhang</td>
<td>Lili</td>
<td>INSERM U1047, University of Montpellier 1</td>
<td>Nîmes</td>
<td>France</td>
<td><a href="mailto:lili.zhang@inserm.fr">lili.zhang@inserm.fr</a></td>
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