

International *Burkholderia cepacia* Working Group Sixth Annual Meeting

ABSTRACTS

KEYNOTE LECTURE

Is Soil Home to *Burkholderia cepacia* ?

James M. Tiedje

Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, USA

This question has two related components: (1) what is proper identification of *Burkholderia cepacia*, and (2) what is the evidence of soil as a suitable home for members of this species cluster. The former question has both scientific and practical components. With regard to the latter, a problem arises from the rather casual identification of a great many soil isolates as *B. cepacia* because of common rather than distinctive traits. This gives those working with the environment the impression that *B. cepacia* is among the most common bacteria in the world, and that each of us encounters thousands per day. Hence, their answer to the title question would be: of course. The scientific question is what is the backbone gene composition that defines this species, how stable is the structure and expression, how much horizontal exchange occurs and how does pathogenicity correlate with the population structure of the group. Assuming we can (at some stage?) properly define what is *B. cepacia*, then do we know soil is a home? This is not an easy question for microbes because it can be difficult to distinguish just visiting and truly living in a sustaining manner, i.e. successfully competing and reproducing over many generations. Soil is also made up of a continuum of many subenvironments such as rhizosphere; decaying plant, animal, insect parts of many chemistries; different pH, moisture, redox, salt and nutrient conditions; different mineral and organic matrices and physical stabilities of these. Because of this, soil can provide almost any niche, and due also due to a very high degree of spatial isolation, even weak competitors can be sustained for very long times. Current evidence would suggest that soil is a great home to at least a number of subgroups within the *B. cepacia* superfamily. The primary evidence is the easy isolation of such strains in soils of all types worldwide. It is time however, for more specific information on this question and with new methodologies.

Detection Of The *Burkholderia Cepacia* Complex In Soil From Urban And Suburban Environments

Suzanne M. Miller¹, Jennifer L. Parke¹, Shane Bies², and **John J. LiPuma**²

¹Department of Crop and Soil Science, Oregon State University, Corvallis, OR and ²Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI U.S.A.

B. cepacia complex (Bcc) bacteria reside in soil, plant rhizospheres, and water, but the prevalence and distribution of Bcc in outdoor environments is not clear. In this study, we sampled a variety of soil and rhizosphere environments with which people may have contact: playgrounds, athletic fields, parks, hiking trails, residential yards and gardens. A total of 91 soil samples was obtained from three large U.S. cities (Philadelphia, PA, Cleveland, OH, and Portland, OR), two of which have a relatively high prevalence of *B. cepacia* colonization among CF patients. In the first phase of the study, putative Bcc isolates were recovered on *Burkholderia cepacia* selective agar (BCSA) and trypan blue tetracycline medium (TBT) and subsequently examined for biochemical reactivity and growth at 32°C and 22°C. Isolates were further examined by polymerase chain reaction (PCR) assays targeting both rRNA and *recA* gene sequences, and genotyped by using RAPD, PFGE and rep-PCR. A total

of 1013 bacterial isolates was examined, and 68 were identified as *B. cepacia* complex (Bcc). The majority of these were genomovar I/*B. pyrrocinia* or genomovar VII (*B. ambifaria*); however, a few genomovar III isolates were also recovered. Among the 68 isolates, genotyping revealed at least 28 distinct strains. Fourteen (15%) of 91 soil samples yielded Bcc isolates. In the second phase of the study, DNA was extracted from 87 of the 91 soil samples and examined with PCR assays targeting Bcc 16S rRNA gene sequences. In spiked soil samples, the detection limits of these assays ranged from 10^4 to 10^5 cfu/g soil dry weight. By using assays developed by LiPuma et al., 82% of the soil samples were positive for at least one Bcc genomovar; whereas, 94% of samples were positive for at least one Bcc species using the Bauernfeind et al. assay system. Assays with a primer pair targeting genomovars I-VII resulted in amplification of DNA from 82% of the soil samples. Amplicons generated from four soil samples were cloned into *E. coli*, and plasmids from multiple transformants (total = 120) were screened by RFLP analysis using the restriction enzyme *Sau96I*. Among the clones evaluated from three of four soil samples, 90% or more had the “*Burkholderia*” RFLP pattern. In the remaining soil sample, only 9.5% of the evaluated clones displayed this profile. Sequence analysis of the 463bp 16S rRNA inserts from eight clones with the “*Burkholderia*” RFLP pattern indicated that all were from members of the Bcc. Four were consistent with genomovar I, three are either genomovar I or *B. multivorans*, and one clone was most consistent with genomovar III. The four soil samples from which these clones were generated did not yield isolates identified as Bcc. This study indicates that genomovar I/*B. pyrrocinia* and genomovar VII are the Bcc species most commonly recovered from environmental sites with which people may have frequent contact; genomovar III and possibly other Bcc species are also present in these sites. Based on PCR detection, Bcc appears to be prevalent in soil from urban and suburban environments. Culture-based recovery of Bcc may underestimate environmental populations.

Isolation Of Soilborne Genomovar I, III And VII *Burkholderia Cepacia* And Lytic Phages With Inter-Genomovar Host Range.

Carlos F. Gonzalez,¹ G. Louise Mark,² Eshwar Mahenthiralingam,³ and John LiPuma,⁴

1 Texas A&M University, 2 Cornell University, 3 Cardiff University, 4 University of Michigan

Burkholderia cepacia and *B. gladioli* are commonly found in highly organic soils. The objective of this study was to determine the range of *B. cepacia* complex species that could be isolated from organic (muck) soils planted to onions. Additionally, soil samples were screened for the presence of *B. cepacia* complex species-associated bacteriophages. Soil samples were collected from six different fields with a long history of planting to onions. Four of the fields were sampled several times during the growing season over a two-year period, whereas the remaining two were sampled several times during the growing season for one year. Sixteen soil samples were taken at each time period at a depth of 7.5 cm along an X transect and mixed to form a composite sample for each of the fields. The samples were processed and plated to selective medium (TBT). Individual colonies were isolated and presumptively identified as *B. cepacia* complex or *B. gladioli* based on morphology and fatty acid methyl ester profiles. Isolates (n=133) were further analyzed to determine species within the *B. cepacia* complex by using rRNA and *recA* gene-targeted PCR. Isolates were also genotyped by using PFGE and RAPD typing. Representative genomovar III isolates were tested for the presence or absence of the BCESM marker by PCR. Six of the 133 presumptive isolates could not be identified as *B. cepacia* complex or *B. gladioli*. Among the remaining 127 isolates, 31 genomovar I, 29 genomovar III, 54 genomovar VII, and 13 probable *B. pyrrocinia* were identified. Ten of 11 genomovar III tested for the presence of the BCSEM marker were positive. To date, soil enrichment experiments have yielded five bacteriophages from genomovar III isolates and one each from genomovar VII and I isolates. Phage susceptibility testing indicates that the genomovar III bacteriophages exhibit a narrow host range. However, two lytic phages (one obtained during another study), recovered from the soil using genomovar I hosts, were also shown to plate on genomovar III and VI isolates. These broad-host range genomovar I bacteriophages were able to form a lysogenic state in a clinical genomovar III isolate (PC184). This study demonstrates that *B. cepacia* complex species co-exist in the natural environment and that bacteriophages with inter-genomovar host-range also exist in the environment. The potential for horizontal gene transfer by *B. cepacia* phages with inter-genomovar host ranges could potentially provide a mechanism for gene transfer in natural environments.

Genomics Of The *Burkholderia cepacia* Complex

¹E Mahenthalingam, ²JRW Govan, ³CA Hart, ⁴P Vandamme, ⁵J Parkhill, ⁵M. Quail, ⁵B Barrell

¹Cardiff University, Cardiff, UK; ²University of Edinburgh, Edinburgh, Scotland; ³Royal Liverpool Hospital, Liverpool University, Liverpool, UK; ⁴Universiteit Gent, Gent, Belgium; ⁵Sanger Centre, Hinxton, Cambridge, UK.

Determination of the complete genome sequence of *Pseudomonas aeruginosa* has generated novel information about its virulence and provided new strategies with which to investigate its ability to cause respiratory infection in patients with cystic fibrosis (CF). In comparison, little is known about the virulence factors associated with the problematic CF pathogen *Burkholderia cepacia*. To improve our understanding *B. cepacia* CF infection, the complete genome sequence of the *B. cepacia* genomovar III strain J2315 (deposited as strain LMG 16656 in the Belgium Co-ordinated Collections of Microorganisms) will be determined. Isolates of *B. cepacia* belong to one of seven closely related taxonomic groups (all of which cause infections in patients with CF) that have been collectively designated the *B. cepacia* complex. New species names have been designated for the following members of this complex: *B. multivorans*, *B. vietnamiensis*, *B. stabilis* and *B. ambifaria*. The remaining distinct taxonomic groups are known as *B. cepacia* genomovars I, III, and VI. A strain from *B. cepacia* genomovar III was chosen for genome sequence analysis based on the following criteria: (i) **major clinical relevance** - *B. cepacia* genomovar III dominates infection of patients with CF and is a highly virulent/transmissible group within the *B. cepacia* complex; (ii) **major epidemic strain** - the strain chosen, J2315, is part of the clonal ET12 lineage which infects a large proportion of CF patients within Canada and the UK. It was recovered from a patient with CF in Edinburgh, Scotland, and it possesses both the cable pilus gene and *B. cepacia* epidemic strain marker (BCESM) DNA; (iii) **known history** - the clinical and storage history of the strains is well documented; (iv) **genetically amenable** - this strain and other clonal strains within this lineage are amenable to genetic manipulation; (v) **preliminary virulence studies** - several investigations into the virulence and pathogenesis of strains within the ET12 lineage have been carried out; (vi) **model system** - sequence analysis of a *B. cepacia* strain provides very interesting model system of an opportunistic pathogen which possesses inherent antibiotic resistance and diverse virulence/metabolic traits; and finally, (vii) **novel challenge** - *B. cepacia* will also provide a novel challenge for sequence analysis, with the genome size of isolate J2315 being approximately 7.6 Mb and consisting of 3 large replicons which are 3.7, 3.1 and 0.8 Mb in size and multiple insertion sequences. The *B. cepacia* genome project will also compliment that of its close relative *Burkholderia pseudomallei* which is already underway at the Sanger Centre. Test sequencing of the *B. cepacia* J2315 genome has begun, with a small amount of data so far available. The project will begin full shotgun soon, and all data will be available immediately from http://www.sanger.ac.uk/Projects/B_cepacia. This project is funded by the Wellcome Trust Beowulf Genomics Initiative.

A Signature-Tagged Mutagenesis Strategy In *Burkholderia cepacia* To Identify Genes Required For *In Vivo* Growth And Survival.

Tracey A. Hunt^{1*}, Cora Kooi², Pamela A. Sokol², and Miguel A. Valvano¹.

¹Dept. of Microbiology and Immunology, University of Western Ontario, London, Ontario

²Dept. of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta

Burkholderia cepacia infections cause elevated morbidity and mortality among cystic fibrosis patients. Patients who develop an infection by this pathogen frequently experience a lethal necrotizing pneumonia termed "cepacia syndrome". The mechanisms by which *B. cepacia* causes disease remain largely unknown. We postulate that many potential virulence genes are preferentially expressed following interactions with the host and prototypical virulence factor identification studies using *in vitro* approaches may be inadequate to identify many of these important virulence factors. The adapted mutagenesis strategy of signature tagged mutagenesis (STM) can be applied to identify bacterial genes that are expressed preferentially *in vivo*. STM uses a pool of genetically engineered transposon mutants each containing a unique oligonucleotide tag incorporated onto the chromosome. While this requires many formidable molecular modifications, it allows each individual mutant to be monitored. Using this negative selection technique, strains harbouring mutations in genes essential *in vivo* will not be detected following infection and can, therefore, be putatively identified as genes required for virulence. A PCR-based STM system developed by Lehoux *et al.* (1999) improves upon the traditional hybridization procedure as it eliminates STM limitations inherent to hybridization, increases specificity during the PCR screening step, and allows for a

more rapid and simpler method of screening. We have adapted the PCR-based technique to construct our own pool of transposons containing 48 unique tags. Transposon mutagenesis was performed to generate 48 groups of *B. cepacia* transposon mutants, each distinguishable by PCR using primers unique to each tag. We have demonstrated that our tags do not cross-react, are specific, and can be detected on the *B. cepacia* chromosome by PCR. Using this modified method of STM, we propose to identify genes important for virulence of *B. cepacia* in mouse and rat models of pulmonary infections.

Identification of Putative Virulence Genes in *Burkholderia cepacia* Complex Genomovar III

Lixia Liu and John J. LiPuma

University of Michigan, Ann Arbor, MI

In order to define factors that contribute to virulence of *B. cepacia* complex (Bcc) genomovar III (GIII), we employed PCR-based subtractive hybridization to identify GIII-specific genes. Several hundred clones were generated after subtracting a pool of genomic DNA of two environmental GIII strains from the genomic DNA of clinical GIII strain AU1482. DNA sequence analysis of three AU1482 specific clones (clones 1, 2, and 3) revealed that they had significant homology to hypothetical protein TP0839 of *Treponema pallidum*, a putative transposase of *Pseudomonas* spp., and a putative virulence gene of *Yersinia enterocolitica*, respectively. The distributions of clones 1 and 2 among Bcc species were determined by dot blot hybridization assay using genomic DNA from 760 isolates recovered from clinical (n=604) and environmental (n=156) sources. Among these isolates, clone 1 hybridized to 116 (35.9 %) of 323 GIII, and 5 (1.1%) of 436 isolates representing the remaining Bcc species. Thus, 95.6% of clone 1 positive isolates were GIII. Similarly, clone 2 hybridized to 109 (33.7%) of 323 GIII, and 28 (6.3%) of 436 isolates belonging to other species. Thus, 79.6% of clone 2 positive isolates were GIII. These results indicate that the cloned genes are relatively specific to GIII isolates, being only infrequently encountered in other Bcc species. As such, they may have roles in the enhanced virulence of GIII strains. These genes are being more completely characterized in ongoing studies. Studies employing both signature-tagged mutagenesis and IVET strategies to analyze putative virulence genes are also currently underway

Functional Genomics Initiatives for CF: Putting Bacterial Genomes To Work.

Chris Penland and **Melissa Ashlock**

Cystic Fibrosis Foundation 6931 Arlington Rd. Bethesda, MD 20814

Below is an introduction to the Cystic Fibrosis Foundation's functional genomics initiative that at present, focuses on *Pseudomonas aeruginosa*. We are considering this project as a stepping stone to discovering new drug targets in CF. If successful, we would like to apply a similar strategy to the study of gene expression from the *B. cepaciae* genome.

Cystic Fibrosis Foundation Therapeutics, Inc. (CFFTI) has contracted with Affymetrix to generate GeneChip® oligonucleotide microarrays for CF-related studies of gene expression from the *Pseudomonas aeruginosa* genome. These microarrays should be available for distribution to investigators interested in CF-related problems in the first or second quarter of 2001. CFFTI is funding the design, development and production of these microarrays so that Qualified Users (users who have submitted an on-line application form and are then approved by CFFTI) can purchase these arrays directly from Affymetrix by e-mail at the substantially discounted rate of \$150 per microarray. This low price reflects the \$200 subsidy that the Cystic Fibrosis Foundation will pay Affymetrix for each microarray.

In addition, the first 25 Qualified Users will be offered a copy of the Microarray Suite software, from Affymetrix Corp., free of charge.

In exchange for these discounted chips, and software when applicable, CFFTI requires that Qualified Users submit their data to a centralized data repository within six (6) months after receiving the microarrays. To facilitate this, CFFTI is funding a Cystic Fibrosis National Bioinformatics Center at UNC-CH and is funding concurrent user licenses to GenoMax™ 3.0.2 software for enterprise bioinformatics, a product of InforMax Inc. This software

package will facilitate data analysis and mining for Qualified Users and includes sequence analysis and gene expression analysis modules. Qualified Users can use GenoMax to analyse their own data and to mine it together with the data of the other Qualified Users who have also submitted data. We anticipate that the database will provide a wealth of information for scientists interested in identifying gene expression patterns in *Pseudomonas aeruginosa*. Our expectation is that this data will be used to develop possible new drug targets for CF patients.

A proposal to Evaluate the Impact of Managed Care on Microbiology Laboratory Referrals in US CF Care Centers

Susanna A. McColley, M.D.

Children's Memorial Hospital Chicago

Background: The US Cystic Fibrosis Foundation provides accreditation and funding to CF Care Centers. In order to qualify for accreditation, centers must meet a number of personnel, facilities, and practice standards. These standards include microbiology laboratory procedures for identification of CF-related organisms. Microbiology laboratories are assessed as part of the review and site visit process for all CF care centers.

While the purpose of microbiology laboratory assessment is to assure that CF patients have access to optimal diagnostic techniques, some patients have restrictions on laboratory access due to insurance company "managed care" programs that allow testing only in contracted laboratories. Contracted laboratories are generally commercial laboratories that do not have specific protocols for evaluation of CF sputum. This puts patients at risk of underdiagnosis of infection, especially *B. cepacia*. The purpose of this study is to evaluate the impact of managed care laboratory restrictions on CF patients.

Method: A brief survey (below) will be sent to all Directors of US CF Centers. Results will be compiled to estimate the impact of managed care on microbiology practices. If appropriate, strategies to increase referral to CF Care Center microbiology labs will be developed.

Managed care and microbiology laboratory: survey

Introduction: The Cystic Fibrosis Foundation recommends specific microbiology protocols for the assessment of respiratory tract flora in CF patients. Microbiology laboratory procedures are reviewed as part of the accreditation of Cystic Fibrosis Centers. However, there is anecdotal information that an increasing number of patients are not allowed to use Center laboratories, but must be referred to commercial labs with managed care company contracts. The purpose of this survey is to estimate the impact of managed care laboratory referral practices on accurate assessment of CF organisms.

1. How many patients are currently followed in your CF Center? _____
2. Of the patients currently followed in your center, what percentage (approximately) are **not** allowed to have respiratory cultures processed in the CF Center microbiology laboratory during outpatient visits?
_____ %

Please complete the remainder of the survey **ONLY** if you answered 2% or more to #2.

3. Approximately how many different outside laboratories are used for specimens?
 - a. 1-2
 - b. 3-5
 - c. more than 5
4. For patients who must use outside microbiology laboratories, do you request an exception from the managed care company?
 - a. yes, always
 - b. no, never
 - c. sometimes
5. If you request exceptions, how often is the request granted?
approximately _____ % of requests
6. To the best of your knowledge, does the outside laboratory used **MOST FREQUENTLY** use the CFF-recommended protocol for ascertainment of CF related organisms such as *B. cepacia*?

- a. yes
- b. no
- c. don't know

Thank you for participating in this survey!

B. CEPACIA: IS IT AN EMERGING PATHOGEN ONLY IN CYSTIC FIBROSIS PATIENTS?

S. Campana, G. Bernini *, S. Audino, G. Taccetti.

Department of Pediatrics, Cystic Fibrosis Center, Meyer Hospital, Florence, Italy. *UO Oncoematologia, Meyer Hospital, Florence, Italy.

B. cepacia has emerged as a major pathogen in patients with cystic fibrosis (CF) and can be associated with a fatal pneumonia. Severe *B. cepacia* infections in immunocompromized hosts, particularly in the hospital setting, have occasionally been described.

Six strains identified as *B. cepacia* with commercial methods were isolated from 170 patients attending the CF Center of Florence (43% of patients were colonized by *P. aeruginosa*, 80% by *S. aureus* and 3.5% by *B. cepacia*).

For epidemiologic purposes all *B. cepacia* strains were analyzed with pulsed field gel electrophoresis (PFGE). The genome fingerprintings of the isolates were different except for 2 strains that showed a high degree of similarity.

In August 1999 an outbreak of *B. cepacia* in the oncologic ward of the same hospital occurred. 8 patients (mean age 8.3 years) with cancer (3 acute lymphoblastic leukemia, 2 rhabdomyosarcoma, 1 Ewing's sarcoma, 1 neuroblastoma, 1 neuroectodermal tumor) undergoing chemotherapy by central venous catheter, developed clinical signs suggesting systemic infection.

All patients experienced septic-type fever and hematological tests documented neutropenia in two of them. One patient showed a thrombosis visible by ultrasound at the apex of central venous catheter. Bacterial cultures indicated the isolation of *B. cepacia* in the blood of all patients. Preliminary PFGE analysis showed that three strains had the same genomic profile, allowing us to hypothesize a single source of infection.

In the CF center cross colonization among patients and contamination from environmental sources do not appear to be a widespread problem. The genomic profiles of *B. cepacia* strains isolated from CF and oncologic patients were different. Yet ward to ward spread of highly virulent *B. cepacia* strains within the same hospital must be taken into account.

Extrapulmonary Complications with *B. cepacia* after Lung Transplantation.

Chaparro C.^{1,2}, Gutierrez C.^{1,2}, Hutcheon M.^{1,2}, Chan C.^{1,2}, Tullis E.^{2,3}, Humar A.^{1,2}, Keshavjee S.^{1,2}

1. Toronto General Hospital, UHN, Toronto, ON, Canada; 2. University of Toronto, Toronto, ON, Canada; 3. The Adult Cystic Fibrosis Program, St Michael's Hospital, Toronto, Ontario, Canada.

Toronto General Hospital 200b Elizabeth Street Eaton North 10-237, Toronto, ON M5G 2C4

Lung transplantation has been demonstrated to be the best treatment for end stage lung disease due to cystic Fibrosis. Unfortunately patients infected with *B. Cepacia* face higher risk of dying do to infection during the first months post-transplantation. Extrapulmonary infections have been found to be part of infections early post-transplant.

This is a retrospective review of *B. cepacia* infected CF lung transplant recipients with extrapulmonary complications. We recorded type of infection, time of presentation after transplantation, treatment and outcome. All CF patients are started in antibiotic therapy immediately post-transplant and were continued on it for at least three to four weeks. Immunosuppression included cyclosporine, azathioprine and corticosteroids.

Between March 1988 and May 2000 we have transplanted 84, 42 of them colonized with *B. cepacia*.

Extrapulmonary complications with *B. cepacia* occurred in 4 patients: 3 male, 1 female (median age: 36, range 31-42). The complications were wound infection with sternal osteomyelitis (3), mastoiditis (1) and prostate abscesses (2). Two of the patients had initially sternal infection followed by the prostate abscess. The median time to infection was 4.1 months (range 1.5-9m). All received treatment with antibiotics that included cephalosporines, aminoglycosides and some chloramphenicol and TMP-Sulfa combined with open drainage of the wound, removal of the sternal wires, and radical mastoidectomy in the patient with mastoiditis. Drainage of the prostate abscess was

performed in two opportunities in one patient. The other one rejected the procedure. The only survivor was the patient with mastoiditis. The deaths occurred 2, 8 and 10 months after the diagnosis despite repeated procedures and antibiotic treatment. Lung infection did not occur in 2 of the patients while having abscesses in other places.

Conclusion: *B. cepacia* extra-pulmonary infections occurred frequently in pre-transplant infected lung recipients and were lethal. Modification in antibiotic and immunosuppression protocols may have favored the appearance of the infections in these locations. Further research in the area is needed.

Cable/Adhesin Positive *Burkholderia Cepacia* Adhere To And Invade Squamous Differentiated Bronchial Epithelial Cell Cultures

Uma Sajjan, Cameron Ackerley and Janet Forstner

Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

Previously we demonstrated that a highly transmissible and potentially virulent genomovar III strain of *B. cepacia* expresses cable pili and an associated 22 kDa adhesin (Cbl/Adh +ve). Adherence of this strain to epithelial cells is mediated by the adhesin, and the major epithelial receptor is cytokeratin 13 (CK 13). CK 13 is expressed abundantly throughout the (chronically damaged) airways of patients with CF, whereas in control lungs CK13 is expressed only in trachea. Lung sections from CF patients colonized with Cbl/Adh +ve *B. cepacia* showed bacteria in epithelia of small bronchioles and in the adjacent parenchyma by immunolocalization, indicating that this strain is invasive. We have now investigated whether binding of Cbl/Adh +ve bacteria to CK 13 of squamous cells enhances the potential of the bacteria to invade and/or damage the epithelium. Bronchial epithelial cells were grown at an air/liquid interface in the presence or absence of retinoic acid to obtain mucocilliary (respiratory) or squamous differentiated cultures, respectively. Squamous, but not respiratory, epithelia expressed abundant CK 13 on the surface. Both types of cultures were exposed to *B. cepacia* for 2h, washed, and the number of bacteria bound to the cultures, their cytotoxicity (LDH release) and pro-inflammatory activity were measured. Invasion of bacteria was determined after 24 h of incubation of cultures containing bound bacteria. Both Cbl/Adh +ve and -ve *B. cepacia* showed minimal binding to mucocilliary differentiated cultures, did not invade the cultures, cause cytotoxicity, or stimulate pro-inflammatory reactions. In contrast, Cbl/Adh +ve, but not Cbl/Adh -ve, *B. cepacia* showed strong binding to squamous cultures, caused cytotoxicity and stimulated the release of IL-8. Immunolocalization of *B. cepacia* and CK 13 showed bacteria to be associated mainly with CK 13-expressing cells. After 24 h of incubation, Cbl/Adh +ve bacteria were recovered from the basolateral chamber, indicating that bacteria had migrated through the multilayered squamous culture. Histological evaluation revealed moderate to severe epithelial damage in proportion to the density of colonized bacteria. By transmission electron microscopy, bacteria were seen at the surface of cells, with some bacteria surrounded by cellular, pseudopod-like protrusions. After 24 h, bacteria were found within and between squamous cells. The intracellular bacteria were in endocytic vacuoles and some were free in the cytoplasm but surrounded by bundles of intermediate (cytokeratin-containing) filaments. These findings imply that once Cbl/Adh +ve *B. cepacia* bind to squamous-type airway epithelia (enriched in CK 13), they can colonize, damage or invade the cells, or move by a paracellular route through the epithelial barrier to reach deeper parenchymal tissues. These observations help to account for the invasiveness, and possibly virulence, of the Cbl/Adh +ve strain of *B. cepacia* in patients with cystic fibrosis.

Quorum sensing systems of *Burkholderia ambifaria* BcF and *B. multivorans* 17616.

H. Zhou, F. Yao, P. Knittle and T.G. Lessie.

We have used PCR probes homologous to the cepI/R region of the genomovar III isolate strain K56-2 to amplify the corresponding bafI and bafR genes of the maize rhizosphere isolate *B. ambifaria* BcF. Strain BcF produces high levels of AHL compared to strain K56-2 and other clinical isolates we have examined. Comparison of the nucleotide sequences of the baf and cep genes and flanking DNA failed to provide any insight into the basis for the differences in amounts of AHL produced. The overall nucleotide sequence of the bafI/R region was 90% identical to that of cepI/R. We have constructed derivatives of strain BcF in which bafI or bafR have been insertionally inactivated. These strains produced only trace amounts of AHL and failed to exhibit exoprotease or anti-yeast activity. Analysis of AHL-deficient strains obtained by transposon mutagenesis revealed that in the

majority of mutants the transposon had inserted into sites outside the *bafI/R* region suggesting the existence of other global regulatory genes governing AHL synthesis. In collaboration with Dr. Dan Roberts at the USDA Plant Protection Lab in Beltsville, MD, we are comparing the ability of wild type BcF and its AHL-negative derivatives to colonize maize rhizosphere. We also are examining the quorum sensing system of *B. multivorans* 17616, a strain that produces only trace amounts of AHL, but which gives rise to variants that produces large amounts of AHL. Our working hypothesis is that the dramatic increase in AHL production by the mutant strains might be due to activation of a cryptic *bmul* gene specifying an autoinducer synthase.

a2-Macroglobulin-Stimulated Secretion Of Cytotoxic Factors Promoting Phagocytic Cell Apoptosis By Clinical Isolates Of *Burkholderia cepacia*

Vasu Punj, Olga Zaborina and **A.M.Chakrabarty**

Dept. of Microbiology and Immunology, University of Illinois

College of Medicine, 835 S. Wolcott Avenue, Chicago, IL 60612, USA

We recently reported (Melnikov et al., Mol. Microbiol. 36, 1481-1493, 2000) that clinical isolates of *Burkholderia cepacia* secrete ATP-utilizing enzymes in their growth medium that appear to activate purinergic receptors on the surface of macrophages and mast cells, leading to their cell death. The secretion of these enzymes, and the consequent phagocytic cell death, was found to be reduced in some environmental isolates. We further demonstrated that secretion of the ATP-utilizing enzymes by strain 38, isolated from a cystic fibrosis (CF) patient, was greatly stimulated in presence of a2-macroglobulin. Fractionation of the growth medium of another CF isolate strain 71 (CEP509) demonstrated not only the presence of ATP-utilizing enzymes that triggered phagocytic cell death in an ATP-inducible manner, but another fraction consisting of two redox proteins that triggered phagocytic cell death through induction of apoptosis. The secretion of ATP-utilizing enzymes and redox proteins by strain 71 was also stimulated in presence of a2-macroglobulin. No such stimulation of secretion by a2-macroglobulin was observed in case of several environmental isolates. Immunoelectron microscopy revealed the presence of receptors for binding a2-macroglobulin on the cell surface of 4 clinical isolates tested but not on the surface of 4 environmental isolates. It remains to be seen if the presence of receptors for a2-macroglobulin might be one way to differentiate clinical isolates of *B. cepacia* from environmental ones, and if the presence of such receptors may correlate with the virulence of the bacteria.

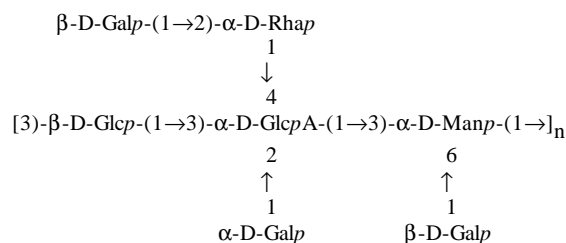
Structural Investigation Of Exopolysaccharides Produced By Different Clinical Isolates Of *Burkholderia cepacia*

Paola Cescutti¹, Silvia Skerlavaj¹, Paola Sist¹, Ranieri Urbani¹, Cristina Lagatolla², Enrico Tonin², Roberto Rizzo¹

¹Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, University of Trieste, Trieste and

²Dipartimento di Scienze Biomediche, University of Trieste, Italy.

When we focussed our attention on *Burkholderia cepacia*, we found out that very little was known about the exopolysaccharides produced by this microorganism; several authors reported that *B. cepacia* does not produce alginate, like *Pseudomonas aeruginosa*, but a polymer composed of rhamnose, galactose, mannose, glucose and glucuronic acid [1]. In collaboration with Prof. Isabel Sá-Correia of the Instituto Superior Técnico in Lisbon, we determined the structure of an exopolysaccharide produced by a strain of *B. cepacia*, named IST408, isolated from a CF patient in Lisbon Hospital [2,3]. The structure of the repeating unit, reported below, is quite complex and uncommon containing a tri-substituted glucuronic acid and a D-rhamnose:



A polysaccharide with the same structure was produced by a clinical isolate of *B. cepacia* from a French CF patient [4], and a recent publication suggested that all clinical isolates of *B. cepacia* produce the same type of EPS [5]. These findings prompted an investigation on several different clinical isolates of *B. cepacia* obtained directly from sputum of CF patients treated at the Regional Centre of Cystic Fibrosis in Trieste, Italy. The strains were characterised by ribotyping and RAPD. The different *B. cepacia* isolates can be classified in three groups based on the type of EPS produced:

- a) EPS identical to IST408
- b) Mixture of two EPS's: one identical to IST408 and another with a completely different structure
- c) EPS constituted of galactose (galactan), with traces of EPS identical to IST408

Moreover, for comparison we examined the strain ATCC 25416 and a strain isolated from a vaginal tampon; the former produced an EPS identical to IST408, the latter strain was ascribable to group b.

The structure-function relationships of the *B. cepacia* IST408 EPS were studied by investigating the solution conformational properties of the polysaccharide. The results showed that the backbone exhibited rather high rigidity and properties very similar to alginate, thus suggesting a possible common role in the virulence for the two polysaccharides.

Our plans for the future are:

- Determination of the structure of *B. cepacia* EPSs;
- Interactions of EPSs with anti-microbial peptides;
- Investigation of mechanism which control the mucoid-non mucoid conversion.

[1] J.R.W. Govan, V. Deretic, *Microbiol. Rev.*, 60 (1996) 539-574.

[2] J. Richau, J.H. Leitão, M. Correia, L. Lito, M.J. Salgado, C. Barreto, P. Cescutti, I. Sá-Correia, *J. Clin. Microbiol.*, 38 (2000) 1651-1655.

[3] P.Cescutti, M. Bosco, F. Picotti, G. Impallomeni, J. H. Leitão, J. Richau, I. Sá-Correia *Biochem. Biophys. Res. Comm.* 273 (2000) 1088-1094.

[4] S. Cérantola, A. Lemassu-Jacquier, H. Montrozier, *Eur. J. Biochem.*, 260 (1999) 373-383.

[5] S. Cérantola, J.-D. Bounéry, C. Segonds, N. Marty, H. Montrozier, *FEMS Microbiol. Lett.*, 185 (2000) 243-246.

Expression of O Antigens from *Burkholderia cepacia* Serogroup O5 and *Pseudomonas aeruginosa* Serogroup O17: Evidence for Horizontal Gene Transfer?

Charles R. Dean and **Joanna B. Goldberg**

University of Virginia

Lipopolysaccharide (LPS) is an important component of the outer membrane of Gram-negative bacteria that is responsible for many of immunostimulatory effects associated with infection. LPS is composed of a variable serogroup-specific O antigen repeating unit, the LPS core, and the endotoxic lipid A. In the case of *Burkholderia cepacia*, there are at least 7 O antigen serogroups that differ in their disaccharide or trisaccharide repeating units. Of particular interest is that the *B. cepacia* serogroup O5 O antigen repeating unit is identical to that of *Pseudomonas aeruginosa* serogroup O17 with the structure [-b-D-ManNAc-(1-4)-a-L Rha-(1-)]. Since *B. cepacia* and *P. aeruginosa* can reside together in the cystic fibrosis lung, it is of interest to determine whether genes, especially those encoding virulence factors, can move between these bacteria. The goal of this study is to identify the genes encoding the O antigen from these two strains, to determine the level of conservation between these loci, and to ascertain the mechanism of gene transfer between these two strains. We first confirmed that commercially available polyclonal antibodies to *P. aeruginosa* serogroup O17 agglutinated *B. cepacia* serogroup O5. A cosmid library was constructed using size-selected DNA in the range of 40 kb from each of these strains. The library was transduced to *Escherichia coli*. Two positive colonies from each library were identified by immunoblot. The cosmids have been isolated and DNA sequence analysis is currently underway.

Quorum Sensing Signal Production In The *B. Cepacia* Complex.

Barbara-Ann D. Conway and David P. Speert

BC Research Institute for Children's and Women's Health
950 West 28th Ave, Vancouver, BC V5Z 4H4, CANADA

Quorum sensing is a method of gene regulation based on population density that is used by a number of bacteria that have both symbiotic or pathogenic and free-living lifestyles. Several gram-negative bacteria, including *Pseudomonas aeruginosa* and *Burkholderia cepacia*, produce a diffusible signal during growth that accumulates in the surrounding medium. When a critical concentration of the acyl-homoserine lactone (acyl-HL) signal is reached, these molecules interact with transcriptional regulators to control expression of certain genes. In *P. aeruginosa*, the acyl-HL signal-dependent *las* and *rhl* quorum sensing systems function as part of a cascade of regulation controlling the expression of certain virulence factors. At least one quorum sensing system has been identified in a *B. cepacia* clinical isolate and has been shown to play a role in the regulation of extracellular protease and siderophore production. We are analysing acyl-HL production by *B. cepacia* isolates from each of the genomovars to determine the nature and quantity of signal molecules produced by members of the *B. cepacia* complex. Preliminary data indicates that at least five of the seven genomovars produce acyl-HLs. Of the isolates studied thus far, octanoyl-HL and decanoyl-HL were the primary signal molecules detected.

Identification and Mutagenesis of the Oxidative Defense Enzymes in a Clinical Isolate of *Burkholderia cepacia*.

M. LEFEBRE* and M. A. VALVANO.

Dept. of Microbiology and Immunology, University of Western Ontario, London, Ontario.

B. cepacia is an aerobic, gram-negative microorganism that was originally described as the cause of soft rot in onions. Over the past 20 years this bacterium has emerged as an important opportunistic pathogen, primarily in cystic fibrosis patients. Our laboratory has shown that *B. cepacia* can survive intracellularly in both macrophages and amoebae. Catalase, catalase/oxidase, and superoxide dismutase (SOD) enzymes are involved in detoxifying toxic oxygen intermediates in both eukaryotic and bacterial systems. We hypothesize that these enzymes are involved in promoting *B. cepacia* survival following exposure to oxidative stress generated by host cells in response to infection. This study focused on the characterization of these oxidative defense enzymes from a genomovar III strain isolated from a cystic fibrosis patient. We have identified open reading frames corresponding to both a bi-functional catalase/oxidase and an iron co-factored SOD. The catalase/oxidase enzyme is encoded by a 2.2 kb gene and produces a protein roughly 85 kDa in size that shows homology to similar proteins from other high G+C bacteria. A catalase/oxidase mutant was generated and the enzymatic profiles of the mutant strain did not appear to be significantly different from the wild type strain (WT). Survival following exposure to hydrogen peroxide (H_2O_2) *in vitro* showed that the mutant and WT strains behaved in a similar fashion when grown in iron rich media. However, the mutant showed a 2 log decrease in survival following H_2O_2 exposure compared to the WT strain when grown in iron-limiting conditions. In accordance to these results, the mutant strain showed a decreased ability to grow under iron limiting conditions as compared to the WT strain. The iron co-factored SOD is encoded by an open reading frame approximately 690 base pairs in length and possess characteristics common to this class of enzyme. We are currently generating an SOD knock out mutant for future analysis. We are also constructing a panel of vectors that will be useful for complementation assays with *B. cepacia*. We have constructed a constitutive complementation vector carrying suitable antibiotic markers and a *B. cepacia* 50s ribosomal protein promoter directly upstream of a multiple cloning site. This vector will allow for stable and constitutive expression of desired genes within a wide range of *B. cepacia* backgrounds. We have also shown that the arabinose inducible promoter under control of *araC* is functional within *B. cepacia*. We are therefore constructing an arabinose inducible expression vector suitable for use in *B. cepacia*. These vectors will provide new opportunities for the complementation assays required to complete this study of the importance of the catalase/oxidase and iron co-factored SOD genes in *B. cepacia* survival.

Antibiotic Resistance In *Burkholderia Cepacia* At Two Regional Cystic Fibrosis Centres In Northern Ireland: An Update

John E. Moore, Mary Crowe, Adrienne Shaw, John McCaughan, Aileen OB Redmond & J. Stuart Elborn
Northern Ireland Regional Adult & Paediatric Cystic Fibrosis Centres, Belfast City Hospital & the Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, BT9 7AD.

Infection with the *Burkholderia cepacia* complex (BCC) continues to present both a diagnostic and management challenge in cystic fibrosis (CF). One difficulty associated with the treatment of infections with this organism with antibiotics has been and remains the acquisition of antimicrobial resistance. Previously, many centres have reported high levels of resistance against several classes of antibiotics. To date, there has been limited data reported on the antimicrobial resistance status of BCC in the Northern Ireland CF population, hence it was the aim of this study to examine susceptibility of wild-type BCC isolates from all infected adult and children to a wide variety of antimicrobial agents. Presently, prevalence rates of BCC within the N. Ireland CF population are 24/109 (22%) adults, with 21 (88%) of these patients being colonised by *B. cepacia* genomovar III and the remaining three patients being colonised by *B. multivorans*. Seven children are infected with this organism in a population of 230 (3%) and all of these children have *B. cepacia* genomovar III. Antimicrobial susceptibilities assays were performed on all recent BCC isolates employing a Modified Stoke's disc diffusion assay on DST agar (Oxoid Ltd., England) supplemented with 5% v/v defibrinated horse blood, with the following antibiotics:- ciprofloxacin (5ug), colistin (10ug), ceftazidime (30ug), azlocillin (75ug), aztreonam (30ug), imipenem (10ug), gentamicin (10ug), tobramycin (10ug), meropenem (10ug), amikacin (30ug), temicillin (30ug), piperacillin/tazobactam (110ug). Antibiotic disc concentrations were similar for adults and children with the exception of colistin, which was 25ug for testing with children. Susceptibility data are shown on Table 1. Overall, the paediatric isolates were more sensitive to several antibiotics in comparison to the adult BCC organisms and this may allow for an opportunity to attempt to eradicate the organism on first isolation from the lung, when the organism is still relatively sensitive. In addition, the *B. multivorans* isolates were more sensitive in the adult patients than *B. cepacia* genomovar III isolates. However, it was noted that 81% of the adults were infected with a pan-resistant genomovar III epidemic organism. Hence, some form of synergy testing, especially testing to examine and prevent antagonism between combinations, should be employed to help guide more efficacious combinations of agents used.

Percentage (%) of isolates

Antibiotic Agent	Resistant	Intermediate	Sensitive
(i). <u>Adults</u>			
[n=21; all BCC* genomovar III]			
ciprofloxacin	100		
colistin	100		
ceftazidime	62	24	14
azlocillin	100		
aztreonam	100		
imipenem	100		
gentamicin	100		
tobramycin	100		
meropenem	95	5	
amikacin	100		
temocillin	100		
tazocin (piperacillin/tazobactam)	71	5	24

No. adult patients with BCC genomovar III strains displaying pan-resistance = 17/21 (81%)

No. adult patients with BCC genomovar III strains displaying multi-resistance = 3/21 (14%)

Antibiotic Agent	Resistant	Intermediate	Sensitive
(ii). Adults			
[n=3; <i>B. multivorans</i> (BCC* genomovar II)]			
ciprofloxacin	100		
colistin	100		
ceftazidime	0	33	67
azlocillin	67		33
aztreonam	67		33
imipenem	100		
gentamicin	100		
tobramycin	100		
meropenem	33	34	33
amikacin	100		
temocillin	33	34	33
tazocin (piperacillin/tazobactam)	0	33	67

No. adult patients with BCC genomovar II strains displaying pan-resistance = 0/3

No. adult patients with BCC strains displaying multi-resistance = 2/3

Antibiotic Agent	Resistant	Intermediate	Sensitive
(iii). Children			
[n=7; all BCC* genomovar III]			
ciprofloxacin	57		43
colistin	80		20
ceftazidime	43		57
azlocillin	43		57
imipenem	33		67
gentamicin	100		
tobramycin	86	14	
meropenem	43		57
amikacin	67	33	
tazocin (piperacillin/tazobactam)	40	20	40

No. children with BCC genomovar III strains

displaying pan-resistance = 3/7 (43%)

Notes:

*BCC, *Burkholderia cepacia* complex

Correlation between *in vivo* and *in vitro* models of invasion in the clinical setting of *Burkholderia cepacia* infections

Martin V. Cieri, Nicole Mayer-Hamblett, Adam Griffith, **Jane L. Burns**,

Children's Hospital and Regional Medical Center, 4800 Sand Point Way N.E., CH-32 Seattle, WA 98105

The lack of a good *in vitro* model of *B. cepacia* lung infection has severely hampered studies of virulence factors in this important CF pathogen. We and others previously reported the use of a modified *in vitro* gentamicin protection assay of invasion in A549 cells. Problems with the assay have included a high degree of day-to-day variability and an inability to validate the model based on the clinical course of infected patients. We have recently modified an *in vivo* mouse agar bead model of chronic lung infection by quantitating splenic invasion, and have been able to correlate, to some extent, splenic invasion and genomovar status. Further analysis of these experiments has identified a correlation between clinical status, A549 cell invasion, and splenic invasion, using strains from the *B. cepacia* experimental panel. A statistical model was developed to compare each strain with positive and negative control strains to first identify which strains showed evidence of being "invasive" or "non-invasive." For the A549 assay, 1/4 of the genomovar I isolates (CEP509, the only CF isolate in this group), 3/8 of the genomovar II isolates (including CEP781, an epidemic CF isolate), 7/10 of the genomovar III isolates (the only non-CF isolate of the 10 was non-invasive), 1/4 of the genomovar IV isolates (one of two CF isolates in this genomovar), and 0/4 of the genomovar V isolates were designated as "invasive". When selected "invasive" and "non-invasive" genomovar II and III isolates from this *in vitro* model were examined in the mouse model, the results correlated. Using the "invasive" strain, CEP781 (a genomovar II epidemic CF isolate), 4/5 mice demonstrated splenic invasion (mean \pm sd, 262.5 ± 415 cfu/mL) and for the "invasive" strain CEP511 (a genomovar III epidemic CF isolate), 3/5 mice demonstrated splenic invasion (13 ± 28.9 cfu/mL). However, using the "non-invasive" isolates, neither the genomovar II laboratory strain, CEP 49, nor the CF isolate, FC475, demonstrated splenic invasion in any of the 8 mice tested. Interestingly, FC475 (AKA BC7) has been reported by other investigators to be non-invasive in the A549 model. The use of this statistical approach and these models may ultimately be very helpful in identifying specific virulence factors of *B. cepacia* and determining their effects on CF lung disease.

Role of type III secretion in the pathogenesis of *Burkholderia cepacia*

Mladen Tomich, Adam Griffith, Christine Herfst, Virginia Cooper, Jane Burns and **Christian Mohr**

Addresses: University of Washington School of Medicine, Seattle, Washington 98195, and Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455.

Type III secretion systems are utilized by a wide range of gram-negative bacterial pathogens to deliver virulence proteins directly into host cells. Using a PCR-based approach we have identified homologs of type III secretion genes in *B. cepacia* strain J2315, a clinical isolate belonging to the ET12 lineage responsible for epidemic outbreaks and mortality in cystic fibrosis patients. One of these genes, designated bscN, encodes a member of a family of ATP binding proteins which likely function in the generation of energy driving virulence protein secretion. A defined null mutation was generated in bscN by allelic exchange mutagenesis and the bscN null strain and wild-type parent were examined for their ability to cause disease in a mouse agar bead model of *B. cepacia*

infection. Quantitative bacteriology of the lungs and spleens of infected C57BL/6 mice revealed that the *bscN* null strain was severely attenuated in virulence when compared to the parent strain, with significantly lower bacterial recovery from the lungs and spleens of infected animals at both three and seven days post infection. Complementation of the *bscN* null strain by providing a wild-type copy of the *bscN* gene alone in trans restored wild-type levels of bacteria in the lungs of infected animals, confirming that the virulence defect was due to disruption of *bscN*. Our findings indicate that bacteria lacking *bscN* are either less likely to establish infection or more likely to be cleared from infected organs. Genetic dissection of the regions flanking the *bscN* gene revealed a locus encoding at least nine type III secretion genes as well as a putative transcriptional regulator involved in the control of type III gene expression. A combination of in vivo and in vitro studies are currently underway to further define the role of type III secretion in the pathogenesis of *B. cepacia*.

The IBCWG website : an interactive research tool ?

Tom Coenye¹, Paul W. Whitby² & John J. LiPuma¹

¹ Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan, USA ² Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

As discussed in a document presented to the International *Burkholderia cepacia* Working Group (IBCWG) at the Fall meeting in Baltimore, the website will have to be moved from its old location (<http://allserv.rug.ac.be/~tcoenye/cepacia>) to a new location. The new location of the website will be on a server at the University of Michigan (Ann Arbor, USA). Since November 2000, the website can also be visited via the URL <http://go.to/cepacia> - since this is only a redirect URL, it will not change when the actual website URL changes and is therefore the preferred designation.

In the past, the website has mainly been used as an on-line archive of minutes and abstracts presented at meetings of the Working Group. In addition, it contains general information on different aspects of the biology of the *B. cepacia* complex and refers visitors to the two largest reference laboratories. It also contains links to other relevant on-line locations (e.g. CF charitable trusts). At the moment of writing, 43 people have requested to be notified when updates are brought on-line.

It is however our feeling that we are not exploiting the full potential of the website and that additional information and links could be added. It should be the responsibility of all the members of the IBCWG to help to keep the site up-to-date and attractive. Additional items that could be added to the site include publication lists from members (including links to full-text articles), consensus statements (eg, infection control policies, identification protocols), and membership contact information.

We may also wish to consider the establishment of a list serve that would be limited to IBCWG members only.

This could provide a forum for rapid dissemination of new information, interactive discussion and communication among Working Group members.

The purpose of this abstract is to stimulate the exchange of ideas with regard to what more should be included on the website, how this should be organized, and to consider establishing a members only list serve.

Unpresented Abstracts

PCR-Based Identification of Bacterial Species Recovered From Cystic Fibrosis Sputum.

Lixia Liu¹, Tom Coenye¹, Peter Vandamme², Jane Burns³, Paul Whitby⁴, Terry Stull⁴, John LiPuma¹

¹ University of Michigan, ² University of Gent, ³ University of Washington, ⁴ University of Oklahoma

PCR assays targeting 16s rRNA genes were developed to identify various genera and species recovered from CF sputum culture. These include certain *Pandoraea* spp, *Alcaligenes xylosoxidans*, and species of the *Burkholderia cepacia* complex. A panel of 94 bacterial isolates that had been previously identified by polyphasic taxonomic analysis was tested for reactivity with each new PCR assay. These isolates were from clinical and environmental sources and included 53 *B. cepacia* complex isolates, 15 *Pandoraea* spp., eight *Pseudomonas aeruginosa*, six *Burkholderia gladioli*, six *Stenotrophomonas maltophilia*, three *A. xylosoxidans*, and three *Ralstonia pickettii*.

Additional relevant strains were included for assessment of specific PCR assays. These included eight Bcc genomovar VI, five *Pandoraea apista*, 46 *Alcaligenes xylosoxidans*, and one strain each of five other *Alcaligenes* spp. The sensitivity and specificity (respectively) of the new assays were: 98% and 100% for *Burkholderia cepacia* complex (genomovars I – VII as a group); 79% and 98% for Bcc genomovar VI; 95% and 99% for *Pandoraea* spp (genus level); 79% and 100% for *P. apista*, and 98% and 100% for *A. xylosoxidans*. The use of these assays has enhanced the identification of bacterial isolates from CF sputum culture.

The *recA* gene as an indicator of diversity in the *Burkholderia cepacia* complex: how far can it usefully go ?

¹E Mahenthalingam, ¹J Fadden, ²T Coenye, ³S Laevens, ²J LiPuma and ³P Vandamme

¹Cardiff University, Cardiff, UK; ²University of Michigan, Ann Arbor, MI, USA; ³Universiteit Gent, Gent, Belgium.

B. cepacia is a very problematic opportunistic pathogen in patients with cystic fibrosis (CF) and many other vulnerable individuals. Isolates of this species were shown to belong to five closely related genetic species (genomovars): *Burkholderia vietnamiensis* (formerly genomovar V), *Burkholderia multivorans* (formerly genomovar II), *Burkholderia stabilis* (formerly genomovar IV), genomovar I and genomovar III. This group of closely related bacteria were collectively designated as the *B. cepacia* complex. Since *B. cepacia* may spread from patient-to-patient and is associated with poor prognosis, rapid identification of strain genomovar is important for the clinical management and treatment of infection. Identification of the different members of the *B. cepacia* complex is not straightforward. Molecular identification approaches based on the *B. cepacia* complex *recA* gene were developed and proved useful for identification of all five original genomovars. However, since these assays were first developed further taxonomic diversity within the *B. cepacia* complex has been identified. *B. cepacia* genomovars VI and VII (*Burkholderia ambifaria*) have been recently described and an eighth genomovar is currently being characterized (see P. Vandamme abstract). *Burkholderia pyrocinnia* also falls within the *B. cepacia* complex by polyphasic analysis and *recA* gene analysis. In addition to these nine taxonomic groups sequence comparison of *recA* genes from remaining “odd” *B. cepacia* complex strains demonstrates the present of at least 3 further putative taxonomic groups. In the wake of all this diversity it is important to ask how far can the *recA* gene go in terms of being of useful diagnostic marker ? The following traits of the *recA* gene diagnostic approach remain constant for the *B. cepacia* complex: (i) amplification of a 1 kb product (the complete *recA* gene) using primers BCR1 and BCR2 is predicative that the isolate is a member of the *B. cepacia* complex; (ii) RFLP analysis of the 1 kb *recA* gene using *Hae*III as a primary analysis and *Mnl* I as a secondary analysis can differentiate all taxonomic groups and overcome pattern overlaps which occur with analysis of just one enzyme RFLP (minor variations in RFLP may however, be very difficult to discern); and (iii) Sequence analysis of the N-terminal encoding 5' 500 bp of the *recA* gene with PCR primers BCR1 and BCR4 is capable of differentiating all current *B. cepacia* complex taxonomic diversity. However, the significance of phylogenetic subgroups within genomovar III and potentially within other genomovars remains to be determined. Identification of *B. cepacia* complex isolates is vital to further research concerning the epidemiology and pathogenesis of this organism in patients with CF and the *recA* gene still forms a useful diagnostic marker despite considerable diversity. This work was funded by grant number PJ472 from the UK Cystic Fibrosis Trust.

Quorum Sensing genes in *B. cepacia* complex

E. Lutter¹, S. Lewenza^{1†}, J.J. Dennis², M.B. Visser¹, And P.A. Sokol¹

Department of Microbiology and Infectious Diseases, University of Calgary Health Sciences Center, Calgary, Alberta, Canada T2N 4N1¹ and Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada²

Burkholderia cepacia has emerged as an important opportunistic pathogen in cystic fibrosis patients. Clinical and environmental isolates of *B. cepacia* have recently been classified into seven closely related species or genomovars referred to as the *B. cepacia* complex. These include *B. cepacia* genomovars I, III, VI and VII, *B. multivorans*, *B. stabilis* and *B. vietnamiensis*. Quorum sensing is a signaling mechanism usually composed of *luxIR* homologues in gram-negative bacteria that is involved in the cell density dependant expression of virulence factors. The *cepIR* quorum sensing genes were recently identified in *B. cepacia* genomovar III, strain K56-2. Random pTnMod

mutagenesis of *B. vietnamiensis* DB01 revealed the presence of a second set of quorum sensing genes, designated *bviIR*, whose products had predicted amino acid sequences 43% identical to CepI and 36% identical to CepR, respectively. The distribution of the *cepIR* and *bviIR* genes within the *B. cepacia* complex was examined by PCR. Both *cepR* and *cepI* were amplified in *B. cepacia* genomovars I, III, *B. stabilis* and *B. vietnamiensis*. *cepR* was also amplified from *B. multivorans* and *B. cepacia* genomovar VI. The *bviI* and *bviR* genes were only amplified from strains of *B. vietnamiensis*. The *cepI* and *cepR* PCR products were cloned and sequenced from one strain of each species. Predicted amino acid sequences were determined and compared to K56-2 CepI and CepR. The percentage identity for CepR ranged from 99% to 92% and CepI ranged from 96% to 90%. Using an *Agrobacterium tumefaciens* reporter assay, representative strains from each genomovar were determined to produce both *N*-octanoyl-L-homoserine lactone and *N*-hexanoyl-L-homoserine lactone. The *B. vietnamiensis* and *B. cepacia* genomovar VII strains produced additional *N*-acyl-L-homoserine lactones detectable by the *A. tumefaciens* reporter. These studies suggest that the *cepIR* genes are conserved among most species of the *B. cepacia* complex and that *B. vietnamiensis* has two sets of quorum sensing genes.

The Role of *rpoS* in Intracellular Survival of *Burkholderia cepacia*.

R.L. Payne and M.A. Valvano

Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada N6A 5C1
mvalvano@uwo.ca

Burkholderia cepacia is a Gram-negative opportunistic pathogen for individuals with cystic fibrosis and chronic granulomatous disease. CF patients infected with *B. cepacia* usually develop "cepacia syndrome", a severe, fatal illness. Previous work in our lab has shown that *B. cepacia* isolates can survive intracellularly in *Acanthamoeba* and macrophages, however they do so with little or no replication. Our results suggest that the intracellular *B. cepacia* may have entered a non-replicating state analogous to stationary phase. We hypothesize that *rpoS*, the alternate primary sigma factor known to regulate stationary phase genes in Gram-negative bacteria, is associated with the ability of *B. cepacia* to survive intracellularly. The *rpoS* gene was cloned from *B. cepacia* strain LB400 by PCR, using primers generated approximately 200 bases upstream and downstream of the open reading frame. The recombinant plasmid complements the *rpoS* phenotype of an *E. coli rpoS::Tn10* mutant. Southern blotting of *B. cepacia* chromosomal DNA confirmed that the gene is present in a single copy in the chromosome of LB400, as well as in other *B. cepacia* genomovars. To mutagenize *rpoS*, a plasmid was constructed using pSUP202, a suicide vector in *B. cepacia*, and a 500 base 5' region of the *rpoS* gene. Conjugation of this plasmid into strain LB400 resulted in a single crossover event that insertionally mutagenized the gene. We will use these mutants to examine intracellular survival of *B. cepacia* in *Acanthamoeba* and macrophages.

***Burkholderia pyrrocinia* and *B. cepacia* genomovar VIII represent two novel members of the *B. cepacia* complex**

Peter Vandamme^{1*}, Deborah Henry², Tom Coenye^{3,4}, Severine Laevens³, John J. LiPuma⁴, David P. Speert² John R.W. Govan⁵ & Eshwar Mahenthiralingam⁶
*Laboratorium voor Farmaceutische Microbiologie*¹, and *Laboratorium voor Microbiologie*³, *Universiteit Gent, Ghent, Belgium*; *Department of Pediatrics, Division of Infectious and Immunological Diseases, University of British Columbia, Vancouver, British Columbia, Canada*²; *Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, U.S.A.*⁴; *Department of Medical Microbiology, University of Edinburgh, Edinburgh*⁵ and *School of Biosciences, Cardiff University, Cardiff*⁶, *United Kingdom*

In on-going multicenter efforts to improve the diagnosis of *B. cepacia* complex infections by studying the taxonomic relationships of large collections of isolates obtained from various ecological niches, we discovered isolates that represented at least two distinct novel genomovars within the *B. cepacia* complex. The methodology used was the polyphasic approach that included whole-cell protein and fatty acid analyses, analysis of the sequences and the restriction fragment length polymorphisms, and specific PCR tests based on the *RecA* and 16S rRNA genes, AFLP fingerprinting, DNA-DNA hybridisation experiments, determination of the DNA base ratio, and an extensive biochemical characterisation.

To date, both groups comprise over 30 isolates obtained from human clinical and environmental sources. One of these groups includes the type strain of *Burkholderia pyrrocinia*, an organism with established biocontrol

properties. Like *B. vietnamiensis*, the latter species was described before it was recognised as a member of the *B. cepacia* complex.

With the inclusion of these novel members, the *B. cepacia* complex presently comprises *B. cepacia* (genomovar I), *B. multivorans* (formerly *B. cepacia* genomovar II), *B. cepacia* genomovar III, *B. stabilis* (formerly *B. cepacia* genomovar IV), *B. vietnamiensis* (also known as *B. cepacia* genomovar V), *B. cepacia* genomovar VI, *B. ambifaria* (*B. cepacia* genomovar VII), *B. cepacia* genomovar VIII and *B. pyrrocinia*.

Epidemiology of *Burkholderia cepacia* in Patients with Cystic Fibrosis in Canada: Geographical Distribution and Clustering of Strains

David P. Speert¹, Deborah Henry^{1*}, Peter Vandamme², Mary Corey³, and Eshwar Mahenthiralingam⁴

Division of Infectious and Immunological Diseases, Department of Pediatrics, University of British Columbia, Vancouver British Columbia, Canada¹; Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Ghent Belgium²; The Hospital for Sick Children, Toronto, Ontario, Canada³; Cardiff School of Biosciences, Cardiff, Wales, United Kingdom⁴

Burkholderia cepacia complex is an important pathogen in patients with cystic fibrosis (CF). There is clear evidence of patient to patient spread, but the microbial factors, which facilitate transmission, are poorly understood. We conducted a prospective evaluation of *B. cepacia* complex isolates from patients with CF from all regions in Canada in an effort to identify microbial clones with enhanced transmissibility. A total of 936 isolates from the *B. cepacia* complex were recovered from 454 patients from eight of the ten provinces. 379 (83.5%) of these patients harbored genomovar III and 48 (10.6%) had *Burkholderia multivorans* (genomovar II). Substantial regional differences in infection prevalence were observed (22% of patients in Ontario, and 5% in Quebec). All isolates were typed by random amplified polymorphic DNA analysis; the only clusters of strain types were from genomovar III. All clustered isolates harbored the *B. cepacia* epidemic strain marker (BCESM), but only those from one lineage carried the gene for cable pilus. We conclude that strains of *B. cepacia* from genomovar III are the most potentially transmissible and that BCESM is a robust marker for transmissibility.

Lori L. Burrows, Ph.D., Assistant Professor, Dept. of Surgery, University of Toronto

My laboratory studies the genetics and prevention of bacterial biofilm formation using the opportunistic pathogen *Pseudomonas aeruginosa* as a model organism. Projects ongoing in the lab range from basic genetic studies of biofilm formation and gene expression in biofilms to applied clinical research on preventing colonization and biofilm formation on medical devices.

On the basic side, we are studying the role of the *P. aeruginosa* cell-surface molecules lipopolysaccharide, flagella and type IV pili in the attachment phase of biofilm formation. We have found that specific O-antigen genes are required for pilin modification and/or function, and that mutants lacking these genes make increased amounts of biofilm. In addition, we have found that hyperpiliated mutants make 10-12 fold more biofilm than wild-type strains although they lack the twitching motility thought to be important for early biofilm formation. In a separate project, we use a modified form of "in vivo expression technology" (IVET) to detect *P. aeruginosa* genes upregulated in the biofilm environment. Using this system, which is based on promoter trapping, we have identified genes induced by oxygen and osmotic stresses, implying these factors are important in *P. aeruginosa* biofilms. We have also identified a novel regulatory gene homologous to the AraC family. Knockout mutants lacking these genes have reduced fitness for biofilm survival compared with the wild type, as direct competition experiments using equal inocula of isogenic wild type and mutant strains yield 4-5 logs less of the mutant compared with the parent after 5 days growth as a biofilm.

On the applied front, we are involved in the development of antimicrobial coatings for medical devices that prevent colonization by killing bacteria on surface contact. These coatings have recently been tested on model peritoneal catheters in a novel rat model of chronic *P. aeruginosa* peritonitis and on Foley (urinary) catheters in pre-clinical human safety studies. In both cases the coated devices have demonstrated a statistically significant ability to prevent colonization compared with uncoated devices.

Estimating The Effect Of *Burkholderia Cepacia* In Population Studies

Annie Dupuis, Mary Corey.

Hospital for Sick Children and University of Toronto

Besides the risk of "*cepacia* syndrome" following infection with *Burkholderia cepacia* (Bc), we have shown increased mortality risk for at least 2 years in those infected with Bc. The Cox proportional hazards regression model is used to evaluate the relative effects of multiple risk factors, including those which can change over time (updated covariates), in a single survival regression model. However, prospective interpretation of these models is complicated by the need to incorporate future changes in the updated covariates. We are working on methods to provide prospective survival estimates based on the current values of updated covariates including FEV₁ and its rate of decline, age, and other concomitant risk factors. These models will provide a cohesive approach to comparing the short and long term risks associated with different genomovars in CF population studies based on the Canadian and US Patient Registries.

Email Addresses

Ashlock, Meissa	MAshlock@cff.org
Burns, Jane L	jburns@chmc.org
Burrows, Lori	Lori.Burrows@uhn.on.ca
Campana, Silvia	campana@dada.it
Paola Cescutti	cescutti@bbcm.univ.trieste.it
Conway, Barbara	conway@cbdn.ca
Chakrabarty, Ananda	ananda.chakrabarty@uic.edu
Cecilia.Chaparro	Cecilia.Chaparro@uhn.on.ca
Chiarot, Josee	jchiarot@cysticfibrosis.ca
Coenye, Tom	tcoenye@med.umich.edu
Corey, Mary	mary.corey@sickkids.on.ca
Dupuis, Anne	annie.dupuis@sickkids.on.ca
Dennis, Jonathan	dennisj@ualberta.ca
Flannagan, Ron	rflannag@uwo.ca
Forstner, Janet	janet.forstner@sickkids.on.ca
Goldberg, Joanna	jbg2b@virginia.edu
Gonzalez, Carlos F.	cf-gonzalez@tamu.edu
Govan, John	jrwg@srv1.med.ed.ac.uk
Lefebvre, Matt	mlefebvre@uwo.ca
Henry, Deborah	dhenry@telus.net
Humar, Atul	C/o Chapparo
Hunt, Tracey	tahunt@uwo.ca
Shaf.Keshavjee	Shaf.Keshavjee@uhn.on.ca
Lessie, Tom	tlessie@microbio.umass.edu
LiPuma, John J.	jlipuma@umich.edu
Liu, Lixia,	Co LiPuma
MacDonald,Noni	Noni.MacDonald@dal.ca
Mahenthiralingam, Eshwar	mahenthiralingame@cardiff.ac.uk
Marolda, Cristina	cmarolda@uwo.ca
McColley, Susanna	smccolley@nwu.edu
Miller, Suzanne	Suzanne.Miller@orst.edu
Mohr, Chris	mohr@lenti.med.umn.edu
Moore, John	jemoore@niph1.dnet.co.uk
Morrison, Cathleen	cmorrison@cysticfibrosis.ca
Nair, Bindu	
Parke, Jennifer	jennifer.parke@orst.edu
Payne, Rebecca	rpayne@uwo.ca
Pritchard, Carolyn	Carolyn.Pritchard@astrazeneca.com
Sajjan, Uma	usajjan@sickkids.on.ca
Sayre, Phillip	sayre.phil@epa.gov
Schwab, Ute	Ute_Schwab@med.unc.edu
Sokol, Pam	psokol@ucalgary.ca
Stull, Terrence L.	terrence-stull@ouhsc.edu
Tiedje, James	tiedjej@pilot.msu.edu
Valvano, Miguel	mvalvano@julian.uwo.ca
Vandamme, Peter	peter.vandamme@rug.ac.be
Whitby, Paul	paul-whitby@ouhsc.edu
Wozniak, Chris	Wozniak.Chris@epamail.epa.gov

