

International *Burkholderia cepacia* Working Group Meeting

April 6-8, 2001

Niagara-on-the-Lake, Ontario, Canada

April 6, 2001

John LiPuma from the University of Michigan presented information on the history of the US *B. cepacia* repository. CFF funding for the laboratory commenced in the spring of 1997. Since then, 5389 isolates have been received. 2914 isolates from 1305 CF patients have been characterized. Of these individuals, 853 from 158 CF centers in 112 US cities have been confirmed to be *B. cepacia*-infected. Data on 606 patients was presented:

Genomovar	# patients (%)	BCESM+	cblA +
I	16 (26)	0	9 (1.4)
II (<i>B. multivorans</i>)	229 (37.8)	0	0
III	303 (50)	141 (23.3)	1 (0.2)
IV (<i>B. stabilis</i>)	1 (0.2)	0	0
V (<i>B. vietnamiensis</i>)	31 (5.1)	0	0
VI	12 (2.0)	0	0
VII	4 (0.7)	0	0
Indeterminate	10 (1.6)	0	0

Other work in the laboratory includes RAPD and PFGE investigation of epidemic lineages. One pattern that has been identified is seeing patients acquiring new strains over time and switching genomovars. REP-PCR evaluation has also been used and has identified evidence of transmission of a single predominant strain from one CF center to another.

Deb Henry from the University of British Columbia presented data from the Canadian repository. The Canadian repository has been functioning since 1994 and currently receives approximately 100 isolates per year. They perform phenotypic characterization, RAPD typing, 16S RNA RFLP, and *recA* PCR typing. She presented epidemiological data from across Canada. There are ~3200 CF patients in Canada, cared for at 36 clinics in 10 provinces. Dividing the country into 4 regions (West, Ontario, Quebec, and East), there were distinctly different patterns:

	# positive patients (%)	% of isolates in repository
West	117 (12)	78
Ontario	285 (22)	86
Quebec	55 (5)	42
East	103 (25)	39

The distribution of genomovars seen in each region was also presented and was distinctly different from region to region.

Genomovar typing on 475 *B. cepacia*-complex infected CF patients was also presented:

Genomovar	# patients (%)
I	1 (0.2)
II (<i>B. multivorans</i>)	48 (10.1)
III	379 (79.8)
IV (<i>B. stabilis</i>)	15 (3.2)
V (<i>B. vietnamiensis</i>)	7 (1.5)
<i>B. fungorum</i>	1 (0.2)
<i>B. gladioli</i>	6 (1.3)
<i>Pandoraea spp.</i>	6 (1.3)
<i>B. cepacia</i> complex	5 (1.1)
Not otherwise identified	

A discussion of the utility and significance of genomovar typing with regard to prediction of clinical syndromes ensued. It was noted that in the Vancouver experience, genomovar IIIA strains were the only ones associated with “cepacia syndrome”. However, several other clinical scenarios were noted where “cepacia syndrome” was seen with infection with other genomovars. The need for definitions of clinical syndromes related to *B. cepacia* infection was discussed. Strain typing using RAPD-PCR and PFGE has identified several patterns within Canada, with the majority of ET 12 (strain type 2) isolates from Ontario. Increased mortality with strain type 4 was also noted.

John Govan presented some data from the UK repository. The repository in Edinburgh has been functioning unofficially since 1980, but was funded last year. They have the advantage of having collected longitudinal strains over 20 years. In addition, they have a number of epidemic clusters. Recently they have focused on determining the genomovar status of isolates, with the collaboration of Peter Vandamme and Esh Mahenthiralingam. They currently receive approximately 80 isolates per year.

Esh Mahenthiralingam presented data about the *recA* diversity of isolates. Current RFLP types are based on *recA* primers with 2 RFLP types in genomovar I, and one each in genomovar II, IV, V, VI, and VII. Genomovar III is more confusing with a IIIA cluster, a IIIB cluster, and a cluster that looks like IIIB, but is PCR negative, using specific primers. Some novel clusters based on *recA* have also been identified. *B. pyrrocinia* cross reacts with genomovar I primers and RFLP group K looks like genomovar I, but does not react with genomovar I primers. Other unique RFLP patterns include H2, S, AP, and the U/T group (which now looks like genomovar VIII).

Peter Vandamme reviewed the taxonomy of *B. cepacia*-complex. The first five genomovars were identified between 1992 and 1997 and 4 of the five have now received species names. Genomovar III remains hard to identify based on biochemical tests. All five of the original genomovars have been isolated from CF patients and from the environment. Some genomovar II-like organisms have recently been identified as genomovar VI which is not yet named, since it is still hard to distinguish from genomovar II. Typing with *recA* has improved the ability to differentiate genomovars I, III and IV, but has defined some additional heterogeneity. Genomovars VII (*B. ambifaria*) and VIII and *B. pyrrocinia* have also been identified from both

CF patients and the environment. He proposed that there are nine genomovars of *B. cepacia*-complex (*B. pyrrocinia* as genomovar IX). The majority of strains are now classified within these groupings.

April 7, 2001

***B. cepacia* and the environment**

Keynote speaker: Jim Tiedje, "Is soil home to *B. cepacia*?"

Do we have the proper identification of *B. cepacia* complex strains from the standpoint of the microbial ecologist? This is both a practical and a scientific issue. Practically, many microbial ecologists use a very casual identification schema for *B. cepacia*. Scientifically, both genetic studies and species descriptions need to be solidified to enable accurate identification.

Is there evidence that soil is a suitable home for *B. cepacia*? Is soil a true habitat where the organism can compete and grow? This would include the rhizosphere, detritus, moisture levels, pH, and nutrients. Is it possible that *B. cepacia* is just a visitor in soil? Indicators of this would be spatial isolation or evidence of being a weak competitor. What is the extent of survival of *B. cepacia* in the soil?

Why are microbial ecologists interested in *B. cepacia*? This is an organism with significant metabolic diversity including its substrate diversity with its abilities to utilize more than 30 different substituted aromatics and to produce oxygenase with strong catalytic capabilities, its tolerance and ability to survive (much better than organisms such as *E. coli*), and its prevalence. It is also an organism with tremendous genomic plasticity, which can play an important role in adaptation. The niche it inhabits is wide. Finally, it can be easily isolated from nature. Logically, given the high soil microbial diversity, if *B. cepacia* is in soil, it should be found in every gram of soil examined.

To examine the degree of endemism of *B. cepacia*, multiple examples of the same types of ecosystems across the world were studied. Natural preserves with little contact with humans were sought and soil was recovered from beneath the surface. The investigators were looking for organisms that could metabolize 3-chlorobenzoate. They isolated organisms with minimal passages and examined 16S RNA profiles. Two thirds of the organisms that metabolized 3-chlorobenzoate were *Burkholderia* species, close relatives of *B. cepacia*-complex. The organisms were also examined by BOX-REP PCR and were not identical.

Models of how to further examine *B. cepacia* in the environment were suggested, based on a study of fluorescent Pseudomonads. In addition, several action items were proposed:

1. Identify 10 strains whose sequences would help define *B. cepacia* from its neighbors and help define differences between environmental and pathogenic lines.
2. Can a *Burkholderia* genome team be identified to organize a larger scale effort for understanding the functions, ecology, and evolution? Can a unique case be made for such an effort?

3. What are the major ecological, evolutionary and systematic questions that need to be answered so that in 5 years unquestionable progress would be widely apparent?

Suzanne Miller presented an abstract entitled: "Detection of the *Burkholderia cepacia*-complex in soil from urban and suburban environments"

Carlos Gonzales presented an abstract entitled: "Isolation of soilborne Genomovar I, III, and VII *Burkholderia cepacia* and lytic phages with intergenomovar host range"

Genomics

Esh Mahenthalingam presented information on the sequencing of *B. cepacia* complex genomovar III strain, J2315, by the Wellcome Trust. The primary effort on the sequencing has not yet begun, but is expected to be in full swing with the majority of work in July, August and September of this year. The annotation should be complete by October 2002. The website for information is: http://www.sanger.ac.uk/Project/B_cepacia

Tracey Hunt presented an abstract entitled: "A signature tagged mutagenesis strategy in *Burkholderia cepacia* to identify genes required for *in vivo* growth and survival"

Lixia Liu presented an abstract entitled: "Identification of putative virulence genes in *Burkholderia cepacia*-complex genomovar III"

Melissa Ashlock from the American CF Foundation presented information on the CFFTI functional genomic initiative including the production and subsidization of gene expression arrays with Affymetrics. The goal of this initiative is to facilitate the use of genomics for drug discovery. A proposed model for *B. cepacia*, based on the work with *P. aeruginosa*, was presented. Based on the *P. aeruginosa* sequencing project, GeneChips are being produced as a tool for transcriptional profiling. These include the 5,549 protein-coding sequences from PAO1, 18 tRNA genes, representatives of the rRNA cluster, and probes from 117 CF-associated genes from strains other than PAO1. There are 13 probe pairs for each gene with each pair consisting of a perfectly matched sequence and one with a single base mismatch. The probes are 25 nucleotides in length and were selected for their uniqueness. The chips are currently being tested in the laboratory of Dr. Steve Lory. This is a web-based initiative with an application process to CFFTI. It includes the ability to receive chips at a subsidized price of \$150, the use of the Infomax/GenoMax software for data analysis and the possibility of "gene mining" from the database. The requirement from the CFF is that researchers apply and are approved and that they agree to provide data to the database at the CF national Bioinformatics at UNC within 6 months. The site for applications for access to GeneChips and the software is: <http://cfgenomics.unc.edu>.

Epidemiology and clinical microbiology

Susanna McColley presented a proposal to evaluate the impact of managed care on microbiology laboratory referrals. An outline of the study and a proposed survey form are in the abstracts.

Sylvia Campana presented an abstract entitled: "*B. cepacia*: is it an emerging pathogen only in cystic fibrosis patients?"

Cecilia Chaparro presented an abstract entitled: “Extrapulmonary complications with *B. cepacia* after lung transplantation”

Pathogenesis

Uma Sajjan presented an abstract entitled: “Cable/Adhesin positive *Burkholderia cepacia* adhere to and invade squamous differentiated bronchial epithelial cell cultures”

Tom Lessie presented an abstract entitled: “Quorum sensing systems of *Burkholderia ambifaria* BcF and *B. multivorans* 17616”

Ananda Chakrabarty presented an abstract entitled: “ α -2 macroglobulin-stimulated secretion of cytotoxic factors promoting phagocytic cell apoptosis by clinical isolates of *Burkholderia cepacia*”

Paola Cescutti presented an abstract entitled: “Structural investigation of exopolysaccharides produced by different clinical isolates of *Burkholderia cepacia*”

Joanne Goldberg presented an abstract entitled: “Expression of O antigens from *Burkholderia cepacia* serogroup O5 and *Pseudomonas aeruginosa* serogroup O17: evidence for horizontal gene transfer?”

Barbara Conway presented an abstract entitled: “Quorum sensing signal production in the *B. cepacia* complex”

Matt Lefebvre presented an abstract entitled: “Identification and mutagenesis of the oxidative defense enzymes in a clinical isolate of *Burkholderia cepacia*”

April 8, 2001

Discussion group presentations

Susanna McColley presented a summary of the Group A discussion (Management, Infection Control, Outcomes)

First she reviewed and reiterated the recommendations from the 2000 meeting:

- Assure CF patients world-wide have access to appropriate laboratory services and culture techniques for *B. cepacia*.
- Support consensus conferences on Infection Control in CF—the US CFF is planning such a conference in May
- Support the idea of national repositories, world-wide
- Identify appropriate resources to evaluate outcomes
- Support the ongoing multicenter trial for antibiotic synergy testing

New recommendations from 2001:

- Appropriate culture techniques of specimens from CF patients are essential for appropriate medical care—due diligence should ensue to see that this is being accomplished
- Assessment of patient outcomes, linking national registry to repository data (while protecting patient confidentiality) is essential
 - Identify mechanisms to increase the percentage of isolates sent to repositories (financial, physician/microbiologist education)
- Improve patient education world-wide by providing facts on transmissibility, virulence and risk reduction
 - There should be an opportunity for this in the context of Infection Control policies
 - Written materials may be particularly helpful
- A question that remains is: Should patients who “clear” *B. cepacia* be “de-isolated” and, if so, when?
 - Perhaps an extra algorithm should be used in the laboratory
 - PCR diagnosis may also be a part of this

Peter Vandamme presented a summary of the Group B discussion (Molecular epidemiology, Microbiology, Taxonomy, Ecology)

He first discussed gaps, priorities and things to do

- Taxonomy: we should continue to monitor the biodiversity of the organism
- Epidemiology:
 - What is the true environmental source of infection?
 - For genomovar III?
 - For genomovar II?
 - What are nosocomial sources
 - Can animals (food) be a source?
 - Why are there geographical differences in patient distribution?
- Ecology:
 - Can we quantitate the organism in soils?
 - Is it a visitor or a native organism?
 - Can it survive at the moisture and pH of soil?
- Diagnosis:
 - Can we develop better tools for the identification of genomovar III strains?
 - Can we improve the isolation and detection of organisms?
 - Is there a role for enrichment?
 - Is the discrepancy between culture and PCR detection genuine or methodological?
 - Minimal standards are not possible for isolation
 - However, for identification we now have good biochemical tests (Henry, JCM, 2001), 16S RNA methods (LiPuma, JCM, 1999; Segonds, JCM, 1999; Mahenthalingam, JCM, 2000), and RecA typing (Mahenthalingam, JCM, 2000)
 - One caveat is that genomovars VI, VII, VIII, and IX are relatively new, so their reactions and profiles are unpublished

With regard to the strain panel:

- The core group of isolates needs to be extended to cover new genomovars and more environmental strains
- A second group of strains that are commonly used need to have unifying information available, perhaps on the web. This should include special lineages (ET-12, the RAPD group strains that have been investigated in Canada, and the dominant US lineage). It would be ideal if this could include references and deposition of these strains in a public collection.

With regard to the genome project and the challenges presented by Dr. Tiedje:

- The group should identify up to 10 strains such that the whole genome comparison will give insight into:
 - Differences between pathogens and non-pathogens
 - The species concept
 - *Burkholderia* as a human, animal and plant pathogen
 - Opportunistic pathogens
 - Environmental species
 - Aspects of biocontrol and bioremediation
 - State of the art phenotypic, taxonomy data on all of the species
- Suggested strains include:
 - BccI (VII, IX)—very similar, just use type strain as choice
 - II (VI)
 - [IIIA]
 - IIIB
 - IV
 - V
 - VII
 - *B. gladioli*
 - [*B. mallei*]
 - [*B. pseudomallei*]
 - [*B. fungorum* (LB400)]
 - *B. sp nov* (root nodule)
- Dr. Tiedje suggested this would be a very reasonable pre-proposal for sequencing

John Govan presented a summary of the Group C discussion (Virulence, Pathogenesis, Animal models, Genomics)

The challenges as of the 2000 meeting in Bethesda:

- To identify “virulent” strains
- To identify “valid” screens and animal models
- To identify virulence determinants

The Niagara challenges included:

- Are all *B. cepacia* complex strains pathogens for:
 - Humans?
 - Plants?
 - Animals?

- Are they opportunistic pathogens?
- What is their potential for virulence?
- Post transplant infection may differ from “lung” infection

Consensus that was reached by the group included:

- Genomovar III is pathogenic (although not necessarily all strains in the genomovar)
- ET 12 is a reasonable candidate in which to identify virulence factors
- Other genomovars are less predictably virulent
 - Genomovar II often is
 - But for other genomovars, there are so few patients it is difficult to determine
 - They can clearly chronically colonize and there is an occasional blood isolate
 - There is not, however, enough data to say they are not pathogenic
- Possible virulence factors include:
 - Colonization (cblA, 22kDa adhesin, BCESM)
 - LPS
 - Antibiotic resistance, including to defensins
 - Efflux pumps
 - Siderophores
 - Catalases
 - Heme binding proteins
 - Invasion
 - Combinations of the above, regulated by quorum sensing
- There are many good models including animal and plant models and there was a consensus that not all models are good for evaluating all potential virulence factors. Investigators need to take into account the likely mechanism of pathogenesis and test in the most appropriate model.
- Current models include:
 - Agar bead/intranasal/aerosol administration
 - Rat/mouse/sheep
 - CF/CGD/neutropenia
 - Post-transplant infections
 - Plant models: bifunctional virulence factors
- Bacterial host interaction must also be considered:
 - ATPase/purinergic receptors
 - Hemolysins
 - Host nucleotide polymorphisms, such as the mannose binding lectin
 - Inflammation synergy and neutrophil priming (polymicrobial influence)

Infection models and antibiotic resistance

John Moore presented an abstract entitled: “Antibiotic resistance in *Burkholderia cepacia* at two regional cystic fibrosis centers in Northern Ireland: an update”

Jane Burns presented an abstract entitled: “Correlation between *in vivo* and *in vitro* models of invasion in the clinical setting of *Burkholderia cepacia* infections”

Chris Mohr presented an abstract entitled: “Role of type III secretion in the pathogenesis of *Burkholderia cepacia*”

Special topics

Tom Coenye: “The IBCWG website: an interactive research tool?”

Paul Whitby: “Development of an IBCWG list server”

Possible sites and dates for next year’s meeting were discussed including San Antonio, Texas and Prague, Czechoslovakia and dates in early April. It was felt that the lead time needed for a meeting in Europe would be too long to plan for 2002, but the possibility of a European meeting in 2003 was entertained—several additional sites were suggested. Carlos Gonzalez had checked into dates at the St. Anthony Hotel in San Antonio and either April 5-7 or 12-14, 2002 were available. A decision was planned for the next two weeks.