SIXTH MEETING OF THE INTERNATIONAL *BURKHOLDERIA CEPACIA* WORKING GROUP

April 9 -11, 1999, Banff Conference Centre, Banff Alberta, Canada

SPONSORED BY:

The MERREM Product Group, Zeneca Pharma Canada Inc.
The Medical Research Council of Canada
The Canadian Cystic Fibrosis Foundation
The US Cystic Fibrosis Foundation
The UK Cystic Fibrosis Trust

PROGRAM:

FRIDAY 9, APRIL 1999

Arrival and check-in for participants (guest check-in at the Professional Development Centre; your accommodation will be either in the Professional Development Centre or Corbett Hall). Meet participants (informal) for drinks (6 pm onwards) at the Sally Borden Recreational Facility Bar.

SATURDAY, APRIL 10, 1999.

7.00 - 8.00 am **BREAKFAST**
(Dining Room adjacent to Donald Cameron Hall)

8.00 - 8.15 am **INTRODUCTION AND AGENDA**
Eshwar Mahenthiralingam and John LiPuma
(Professional Development Centre, PDC103, Amoco Canada Petroleum Co. Room; all sub-group meetings to held held in rooms within this block too)

8.15 - 8.45 am **PROGRESS SINCE MIAMI:**
(a) US *B. cepacia* Referral Laboratory - Dr. John LiPuma (10 mins)
(b) Canadian *B. cepacia* strain Repository - Deb Henry (10 mins)
(c) International Standardization of Strain Typing - Dr. John Moore (10 mins)

8.45 - 10.00 am ***B. CEPACIA* - PLANT PATHOGENESIS, PLANT PROTECTION AND LEGISLATIVE ASPECTS OF COMMERCIAL USE**

(a) “"Understanding the pathogenic process in phytopathogenic *B. cepacia*" - Dr. Carlos Gonzalez, (25 mins)

(b) "*B. cepacia* in biological control of plant diseases" - Dr. Jennifer Parke, Oregon State University (25 mins).

(c) “Risk Assessment and Regulation of Microbial Biopesticide Opportunistic Human Pathogens” - Dr. Doug Gurian-Sherman and Dr. William Yan (25 mins) - US Environmental Protection Agency & Health Canada Pest Management Regulatory Agency (25 mins)

10.00 - 10.30 am **AM NUTRITIONAL BREAK**

10.30 - 11.30am **SELECTED SHORT PRESENTATIONS FROM OTHER NEW PARTICIPANTS:**

(a) “Quorum Sensing in *B. cepacia*” Dr. Barbara Conway, University of Iowa (15 mins).

(b) "Antibiotic Resistance in *B. cepacia*"- Dr. Keith Poole, Queens University (15 mins).

(c) "Antibiotic synergy testing for CF exacerbations caused by multi-resistant *B.cepacia* and *P. aeruginosa* organisms." - Dr. Shawn Aaron, Adult CF Clinic Director, Ottawa General Hospital (15 mins).

(d) “Survey of resistance and epidemiology of *B.cepacia* in cystic fibrosis patients in a Czech hospital” Dr. Otakar Nyé, Dept. of Clinical Microbiology FN Motol, Prague, Czech Republic (15 mins).

11.30 am - 12.30pm ***B. CEPACIA* INFECTION AND LUNG TRANSPLANTION IN PATIENTS WITH CYSTIC FIBROSIS**

(a) The University of North Carolina Experience: Dr. Peter Gilligan (20 mins).

(b) The Toronto experience: Dr. Mary Corey/Dr. Janet Forstner/ Dr.Uma Sajjan/Dr. Elizabeth Tullis and Dr. Cecilia Chaparro (40 mins)

12.30 pm - 12.45 pm. INTRODUCTION OF DISCUSSION TOPICS AND OBJECTIVES OF BREAK-OUT GROUPS

Topic 1 - Identification and molecular epidemiology of *B. cepacia* (Chair - John LiPuma, Rm. PDC 103)

Topic 2 - Virulence, Pathogenesis and Models of *B. cepacia* infection (Chair - David Speert, Rm. PDC 102)

Topic 3 - Transplantation, Therapy and Clinical Practices in relation to *B. cepacia* infection (Chair - Debbie Toder, Rm. PDC 104)

12.45 - 2.00 pm GROUP LUNCH (Dining Room adjacent to Donal Cameron Hall)

2.00 - 4.00 pm AFTERNOON BREAK

4.00 - 4.30 pm MEET FOR EVENING SESSION AND DISCUSSION GROUPS (PM Nutritional Break)

4.30 - 6.30 pm - Discussion groups in break-off rooms

6.30 - 7.00 pm - Break and allow mixing between groups

7.00 - 8.30 pm - Continue discussion groups in break-off rooms

8.30 pm **END SATURDAY SESSION**

9.00 pm INFORMAL EVENING FUNCTION AT ST. JAMES GATE OLDE IRISH PUB (optional for all participants; Tel: 1 403 762-9355, Address: 205 Wolf St Banff Alberta)

SUNDAY APRIL 11, 1999

8.00 - 9.00 am BREAKFAST
(Dining Room adjacent to Donal Cameron Hall)

9.00 - 9.40 am Wrap-up discussion topics in breakout groups.

9.40 -10.10 am Meet as one group, present and discuss conclusions for TOPIC 1
Identification and molecular epidemiology of *B. cepacia* (Chair - John LiPuma)

10.10 - 10.40 Present conclusions for TOPIC 2
Virulence, Pathogenesis and Models of *B. cepacia* infection (Chair - David Speert)

10.40 - 11.10 - AM NUTRITIONAL BREAK

11.10 - 11.40 Present conclusion for TOPIC 3
Transplantation, Therapy and Clinical Practices in relation to *B. cepacia* infection (Chair - Debbie Toder)

11. 40 - 12.30 pm CONCLUDE FORMAL MEETING

(a) General Discussion - set objectives/agenda/venue for next IBCWG meeting.

(b) Acknowledge Sponsors

12.30 - 1.30 pm GROUP LUNCH (Dining Room adjacent to Donald Cameron Hall)

4.00 - 4.30 pm — Remaining delegates to meet for afternoon/evening presentation session
PDC 103, PM Nutritional Break

4.30 - 8.00 pm — **INFORMAL PRESENTATION AND DISCUSSION SESSION (12 x 15 min. Talks)**
Chairs: John LiPuma and Eshwar Mahenthiralingam

VIRULENCE DETEMINANTS OF *B. CEPACIA:*

1. **Pam Sokol:** **Virulence Factors of *Burkholderia cepacia***
Authors: Sokol, P.A., P. Darling, S. Lewenza, C.R. Corbett, C. Kooi and M. Visser.

2. **Shawn Lewenza:** **The role of quorum sensing in the production of virulence factors in *Burkholderia cepacia***
Authors: Lewenza, S. and Sokol, P.A.

3. **Jane Burns:** **Antibiotic efflux in *B. cepacia***

Short Break

4. **Miguel Valvano:** **Intracellular survival of *B. cepacia* in macrophages and amoebae**

5. **Uma Sajjan:** **Interaction of *B. cepacia* isolates with human bronchial epithelial cells**
Authors: Uma Sajjan and Jan Forstner

6. **Karen Chu:** **Mechanism by which *B. cepacia* establishes a persistent infection in the mouse**

7. **Jacqueline Chung**: **The identification of the virulence determinants of *Burkholderia cepacia***

Short Break:

TAXONOMY, IDENTIFICATION, AND GENETICS OF *B. CEPACIA* AND RELATED CYSTIC FIBROSIS PATHOGENS:

8. **Tom Coenye**: **Increasing bacterial biodiversity in cystic fibrosis patients description of *Pandoraea gen. nov.***

9. **Paul Whitby**: **Species specific PCR for *Burkholderia gladioli***
Authors: P.W. Whitby, T.L. Stull, and J. LiPuma

10. **Tom Lessie:** **Characterization of a biocontrol strain of *Burkholderia vietnamiensis***
Authors: T.G. Lessie, H. Zhou, and F. Yao. Univ. Massachusetts, Amherst, USA

11. **Eshwar Mahenthiralingam:** **Phylogenetics of the *B. cepacia* complex**
Authors: Eshwar Mahenthiralingam, Jocelyn Bischof, Sean K. Byrne, Peter Vandamme, Christopher Radomski, Yossef Av-Gay and Julian E. Davies.

12. **Stuart Elborn:** **Speciation of the *B. cepacia* complex directly from crude CF sputum**
A. McDowell, K. Dunbar, J. E. Moore, K. Webb, E. Mahenthiralingam and J. S. Elborn

FINAL DISCUSSION, CONCLUSION OF SESSION AND MEETING

ABSTRACTS (alphabetical by author):

Name: **Shawn Aaron, MD, MSc., FRCP**

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Abstract: **Antibiotic synergy testing for CF exacerbations caused by multi-resistant *B. cepacia* and *P. aeruginosa* organisms**

Authors: S. Aaron, N. MacDonald, W. Ferris. University of Ottawa

Background: Combination antibiotic therapy has been shown to be beneficial in the management of pulmonary exacerbations in cystic fibrosis (CF). When *Pseudomonas aeruginosa* or *Burkholderia cepacia* are multiresistant, ie. resistant to two or more antipseudomonal agents, selection of an antibiotic combination can be problematic. Conventional in vitro methods such as time kill curve (TKC) determination of antibiotic synergy or antagonism of antibiotic combinations are labour intensive, expensive, and may not provide an answer in a clinically useful time period. We have developed a modified TKC protocol (called MCT) for evaluation of multiple antibiotic combinations for multiresistant *P. aeruginosa* and *B. cepacia* isolates from CF patients which provides information on bactericidal combinations in a clinically relevant timely fashion.

Methods: Our current template for *P. aeruginosa* (PA) MCT testing utilizes 13 antibiotics in 377 potential combinations. The current template for *Burkholderia cepacia* (BC) MCT testing uses 12 antibiotics in 298 potential combinations. Results of MCT tests are available within 48 to 72 hours.

Results: To date, 89 isolates of multiresistant PA from 42 patients and 111 isolates of multiresistant BC from 48 patients have undergone MCT testing in the microbiology laboratory at The Children's Hospital of Eastern Ontario over an eleven year period. For all strains tested we have been able to find at least one bactericidal three antibiotic combination. Antagonism (growth of an organism when a third antibiotic was added to a bactericidal combination of two antibiotics) has been a relatively frequent finding, and has been seen in up to 45% of PA isolates tested and in 17% of BC isolates tested. We have been routinely providing results of the MCT testing to clinicians so that they can change their patient's usually empirically chosen antibiotic therapy over to the appropriate bactericidal MCT combination.

Future research objectives: Unfortunately, we do not have objective, prospectively-collected data available to suggest that modification of the patient's antibiotic regimen based on the results of MCT testing results in improved bacteriologic or therapeutic outcomes for the patient. A protocol for a randomized, double-blind, clinical trial to evaluate the effects of MCT testing on bacteriologic and clinical outcomes will be presented to IBCWG meeting participants at Banff, Alberta on April 10,1999.

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Abstract: **Antibiotic efflux in *B. cepacia***

Multiple antibiotic resistance is an important problem in the treatment of CF lung infections caused by *B. cepacia*. Using resistance to chloramphenicol as a prototype, we have cloned and sequenced 5 genes that appear to encode an antibiotic efflux operon. They encode resistance to chloramphenicol, trimethoprim and ciprofloxacin, three agents that may have clinical utility in managing *B. cepacia*

infections. The genes we have identified include a periplasmic link protein (CeoA), a cytoplasmic membrane protein (CeoB), an outer membrane lipoprotein (OpcM), a presumed regulator (CeoR) and an open reading frame of unknown function (ORF1). *ceoABoprM* is most homologous with the *mexEFoprN* efflux pump reported in *P. aeruginosa*. *ceoR* appears to be a regulator of the operon and is homologous with the LysR family of transcriptional regulators. Regulation appears to be iron-mediated, via salicylate, which also serves as a siderophore for the majority of CF isolates of *B. cepacia*. If multiple antibiotic efflux is up-regulated by low iron conditions this would be a unique situation in which the environment present in the lungs of patients with CF would be responsible for inducible antibiotic resistance without the requirement for antibiotic selective pressure.

Name: **Karen Chu, PhD Student, Speert Lab**

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Abstract: **Mechanism by which *B. cepacia* establishes a persistent infection in the mouse**

Previous work in David's lab has produced evidence that *B. cepacia* persists for up to 8 weeks in the murine spleen with no apparent pathology to the infected tissues. The exact site(s) and mechanism of this persistence are unknown. We hypothesize that this important agent of infectious disease persists in the host by virtue of its ability to circumvent host defenses and establish an intracellular infection. The goal of my studies is to establish that *B. cepacia* is persistent in the mouse, to determine the exact site(s) of persistence and to elucidate the mechanism by which *B. cepacia* lingers in this animal. Mice are inoculated with a *B. cepacia* strain FC147 (JTC), and spleen is harvested at various time points for analysis. Homogenisation of organs and bacteriology are performed to determine colony-forming units/gram tissue over time. A polyclonal sera against strain JTC is used to conduct indirect immunofluorescence in spleen sections to determine cellular type and site of persistence. Electron microscopy will also be employed to this end. Efforts to engineer strain JTC to constitutively express the green fluorescent protein (GFP) are also under way. Mice will be inoculated with GFP-expressing strains of *B. cepacia*, and spleen will be harvested for analysis: fluorescence-activated cell sorting may be used to isolate host cell populations infected with *B. cepacia* and these same populations may be subjected to confocal microscopy to determine cellular site of persistence. The results from these *in vivo* experiments will hopefully provide a background for the future in vitro examination of the mechanism by which this organism is able to circumvent the host immune response to establish long-term infection.

Name: **Jacqueline Chung, PhD Student, Speert Lab**

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Abstract: **The identification of the virulence determinants of *Burkholderia cepacia***

Burkholderia cepacia is an opportunistic pathogen well recognized in the cystic fibrosis (CF) community due to its devastating effects on the clinical outcome of patients with CF. Poor prognosis and the potential for septicemia and invasion are what makes *B. cepacia* a prominent threat to not only CF patients but also to patients with chronic granulomatous disease (CGD) and other immunocompromised individuals. In addition, multi-drug resistance and an ability to spread among CF patients has made *B. cepacia* a formidable infection difficult to treat and control. The mechanisms by which this bacterium is able to colonize and rapidly debilitate some patients are poorly understood. In trying to understand the *in vivo* effects of *B. cepacia*, our lab has developed an animal model using intraperitoneal (i.p.) injection with C57/Bl6 mice to establish a systemic infection. The persistence and survival of bacteria are monitored through viable counts within the lung, liver and spleen. To date we have been able to establish splenic persistence for up to 8 weeks with a clinical *B. cepacia* strain, JTC; strains of *Pseudomonas aeruginosa* and one environmental strain of *B. cepacia* were rapidly cleared within 7 days. The basis of this model will assist in providing some insight into *B. cepacia's* ability to establish an infection and persist *in vivo*. Our primary approach to identifying those genes which play a role in strain JTC's persistence is through

genetic complementation. The strategy involves transforming a non-persistent host strain with the genes of a persistent strain and observing the survival of recombinant clones within the mice. A cosmid library of strain JTC has already been constructed and we are now in the process of screening putative host strains for transformation. Once appropriate host strains have been chosen and transformed with the JTC library, they will be injected into C57/Bl6 mice in our i.p. model. These recombinant clones will be closely monitored for any enhanced survival conferred by the DNA of strain JTC and will be passaged again through the mice to enrich for persistence. Recovered clones will be analyzed to identify the molecular determinants that play a role in strain JTC's survival and persistence. These results will provide insight into the virulence of *B. cepacia.*

Name: **Martin Cieri, MD, Fellow in the Division of Infectious Disease**

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Abstract: **Determinants of Invasion in *Burkholderia cepacia.***

Virulence factors in *B cepacia* which are required for intra-epithelial invasion have not been characterized. Studies of intra-cellular invasion by *B cepacia* are conducted both in vitro (A549 cells, Caco2 cells) and in vivo (C57/B6 mice) in our lab to characterize invasive phenotypes in clonal strains. K61-3 is a Genomovar III clinical isolate with a well characterized invasive phenotype, which is being interrogated for genetic elements that are related to an invasive phenotype. A hemolysin deficient mutant of *B cepacia* strain 69 (69NH) exhibits a 10 fold decrease in invasion relative to both it's parent and to K61-3. Current strategies include both 1) complementation of 69NH with a cosmid library constructed from K61-3, and 2) transposon mutagenesis of K61-3 screened for loss of invasive phenotype.

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Abstract: **Increasing bacterial biodiversity in cystic fibrosis patients description of *Pandoraea gen. nov.***

In CF microbiology, the separation of members of the *Burkholderia cepacia* complex from unrelated but phenotypically similar bacteria is of utmost importance. In the present study we examined a collection of strains isolated from CF patients in Denmark, the U.K., Canada and the U.S.A, which were tentatively identified as *Burkholderia cepacia*-like, *Ralstonia pickettii*-like or as CDC group IVc-2, by means of a polyphasic taxonomic approach (including 16S rDNA sequence analysis, SDS-PAGE of whole-cell proteins, fatty acid analysis, AFLP fingerprinting, DNA-DNA hybridisations and biochemical characterisation). Our results demonstrate that these strains belong to several new species which form a separate novel genus in the group of the *b-Proteobacteria*, for which we propose the name *Pandoraea*. The closest relatives are the genera *Burkholderia* and *Ralstonia*. Characteristics which distinguish these new species from each other and from *Burkholderia* and *Ralstonia* species will be presented. Present clinical data indicate that at least some of these organisms may cause chronic infection and can be transmitted amongst CF patients.

Name: **Barb Conway, Postdoctoral Associate**
(Supervisor: E.P. Greenberg)

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Abstract: **Role of quorum sensing in the regulation of gene expression in *Burkholderia cepacia***

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. An autoinducer molecule is synthesized that interacts with a transcriptional activator to control specific genes once a critical concentration of the autoinducer is reached. In *Pseudomonas aeruginosa*, the las and rhl quorum sensing systems function as part of a cascade of regulation controlling the expression of certain virulence factors. A number of virulence factors produced by *Burkholderia cepacia* have also been shown to respond to the addition of exogenous autoinducer from *P. aeruginosa*. We are interested in studying the role of quorum sensing in gene regulation in both environmental and clinical isolates of *B. cepacia*.

We have identified a number of acyl-homoserine lactone signal molecules from cell-free culture supernatants from the environmental strain *B. cepacia* G4. A collaboration with Pam Sokol at the University of Calgary has allowed us to identify a signal molecule produced by the clinical strain *B. cepacia* K56-2, as well as to compare autoinducer production in a number of different clinical isolates. The genes responsible for synthesis of the autoinducer molecules in both G4 and K56-2 have been cloned and sequenced. They encode proteins with homology to the LuxI family of autoinducer synthases and the LuxR family of transcriptional regulators. In K56-2, mutations interfering with autoinducer production have been shown to result in a loss of extracellular protease activity and hyperproduction of siderophore. These data suggest a role for quorum sensing in virulence gene regulation in *B. cepacia*.

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Abstract: **Epidemiology of CF**

B. cepacia is a consistently significant factor in statistical models of the disease process in CF. In addition to the immediate and dramatic effect of "*cepacia* syndrome", *B. cepacia* increases mortality risk over periods of 1 to several years. *B. cepacia* is also associated with poorer pulmonary function, but this association is extremely variable. In some patients Bc colonization appears to have very little if any effect on declining pulmonary function over perids from one to 15 years. All of these studies have been done in the Toronto CF patient group, which generally harbours a single strain of Bc. More refined classification of Bc organisms, and their epidemic and virulence factors, may help to explain the extreme variability in clinical course in future analyses. Other factors which are included in regression models of survival and pulmonary function include: CFTR mutation group, sex, nutritional status, pancreatic function, and infection with *Pseudomonas aeruginosa* and other organisms. Interactions between Bc and other infecting organisms and the role of antimicrobial therapies are also of major importance in modelling CF lung disease.

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Abstract: **Pathogenicity factors of phytopathogenic *Burkholderia cepacia***

Authors: Carlos F. Gonzalez and Enrique G. Medrano

Burkholderia cepacia was first identified as a phytopathogen of onions. This plant pathogen is now recognized as a significant pathogen in nosocomial infections and in patients with cystic fibrosis. *B. cepacia* has now also become an important bioremediation agent because of its catabolic ability and a potential biocontrol agent for plant pathogens. Recent studies have identified five genomic species (genomovars) of this heterogenous microorganism. Our laboratory has reported that the gene encoding polygalacturonase, pehA (pectic enzyme hydrolase), in plant pathogenic *B. cepacia* is plasmid determined. We have mobilized the Tn5-Mob tagged Peh-encoding plasmid (pPEC321) into cured derivatives of the parental isolate, isolates of clinical origin, and non-phytopathogenic isolates of soil origin. All transconjugants expressed Peh activity but only the plant and clinical transconjugants containing plasmid pPEC321 expressed a Peh+ phenotype and plant macerating activity. The fact that the soil isolate produced and exported the Peh, but did not macerate plant tissue has been associated with its inability to cause water-soaking (wts) in plant tissue. In plants, the wts reaction that is observed is the result of plant cell electrolyte leakage. We have observed that plant isolates that have been cured of the Peh-encoding plasmid or that have a 35 kb deletion of the plasmid do not macerate plant tissue but still cause a wts reaction. This indicates that both wts and Peh activity may be necessary for plant tissue maceration. Isolates representing the five genomovars were obtained from the IBCWG collection (E. Mahenthiralingam). We also obtained genomovar III isolates from the collection of J. R. W. Govan. The isolates from both collections were evaluated for the wts phenotype, Peh activity, and the presence of the pehA gene cloned from the Peh-encoding plasmid. We have observed the wts phenotype in 16/16 genomovar III isolates tested. Genomovar II isolates did not express a wts phenotype, whereas representatives of genomovars I,IV, and V showed variable results. Culture supernatants of a Peh-negative phytopathogenic isolate and a genomovar III isolate indicate that an inducible extracellular product is involved in the production of the wts phenotype in plant tissue. We observed no Peh activity in the genomovar IV and V isolates tested. Peh activity was observed in some genomovar I, II, and III isolates. Southern blot hybridization studies indicate that of the genomovar I, II, and III isolates that expressed Peh activity, none of the resident plasmids from these isolates appeared to have homology to the pehA gene from the Peh-encoding plasmid. PCR analyses with specific primers for the cloned plasmid-encoded pehA gene, employing the same isolates, did not produce an amplified product. Isoelectric focusing of culture supernatant concentrates will determine if a different isozyme(s) of Peh is produced by these isolates.

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Abstract: **Risk Assessment and Regulation of Microbial Biopesticide Opportunistic Human Pathogens**

Doug Gurian-Sherman and William Yan

Microbes which are sold for the purpose of controlling pests must be registered at EPA in the United States and PMRA in Canada. Some of the organisms registered, or proposed for registration, are recognized opportunistic human pathogens, of which *Burkholderia cepacia* is the most prominent example. Registration requires human and environmental risk assessment. We will discuss this risk assessment process with emphasis on opportunistic human pathogens, and *B. cepacia* in particular. Aspects of the risk assessment process discussed will include: human toxicity and pathogenicity testing using animal models, taxonomy of clinical compared with biopesticide strains, consideration of virulence factors and host susceptibility, biopesticide usage patterns and exposure of susceptible populations.

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Abstract: **Epidemiology of *Burkholderia cepacia* in Canadian Cystic Fibrosis Patients**

Deborah Henry, Eshwar Mahenthiralingam, Jocelyn Bischof, Maureen Campbell, Peter Vandamme and David P. Speert

Our laboratory has been collecting *Burkholderia cepacia* complex isolates and closely related species which have been recovered from patients with CF in Vancouver, British Columbia, since 1981. During the last five years, we have also received isolates recovered from CF patients in the following provinces, British Columbia, Alberta, Manitoba, Ontario, Quebec, Newfoundland, Prince Edward Island, Nova Scotia and New Brunswick. Genetic typing of these bacteria has been performed by Random Amplified Polymorphic DNA (RAPD) analysis and recently confirmed by pulsed-field gel electrophoresis (PFGE). We have also evaluated these strains for the presence of the cable pilin subunit gene and *B. cepacia* epidemic strain marker (BCESM). The genomovar status of several strains has also been determined by whole-cell protein profile analysis and by a phylogenetic approach based on DNA sequence variation within the *recA* gene. Using these multiple approaches we have examined the epidemiology *B. cepacia* in the Canadian CF population (as measured by the *B. cepacia* complex strains submitted to our collection).

A total of 866 isolates recovered from 448 patients have been examined. *B. cepacia* genomovar III strains were the most prevalent genomovar recovered (82.6% of patients). *B. multivorans* was present in 8.2% of patients and the remaining genomovars (I, IV and *B. vietnamiensis*) accounted for 4.7% of patients . Examples of single strain types which were recovered from multiples patients were common in genomovar III, indicative of acquisition by patient-to-patient spread. The most common epidemic genomovar III strains were as follows, strain type 02 (74.9% of patients), strain type 04 (14.1%), strain type 01 (4.6%); all these latter strains encode the BCESM, but only strain type 02 encodes the cable pilus subunit gene. Approximately 25% of CF patients in Alberta from whom *B. cepacia* complex bacteria were recovered carried a single clone of *B. cepacia* genomovar IV, strain type 16. Instances of multiple patients sharing the same clone of *B. multivorans* were rare. Patients sharing a single clone of *B. cepacia* genomovar I or *B. vietnamiensis* has not been observed among Canadian CF patients.

In Vancouver, there have been sufficient numbers of CF patients infected with *B. cepacia* genomovar III (n = 43) and *B. multivorans* (n = 19) to statistically examine the clinical outcome associated with colonization of each of the latter organisms. Acqusition of *B. cepacia* genomovar III was associated with a 46% mortality rate, while *B. multivorans* colonization was associated with a 15% mortality rate. Evidence of patient-to-patient spread was apparent for *B. cepacia* genomovar III but not *B. multivorans*. Six cases of genomovar III strains replacing infection with *B. multivorans* were also encountered. Improved infection control policies and the separation of patients colonized genomovar III and *B. multivorans* appear to have reduced the spread of epidemic genomovar III strains at out treatment centres over the last two years. These studies were funded by grants from the Canadian Cystic Fibrosis Foundation.

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Abstract: **Characterization of a biocontrol strain of *Burkholderia vietnamiensis***
Authors: T.G. Lessie, H. Zhou, and F. Yao. Univ. Massachusetts, Amherst, USA

B. vietnamiensis BcF, a maize rhizosphere isolate demonstrated at the USDA Plant Protection Laboratory in Beltsville, MD, to confer resistance to fungal infections, was sent to us for genomic analysis by Dr. Dan Roberts. Analysis of macrorestriction fragments and randomly linearized replicons indicated this strain had a 7.5-Mb genome comprised of three large replicons of 3.5, 2.7, and 1.3 Mb. All three replicons appeared to contain *rrn* genes. The sum of the molecular weights of fragments generated by treatment of the DNA with *Ceu*I (which cleaves within 23S *rrn* genes) was equal to overall genome size. Preliminary experiments with a Tn*5* derivative carrying a *Swa*I site, suggest that it will be a suitable transposon for mapping the chromosomal distribution of key genes. Macrorestriction fragment analyses have revealed a significant background of genomic rearrangements in populations of this strain.

It seemed reasonable that analysis of functions related to plant colonization might provide insights into global regulatory mechanisms important for adaptation of *Burkholderia* species from a free-living to a host-associated state including their ability to function as an opportunistic pathogen for humans. Our initial strategy has been to screen for mutants blocked in extra-cytoplasmic functions. We have screened for mutants which fail to exhibit exoprotease or anti-yeast activity. One class of such mutants was altered with respect to cell-density-dependent expression of such functions.

Strain BcF produced at least two N-acyl-homoserine lactones which presumably govern the expression of different sets of genes. Two different bioassays were used to detect such autoinducers: *i*) ability of strain BcF to cross feed and restore pigment formation by an autoinducer-deficient mutant of *Chromobacterium violaceum*, and *ii*) ability of culture filtrates to elicit b-galactosidase formation by an autoinducer-deficient strain of *Agrobacterium tumefaciens* bearing a *traG*:*lacZ* fusion. The *Chromobacterium* assay presumably detects HHL, an autoinducer with a six-carbon side chain, and the *Agrobacterium* assay OHL, an autoinducer with an eight-carbon side chain. We have isolated mutant strains which appear to be blocked in formation of HHL. These strains failed to exhibit significant exoprotease or anti-yeast activity and appeared to overproduce siderophore(s). Culture filtrates of such mutants were still

able to elicit b-galactosidase formation suggesting that formation of OHL was unaffected.

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Abstract: **The role of quorum sensing in the production of virulence factors in *Burkholderia cepacia***
Authors: Lewenza, S. and Sokol, P.A.

We have recently identified the *cepIR* quorum sensing system in *Burkholderia cepacia*. Quorum sensing is a regulatory mechanism that couples gene expression to cell-density in response to a threshold concentration of signaling molecules or autoinducers. This phenomenon has been shown to regulate a variety of bacterial phenotypes including virulence factor production, conjugal plasmid transfer and bioluminescence.

Among the extracellular virulence factors produced by *B. cepacia*, the *cepIR* system has been to shown to play a role in siderophore and protease production but not lipase production. *B. cepacia* produces four types of siderophore molecules: ornibactins, salicylic acid, pyochelin and cepabactin. To date, we have shown that ornibactins are hyperproduced in *cepIR* mutants while salicylic acid and pyochelin production are unaffected in *cepR* mutants. The role of *cepR* in the regulation of cepabactin production has yet to be determined. In contrast, protease production is decreased in *cepR* mutants. Interestingly, the *cepIR* quorum sensing system appears to play both positive and negative regulatory roles in controlling virulence factor production.

Although the protease gene(s) has not been identified, our laboratory has recently identified two genes involved in the biosynthesis of ornibactin. We are currently investigating the regulation of these putative target genes to determine if *cepR* acts as a transcriptional regulator directly or whether it acts through an intermediary protein. The regulation of genes involved in iron acquisition is complex and often involves multiple layers of negative and positive regulatory elements.

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Abstract: ***Burkholderia cepacia* Research Laboratory and Repository**

The *B. cepacia* Research Laboratory and Repository, funded by the Cystic Fibrosis Foundation, was established in Spring 1997 to provide (a) confirmation of species identification of putative *B. cepacia* and (b) cataloging and storage of *B. cepacia* isolates recovered from persons with CF. The Lab has since expanded its role to provide genomovar and genotyping analyses to referring clinical microbiology laboratories. In collaboration with Drs. Peter Vandamme and Tom Coenye of the University of Gent, Belgium, advanced taxonomic analyses are performed on select isolates. These studies have contributed to a clearer understanding of the taxonomy of the *B. cepacia* complex (Bcc) and have identified several potential novel taxa among CF isolates.

Since June 1997 the Lab has received nearly 1100 isolates recovered from 648 patients receiving care in 116 treatment centers. A polyphasic evaluation including both phenotypic (selective media, biochemical tests and commercial test kits) and genotypic (genus- and species-specific PCR) analyses is used to confirm species. Among the 1012 isolates for which analyses are complete at this writing, 737 were identified as *B. cepacia* by the referring lab and 275 were either identified as another species or unidentified.

Among the 737 isolates referred as *B. cepacia*, 661 were confirmed as Bcc; the remaining 76 (10%) were NOT Bcc (60 were identified as belonging to one of 9 other species and 16 remain unidentified). Among the 281 isolates referred as unidentified or as another species, 175 (36%) were, in fact, identified as Bcc. Because this latter group of isolates represents a biased sample, we have undertaken a study in which ten participating clinical microbiology laboratories are referring *all* nonfermenting, nonenteric, gram negative bacteria cultured from CF sputum to us for analysis. This will provide a more reliable assessment of the rate with which Bcc may be misidentified as other species.

We have also begun an analysis of identification methods used by referring laboratories in order to determine the accuracy with which these identify Bcc. At least 10 commercial systems are used by the 116 referring laboratories; only a few laboratories use conventional biochemical tests to augment commercial systems. An analysis of the referring laboratories' methods as compared to our results will determine the positive predictive value of commercial systems in identifying Bcc. The results of this analysis may allow clinical laboratories to modify their current protocols for evaluation of CF sputum isolates.

Among the 648 patients from whom sputum isolates have been received, Bcc has been identified in 427. Among these, 249 (58%) are in genomovar I/III/IV, 156 (37%) are *B. multivorans*, 14 (3%) are *B. vietnamiensis*, and 8 (2%) are in Vandamme's "Group 13." Preliminary analysis indicates that the great majority of the isolates in the first category are, in fact, genomovar III. Thus, it appears that genomovar III and *B. multivorans* may account for 90% to 95% of Bcc recovered from CF sputum in the U.S. Data from the CF National Patient Registry will be used to investigate the relationship between genomovar and clinical outcome.

An analysis of isolates for the presence of putative virulence and/or transmissibility markers has been undertaken. *B. cepacia* epidemic strain marker (BCESM) and cable pili gene (*cblA*) have been sought among referred isolates by using both PCR and dot blot assays. Among the confirmed Bcc from 331 patients attending 85 centers, BCESM has been identified in 67 (34%) of 197 genomovar I/III/IV isolates; this marker has not been found in 130 *B. multivorans* nor in 4 *B. vietnamiensis*. No BCESM-positive Bcc were found among isolates received from 54 (63%) of the 85 centers; in 31 (28%) centers we detected a mix of BCESM-positive and -negative Bcc, while in the remaining 8 centers all Bcc were BCESM-positive. *cblA* has been detected only in the Edinburgh Toronto (ET12) clone.

We have provided genotyping analysis of confirmed Bcc for 36 centers. Nearly 250 isolates have been typed by using random amplified DNA (RAPD) typing and/or pulsed field gel electrophoresis (PFGE). We have identified a few large treatment centers in which the same "epidemic" Bcc clone is recovered from *all* colonized patients; other centers demonstrate a heterogenous population of Bcc. Most epidemic Bcc are BCESM-positive, although at least one epidemic clone affecting most patients at two large centers is BCESM-negative. We have also detected several CF patients at one center who appear to be colonized with the same *B. multivorans* clone. Another *B. multivorans* clone has been found to infect several newborn infants in a neonatal intensive care unit.

Note: Contributors to these studies include: Drs. Peter Vandamme, Tom Coenye, Esh Mahenthiralingam, Terry Stull, Paul Whitby, Preston Campbell, and Janet Shen. Other contributors include: Jennifer McMenamin, Theresa Zaccone, Lara Cartaya, Ahn Dang, Deborah Blecker-Shelly, and Jocelyn Bischof. The *B. cepacia* Research Laboratory and Repository and the studies detailed above are supported by Cystic Fibrosis Foundation (US).

Commercial Use of *Burkholderia cepacia*

To the Editor: In their review of the potential threat to human health by the commercial use of *Burkholderia cepacia*, Holmes *et al.* (1) focus on the biopesticidal uses of this bacterium in agriculture. By virtue of its ability to antagonize a number of soilborne plant pathogens, B. cepacia is an attractive natural alternative to currently used chemical pesticides, such as captan, mancozeb, and metalaxyl. The replacement of these highly toxic agents, which are among the mainstays of crop protection chemicals, by safer products is a laudable goal. However, despite being nonpathogenic to healthy humans (and thus classified as a Biosafety Level 1 species), *B. cepacia* can cause life-threatening pulmonary infection in persons with cystic fibrosis. Holmes *et al.* call for a moratorium on the use of *B. cepacia* in agriculture until more is known about risks from such use.

Perhaps of greater concern than agricultural use is *B. cepacia's* use as a bioremedial agent. Holmes et al. only briefly refer to the capacity of this species to degrade chlorinated aromatic substrates such as those found in certain pesticides and herbicides. By virtue of its extraordinary metabolic versatility, *B. cepacia* can use such compounds as nutrient carbon energy sources. In addition, some strains produce enzymes capable of degrading nonnutritive substrates, such as trichloroethylene (TCE), a major ground water contaminant used in the dry cleaning industry and in degreasing solvents.

The degree to which *B. cepacia* is being used in bioremediation products is unknown; however, the species has been used extensively to degrade ground water TCE contamination in at least one large U.S. city. A number of environment-friendly bioremediation products containing only naturally occurring, nonpathogenic bacteria are being marketed for use in drain opening and grease eradication systems. Because their formulations are proprietary, it is not known if these products contain *B. cepacia*; however, franchises that distribute such totally natural, noncorrosive, nontoxic products specifically target fast-food restaurants, photo processing facilities, and hospital radiology departments.

In the United States, the biopesticidal use of microorganisms such as *B. cepacia* is regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act; however, the use of naturally occurring, nonpathogenic bacteria as bioremedial agents is essentially unregulated. Only new microorganisms (i.e., intergeneric or formed by combining genetic material from organisms in different genera) are regulated by EPA under the Toxic Substances Control Act

(TSCA) (2). Ironically, TSCA regulations provide a strong disincentive to the development of safer microbiologic alternatives for use in bioremediation. For example, although the genetic elements responsible for TCE degradation by *B. cepacia* have been cloned, their recombination into another nonpathogenic bacterial host (e.g., *Escherichia coli*) would constitute a new microorganism, the licensure of which would be considered prohibitively time-consuming and expensive by many companies.

In Canada, biopesticidal uses of microorganisms are regulated by the Pest Management Regulatory Agency of Health Canada, under the Pest Control Products Act (PCPA); bioremedial uses are regulated by Environment Canada under the Canadian Environmental Protection Act (CEPA) (3). Both naturally occurring and genetically engineered microorganisms are strictly controlled under these acts. However, accurate species identification is the cornerstone of all notification of products under the Canadian regulations. This presents a further dilemma. At least five genomovars (discrete species) constitute what has recently been designated the "*B. cepacia* complex" (4). Insofar as the taxonomy of this group is poorly defined, there are no conventional taxonomic designations to distinguish pathogenic from nonpathogenic species. At present, it appears that all five *B. cepacia* genomovars are capable of causing infections in vulnerable persons (4).

Because the epidemiology of *B. cepacia* complex infection in humans is incompletely understood, the threat posed by the inclusion of this species in biopesticides and bioremedial products is difficult to quantify. However, we agree with Holmes *et al.* (1) that such use should be approached with considerable caution. In a broader context, the commercial use of *B. cepacia* illustrates our incomplete understanding of nonpathogenic bacteria and their potential to cause human disease. Regulations governing the use of microorganisms in industry must constantly adapt to keep pace with the emergence of infections due to nonpathogens and limit risk to human health.

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(A Letter to the Editor, Emerging Infectious Diseases, Vol. 5 (2), March-April 1999 [http://www.cdc.gov/ncidod/eid/index.htm])

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Abstract: **Phylogenetics of the *B. cepacia* complex based on the gene encoding RecA**
Authors: Eshwar Mahenthiralingam, Jocelyn Bischof, Sean K. Byrne, Peter Vandamme, Christopher Radomski, Yossef Av-Gay and Julian E. Davies.

Identification of *Burkholderia cepacia* is not straightforward and recent taxonomic studies demonstrated that the species is composed of five different genomovars (two of which have been proposed as new species: *Burkholderia vietnamiensis* and *Burkholderia multivorans*). As a collective these bacteria have been designated the *B. cepacia* complex. We are developing rapid molecular tests for speciation of these bacteria based on DNA sequence variation within genes that are shared by all members of the *B. cepacia* complex. Such tests are urgently needed by the clinical and research communities to determine the epidemiology of each *B. cepacia* genomovar. In addition, comparison of phylogeny of biocontrol/bioremediation strains with clinical strains may also assist in assessing the potential health risks of widespread commercial use of *B. cepacia*.

Genes encoding the RecA protein (*recA*) and 16S ribosomal RNA (16S rDNA) have been examined. The 16s rRNA gene demonstrated sufficient sequence variation for differentiation of *B. multivorans* and *B. vietnamiensis*, but not genomovars I, III, and IV. Analysis of *recA* revealed sufficient DNA sequence variation to identify all five current genomovars and also detect further subgroups within the *B. cepacia* complex. The complete DNA sequence of 20 *recA* genes from strains representative of all five current genomovars has been determined. Nucleotide sequence analysis has been performed by direct sequencing of PCR products. This state-of-the-art approach obviates errors which may be introduced by *Taq* polymerase since only the majority of PCR product templates, which are correct, will contribute nucleotide sequence detected (about 1 in 10,000 PCR products may have a single base error for a 1 kb amplicon and these incorrect products are too dilute to contribute to a signal in the sequencing reactions). From alignment of these sequences genomovar specific PCR primers have been designed and a phylogenetic tree of the *B. cepacia* complex constructed. The *recA* gene appears to be good target for simple molecular identification assays. Amplification has been 100% successful from over 300 *B. cepacia* complex isolates tested suggesting the gene is present in a very stable region of the *B. cepacia* genome which is not lost as a result of genomic plasticity of the species. It is a single copy gene and present on the largest chromosome of all *B. cepacia* strains tested (a previous map of strain ATCC 25416 incorrectly indicated two copies were present) and thus perhaps less susceptible to variation due to cross-over of multi-copy genes between chromosomes. Genomovar specific *recA* PCR primers have been designed and are currently being evaluated. The relevance of new groups detected by phylogenetic analysis of *recA* is also being investigated. To date no conflicting gene profiles have been detected (for example a 16s rDNA RFLP characteristic of *B. vietnamiensis* from a strain with a *recA* of genomovar I) suggesting that loss, gain or exchange of chromosomes are probably not mechanisms by which the *B. cepacia* genomovars have evolved. This work was funded by a grant from the Canadian Cystic Fibrosis Foundation.

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Abstract: **Speciation of the *B. cepacia* complex directly from crude CF sputum**

A. McDowell, K. Dunbar, J. E. Moore, K. Webb, E. Mahenthiralingam and J. S. Elborn*
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Our research interests have focused in part on the speciation of the *Burkholderia cepacia* complex. We have been using restriction fragment length polymorphism (RFLP) analysis of the PCR amplified *recA* gene sequence to identify genomovars in environmental and CF isolates and those present within crude CF sputum. The technique has proved both successful and reproducible and is now used routinely within our laboratory.

The method can be applied directly to DNA prepared from crude CF sputum which greatly reduces laboratory processing time required for identification of the particular organism. We have utilised this technique for the analysis of sputum from 100 patients attending a CF clinic in Manchester. Of all the samples analysed 13 were found to be positive for *B. cepacia*. These samples were then examined for genomovar status using the *recA* method. All were found to contain genomovar III strains (GIII; epidemic strains). However, samples were found to differ with respect to GIII phylogenetic cluster. Approximately 70% had GIII cluster A while the remainder were positive for GIII cluster B. The cable pili subunit gene (virulence determinant) was detected in many of the GIII A patients but none of the GIII B. In addition, *B. cepacia* epidemic strain marker (BCESM) was also found predominantly within the GIII A group. These results therefore demonstrate the potential of this technique for quick and routine analysis of sputum samples for genomovars. More specifically, since the genomovars differ with respect to virulence and transmissibility the results highlight the capacity of this method to provide information which may be crucial in the analysis of patient prognosis as well as potential for cross-infection within the CF centre.

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Project: **Participation with the Centre for Cystic Fibrosis in Children's Hospital in Prague**

As microbiologist with specialisation in antimicrobial therapy I am interested in the monitoring susceptibility bacterial pathogens occuring in cystic fibrosis patients, including *B. cepacia.*

On the basis our findings I provide also consultation in antimicrobial therapy. Ours laboratory experience especially with fluorochinolones, will be presented in the short lecture (Saturday's program).

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Project: **Biological control of plant diseases with *B. cepacia***

Burkholderia cepacia is naturally present in soil, water, and on plant surfaces. It is distinctive in its ability to metabolize a broad range of organic compounds as carbon and energy sources, an attribute which has spurred the development of *B. cepacia* for use in bioremediation of soil and groundwater contaminated with chlorinated hydrocarbons and herbicides. *B. cepacia* has also been the focus of considerable research by plant pathologists who have shown it to be an effective biocontrol agent against soilborne, foliar, and post-harvest diseases of plants. For example, certain strains of *B. cepacia* isolated from the root zone of plants are highly effective in suppressing Pythium damping-off disease when applied to seeds of vegetable crops before planting. Many strains of *B. cepacia* produce one or more antibiotics active against a broad range of plant pathogenic fungi. These antibiotics appear, in many cases, to be important for disease suppression. Biocontrol with *B. cepacia* can be an effective substitute for chemical pesticides which may pose risks to human health and the environment. *B. cepacia* strains also provide control of diseases that are not controlled by chemical pesticides. Three *B. cepacia* type Wisconsin strains are currently registered by the U. S. EPA for use as microbial pesticides (biological control agents). The products include Blue Circle and Deny (Stine Microbial Products). EPA is proceeding cautiously on applications for experimental use permits or registration of new *B. cepacia* strains for biocontrol because of the uncertainties regarding its potential risks. They have also required label revisions and use restrictions for current registrations. For example, labels of microbial pesticide products that contain *B. cepacia* have been revised to eliminate or greatly reduce inhalable aerosols and exposure to at-risk populations. Application to turf (previously the only spray application) has been eliminated altogether. Questions to be considered before additional strains of *B. cepacia* are approved for biocontrol of plant diseases include the following: 1) Is it possible to differentiate between non-pathogenic or biocontrol strains vs. human pathogenic strains? 2) Do naturally-occurring populations of *B. cepacia* in soil, water, and plant surfaces pose a hazard to CF patients? 3) Does use of biocontrol strains in agriculture increase the exposure of CF patients to *B. cepacia*? and 4) What label restrictions should be added to reduce potential risks associated with the use of *B. cepacia* in agriculture?

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Abstract: **Mechanisms of intrinsic and acquired multiple antibiotic resistance in *Burkholderia cepacia***
Authors: K. Poole, P. Segal, and R. Srikumar

Clinical strains of *B. cepacia* display high-level resistance to multiple antibiotics, a phenotype dubbed intrinsic multidrug resistance (MDR). While this complicates antimicrobial chemotherapy, the mechanisms that contribute to this innate resistance are, as yet, poorly defined. Other non-fermenting Gram-negative rods such as *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* make use of multidrug efflux systems which contribute to both intrinsic and acquired MDR in these organisms. Such systems have been described in the Burkholderia, including *B. cepacia*, where a three component MDR efflux system showing homology to the MDR efflux systems of *P. aeruginosa* and *S. maltophilia* has been described (CeoAB-OpcM). Using comparatively drug sensitive clinical strains of *B. cepacia* Genomovars III and IV, we have isolated MDR strains exhibiting elevated levels of resistance to several

agents. Using a strategy employed in *S. maltophilia* and *P. aeruginosa*, these were selected on fluoroquinolone antibiotics, which seem to be particularly adept at selecting strains exhibiting cross-resistance to multiple agents. Two classes of MDR strains were obtained. Class I MDR mutants exhibit enhanced resistance to quinolones, chloramphenicol, trimethoprim and tetracycline, with no change in resistance to b-lactams, aminoglycosides or macrolides. Class 2 MDR mutants showed enhanced resistance to quinolones and b-lactams, including the newer cephems. The later resistance was not attributable to changes in b-lactamase. We are currently assessing the possibility that these two mutant classes result from expression of two distinct MDR efflux systems which differing substrate specificity. Using an in vitro constructed deletion of the ceoAB-opcM MDR operon, we will be constructing knockouts in *B. cepacia* to assess the contribution of this efflux system both to intrinsic MDR and the MDR of class I mutants, whose resistance profile parallels that previously attributed to CeoAB-OpcM. Attempts are currently underway to clone the gene(s) responsible for the quinolone-b-lactam resistance of the class 2 mutants.

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Project: **Interaction of *B. cepacia* isolates with human bronchial epithelial cells**

We would like to present the interaction of *B. cepacia* isolates with human bronchial epithelial cells (NHBE) grown in multilayers at the air/liquid interface. We have compared the adherent, invasive and destructive properties of a CF clinical isolate, BC7 (genomovar III, RAPD 2, and Cbl+ve) with an environmental isolate ATCC 25416 (genomovar I, RAPD-?, Cbl-ve).

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Abstract: **Virulence Factors of *Burkholderia cepacia***
Authors: Sokol, P.A., P. Darling, S. Lewenza, C.R. Corbett, C. Kooi and M. Visser.

Our laboratory is interested in virulence mechanisms of *Burkholderia cepacia* and their role in lung disease. Our recent studies have focussed on siderophore mediated iron transport systems. We have determined that ornibactins, salicylic acid and pyochelin are the predominant siderophores produced by *B. cepacia* respiratory isolates and that cepabactin is not commonly produced by clinical isolates. Siderophore production profiles do not correlate with genomovar groupings. We have used a transposon mutagenesis strategy to identify a series of genes involved in ornibactin biosynthesis and uptake. Mutants deficient in ornibactin biosynthesis (*pvdA*-mutants) have been shown to be less virulent in both acute and chronic respiratory infection models. These infection model studies have shown that the ability of *B. cepacia* to produce and take up ornibactins is important for colonization and persistence in lung infections and also contributes to the pathological damage that occurs during these infections.

We have also identified and characterized a quorum sensing system in *B. cepacia* and determined that it is involved in the regulation of siderophores and proteases in this organism. The *cepIR* genes have been sequenced and allelic exchange or transposon mutants constructed in each of these genes. *CepIR* mutants hyperproduce ornibactins and have decreased protease production. This quorum sensing system does not appear to regulate lipase production. The *cepIR* genes are present in strains from all five genomovars of the *B. cepacia* complex. We are currently investigating the role of *cepIR* in the regulation of other potential virulence factors and examining in more detail their specific effects on ornibactin biosynthesis and uptake genes.

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Abstract: **Intracellular survival of *B. cepacia* in macrophages and amoebae**

We recently discovered that *B. cepacia* can survive within amoebae and monocytic macrophages. A remarkable feature with *B. cepacia* is that their survival could be quite prolonged (up to 17 days within amoebae and up to 6 days within macrophages) in the absence of substantial intracellular bacterial replication. This feature distinguishes *B. cepacia* from many other intracellular bacteria that in general replicate well within host cells. Our hypothesis is that *B. cepacia* have the property to resist intracellular killing, and that this feature is important in pathogenesis. *B. cepacia* strains are naturally resistant to non-oxidative killing mechanisms, and also they produce high levels of catalase/peroxidase, which may contribute to resist oxidative damage. We would like to gain insight into the genetics of *B. cepacia* pathogenicity, which according to our hypotheses, may be related to the distinctive properties of its outer membrane permeability and/or the ability of isolates to resist oxidative-killing. For this purpose, we are investigating the ability of clinical isolates of *B. cepacia* to resist oxidative damage and the role of this property in bacterial survival from intracellular killing by phagocytic cells. We also have constructed a library of polymyxin B (PmB)-sensitive transposon mutants. PmB is a cationic peptide that interacts with lipopolysaccharide (LPS). Some of these mutants exhibit a killing-sensitive phenotype upon infection of macrophages and amoebae, and they display defects in the LPS. We have sequenced the mutated genes and work is ongoing to characterize their functions.

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Abstract: **Species specific PCR for *Burkholderia gladioli***
Authors: P.W. Whitby, T.L. Stull, and J. LiPuma

A review of the CF sputum isolates forwarded to the U.S. *B. cepacia* Research Laboratory and Repository (BcRLR) has indicated a high percentage of isolates mis-identified by the submitting clinical laboratory. Since many CF centers cohort *B. cepacia* positive patients, incorrect identification of the isolate potentially exposes non-*B. cepacia* colonized individuals to patients colonized with *B. cepacia*. This is a major concern with *B. gladioli* since *B. gladioli* is commonly identified as *B. cepacia* in clinical laboratories. Previous attempts to distinguish these species have used a series of biochemical tests. These tests however do not reliably differentiate the species. Previous success of molecular approaches to distinguish the five "genomovars" of the *B. cepacia* complex led us to examine the possibility of a molecular analysis for the identification of *B. gladioli*. We have cloned three independent copies of the 23S rRNA loci from three independent clinical isolates. Each isolate was derived from sputum of patients with CF and have been phenotypically characterized by the BcRLR. We chose to derive clones from separate isolates to distinguish isolate-specific and species-specific regions of heterogeneity. Partial sequencing of the clones has indicated two regions that are putatively specific for *B. gladioli*, and opposing primer pairs have been manufactured. These were named LP1 and LP4. Preliminary studies have been performed using this primer pair in PCRs with templates from 45 isolates of bacterial species known to colonize CF patients and other Pseudomonas species of medical importance. The following microbial species were tested: *P. aeruginosa*, P. fluorescens ATCC13525, P. stutzeri ATCC17588, A*cinetobacter anitratus, Klebsiella pneumoniae* ATCC13883, Proteus mirabilis ATCC29906, *Stenotrophomonas maltophilia* ATCC13637, *Moraxella catarrhalis* ATCC25238, *B. gladioli* ATCC strains 10854, 19302, 10248, *Ralstonia solanacearum* ATCC10692, 11696, and *B. caryophylli* ATCC25418, 11441. In addition 16 clinical *B. gladioli* and 13 clinical *B. cepacia* strains were analyzed. All clinical isolates of *B. gladioli* and *B. cepacia* were phenotypically typed at the BcRLR. The results indicated both sensitivity and specificity of 100% for *B. gladioli*. Using this PCR method a number of strains previously published by other workers were examined. Several previously identified *B. gladioli* strains were obtained from the culture collection of the Clinical Microbiology Laboratories of the University Hospitals, Oklahoma City, OK. These represented several New Zealand isolates discussed by Wilsher et al. in a report detailing the nosocomial acquisition of *B. gladioli* {Wilsher Am. J. Resp. Crit

Care,1997}. These isolates were forwarded to BcRLR for phenotypic and biochemical analysis. PCRs were performed, blinded, with the primer pairs LP1 and LP4. Of the nine isolates previously identified as *B. gladioli*, only two were confirmed by the BcRLR as *B. gladioli*. The other isolates were *B. cepacia*, and in one instance *P. aeruginosa*. In concordance with this the PCRs detected the two confirmed *B. gladioli* isolates and were uniformly negative for the other isolates. We conclude that detection and identification of *B. gladioli* by molecular analysis may contribute significantly to the clinical care of patients colonized with *B. gladioli* and species with similar phenotypes.

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Abstract: **Risk Assessment and Regulation of Microbial Biopesticide Opportunistic Human Pathogens**
Doug Gurian-Sherman and William Yan (abstract listed under Doug Gurian-Sherman)

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Standardisation of Pulsed-Field Gel Electrophoresis (PFGE) for B. cepacia

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Following the last meeting of the IBCWG in Montreal in October 1998, one of the points detailed for further investigation was that relating to the standardisation of genotyping methods for B. cepacia. At this meeting, it was proposed that PFGE was the most suitable method for genotypic analysis of these organisms and that there should be an eventual move towards the harmonisation and standardisation of these methods in laboratories typing B. cepacia organisms. Employment of PFGE methodology may therefore be the most appropriate technique to further develop, in that it is largely a reproducible and repeatible method, which has been widely accepted and adopted into laboratories providing molecular epidemiological analysis of organisms. The diagnostic methodologies relating to the B. cepacia complex organisms is rapidly evolving in order to help provide answers to important clinical concerns over patient management and in particular, infection control issues. Presently we are experiencing exciting developments in (a). Primary Diagnostics through the development of new selective media (Henry et al) and employment of specific B. cepacia complex primers (Campbell et al., Whitby et al), (b). Genomovar typing by recA analysis (Mahenthiralingam et al) and by 16S-23S rRNA analyses (Lipuma et al; Whitby et al), © Virulence/Transmissibility Determinant Characterisation. It is therefore timely that we continue to focus attention at developing standard PFGE typing methods to aid with the epidemiology of these organisms at the sub-genomovar level.

After examining the PFGE protocols that various groups have submitted, it is apparent that there exists a large degree of variation with methods, protocols, reagents restriction enzymes used (& manufacturer), DNA extraction procedures, type of agarose employed, hardware used (& manufacturer), PFGE conditions, molecular weight markers used, internal standards used, as well as the software employed for cluster analysis of the resulting profiles. Therefore it could be postulated that with such variation in these parameters, this may be translated into variation with the resulting PFGE profiles and hence the genotypes attributed to such profiles. With this degree of variability, it would therefore be difficult to compare PFGE types from laboratories employing different methodologies, without first defining how such deviations from a consensus protocol may tangibly influence the data.

Consequently, it may be proposed that as various groups already have established PFGE as a genotyping technique in their laboratories and are further constrained by equipment type, purchasing agreements from consumable suppliers, etc., that it may not be feasible to draft an extremely rigid consensus protocol that could be fully adopted by everyone, but first examine how robust the methods are by carrying out a laboratory-based ring trial, employing an agreed basic protocol. To this end and to the eventual adoption of a consensus protocol, we could collectively agree a consensus protocol on the major issues associated with PFGE typing including macro restriction enzymes to be used, control strains, molecular weight standards, etc and propose a small study to examine this, as one of the outputs from Banff.