

Diagnostically and Experimentally Useful Panel of Strains from the *Burkholderia cepacia* Complex

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Two new species, *Burkholderia multivorans* and *Burkholderia vietnamiensis*, and three genomovars (genomovars I, III, and IV) currently constitute the *Burkholderia cepacia* complex. A panel of 30 well-characterized strains representative of each genomovar and new species was assembled to assist with identification, epidemiological analysis, and virulence studies on this important group of opportunistic pathogens.

The gram-negative bacterium *Burkholderia cepacia* is a problematic pathogen in patients with cystic fibrosis (CF) (18) or chronic granulomatous disease (CGD) (28) and in other vulnerable individuals (31). At least five genomovars constitute isolates which were previously classified as *B. cepacia*, and these strains have been collectively designated the *B. cepacia* complex (30). Bacteriological identification, epidemiological tracking, and virulence studies will all benefit from the use of a defined set of strains representative of each genomovar.

Assembly of a strain panel. A *B. cepacia* complex strain panel consisting of 30 strains representative of all five currently defined genomovars was assembled (Table 1). Strains were cultured as described previously (4, 11, 30) and deposited in the Belgium Coordinated Collections of Microorganisms/Laboratorium Microbiologie Ghent (BCCM/LMG) (<http://www.belspo.be/bccm/>) bacterial collection at the University of Ghent, Ghent, Belgium.

Genomovar analysis and strain typing. Genomovar testing was performed by whole-cell protein profile analysis as described previously (30). In addition, amplified fragment length polymorphism analysis (4) and sequence analysis of the *recA* gene (21) were used to confirm the classifications obtained by conventional analysis (30). Genetic typing of each strain was performed by random amplified polymorphic DNA (RAPD) analysis (19) and by pulsed-field gel electrophoresis (PFGE) (25) as described previously. The presence of the cable pilus subunit gene (*cblA*) (26) and *B. cepacia* epidemic strain marker (BCESM) were determined as described previously (20).

Genetic manipulation. Susceptibility to trimethoprim and transformation by electroporation with the broad-host-range vector pUC29T (32) were carried out as described elsewhere (1).

***B. cepacia* genomovar I.** Four strains representative of this genomovar were included in the panel (Table 1). Strain ATCC 25416^T, isolated from onions, has been genetically mapped (25) and well characterized phytopathologically (9). Strain ATCC 17759, also an environmental isolate, has been studied for its autoinducer production and potential for interspecies

signalling (22). Strain CEP509 was recovered from a patient with CF in Sydney, New South Wales, Australia (Table 1); isolates with RAPD fingerprints identical to those of strain CEP509 were recovered from three other CF patients attending this treatment center. Strain LMG 17997 was isolated in 1976 from human urine and persisted in the urinary tract of this patient for 10 years with no clinical symptoms of infection (Table 1).

***B. multivorans* (formerly *B. cepacia* genomovar II).** Eight *Burkholderia multivorans* strains were included in the panel (Table 1). Strain C5393 was recovered from a CF patient in Vancouver, Canada, and was not associated with patient-to-patient spread (19). Strain LMG 13010, the type strain of *B. multivorans*, was recovered from a Belgian CF patient and was also not associated with epidemic spread (24). Strain C1576 was recovered from a CF patient in Glasgow, Scotland, and was the index strain in an outbreak among 17 pediatric CF patients attending a treatment center in which five children died after colonization (33). Strain CF-A1-1 is a representative of an outbreak among four adult CF patients in Cardiff, Wales (23). Strain JTC was recovered from a patient with CGD and has been demonstrated to be resistant to nonoxidative killing by human neutrophils (28). Strain C1962 caused multiple brain abscesses in an immunocompetent individual (12). Strain ATCC 17616 is a soil isolate from the United States (29) and has been well characterized with regard to its metabolism, genetics, and genome structure (3, 6, 29). *B. multivorans* 249-2 was derived in the laboratory from ATCC 17616; it has suffered a genomic deletion resulting in a number of phenotypic alterations, including susceptibility to gentamicin (6), which is not a characteristic trait for strains of the *B. cepacia* complex (11).

***B. cepacia* genomovar III.** Ten genomovar III strains were included in the panel (Table 1). Four strains from the major transmissible lineage known as ET12 (14), the *cblA*⁺ strain (26), or RAPD type 2 (19, 20) were included. Strain J2315 was the index strain from which patient-to-patient spread of this lineage was first reported in Edinburgh, Scotland (10). This strain also produces a hemolysin capable of inducing apoptosis and degranulation in human neutrophils (13). Strain BC7 was recovered from a CF patient in Toronto, Ontario, Canada, and has been studied extensively with regard to binding to mucins or respiratory epithelial cells and cable pilus virulence factor (26). Strain K56-2 was also recovered from a CF patient in Toronto and has proven to be highly amenable to genetic

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TABLE 1. The *B. cepacia* complex strain panel

Strain name	Accession no. from BCCM/LMG Culture Collection	Source and location ^a	Strain type ^b	Presence of:		Transformation rate ^c	Reference(s)
				BCESM	<i>cblA</i>		
<i>B. cepacia</i> genomovar I							
ATCC 25416 ^T	LMG 1222 ^T	Onion, USA	01	–	–	10 ²	9, 22, 38
ATCC 17759	LMG 2161	Soil, Trinidad	15	–	–	10 ²	29
CEP509	LMG 18821	CF, Australia	18	–	–	10 ⁴	This study
LMG 17997	LMG 17997	UTI, Sweden	19	–	–	10 ²	This study
<i>B. multivorans</i>							
C5393	LMG 18822	CF, Canada	03 ^d	–	–	Negligible	19
LMG 13010 ^T	LMG 13010 ^T	CF, Belgium	09	–	–	10 ⁵	24, 30
C1576	LMG 16660	CF-e, UK	10	–	–	10 ⁴	30, 33
CF-A1-1	LMG 18825	CF-e, UK	11	–	–	Negligible	23
JTC	LMG 18824	CGD, USA	12	–	–	10 ⁵	28
C1962	LMG 16665	Clinic, UK	24	–	–	10 ⁶	12
ATCC 17616	LMG 17588	Soil, USA	14	–	–	10 ³	3, 6, 29, 30
249-2	LMG 18823	Laboratory, USA	14	–	–	10 ³	6
<i>B. cepacia</i> genomovar III							
J2315	LMG 16656	CF-e, UK	02 ^d	+	+	Negligible ^e	10, 13
BC7	LMG 18826	CF-e, Canada	02 ^d	+	+	Negligible ^e	26
K56-2	LMG 18863	CF-e, Canada	02 ^d	+	+	10 ⁴	5, 16
C5424	LMG 18827	CF-e, Canada	02 ^d	+	+	10 ^{3e}	19, 20
C6433	LMG 18828	CF-e, Canada	04 ^d	+	–	10 ^{3e}	19, 20
C1394	LMG 16659	CF-e, UK	13 ^d	+	–	10 ^{3e}	19, 27
PC184	LMG 18829	CF-e, USA	17 ^d	+	–	10 ^{5e}	17
CEP511	LMG 18830	CF-e, Australia	05	+	–	10 ⁶	19
J415	LMG 16654	CF, UK	23	–	–	10 ⁵	8
ATCC 17765	LMG 18832	UTI, UK	06	+	–	10 ⁵	29
<i>B. cepacia</i> genomovar IV							
LMG 14294	LMG 14294	CF, Belgium	16 ^d	–	–	10 ^{2e}	24
C7322	LMG 18870	CF, Canada	16 ^d	–	–	10 ³	19
LMG 14086	LMG 14086	Respirator, UK	16 ^d	–	–	10 ³	4
LMG 18888	LMG 18888	Clinical, Belgium	07	–	–	10 ³	31
<i>B. vietnamiensis</i>							
PC259	LMG 18835	CF, USA	08 ^d	–	–	10 ³	2, 15
LMG 16232	LMG 16232	CF, Sweden	20	–	–	Negligible	30
FC441	LMG 18836	CGD, Canada	21	–	–	10 ⁴	This study
LMG 10929 ^T	LMG 10929 ^T	Rice, Vietnam	22	–	–	10 ⁶	7, 30

^a CF, infection of a CF patient; CF-e, strain that has spread epidemically among patients with CF; CGD, infection of a CGD patient; UK, United Kingdom; UTI, urinary tract infection.

^b Strain types observed by RAPD and PFGE fingerprinting correlated for isolates examined in the panel; hence, each strain was given a single numerical strain type.

^c Transformation rate per microgram of DNA electroporated into competent cells is the average number of Tp^r colonies obtained from two independent experiments rounded up to the nearest log 10. Strains producing fewer than 100 Tp^r colonies per electroporation were considered as having a negligible transformation rate.

^d Strain type assigned for this study correlates to the numerical RAPD type assigned previously to this fingerprint pattern (19).

^e Strains requiring selection on LB agar containing 400-µg/ml trimethoprim sulfate to overcome background due to intrinsic resistance; pUCP29T transformants from all other strains were selected on Luria-Bertani agar containing 100-µg/ml trimethoprim sulfate.

manipulation, enabling characterization of siderophore production (5) and genes involved in quorum sensing (16). Strain C5424 was recovered from a CF patient in Vancouver (19) and was the isolate from which the BCESM DNA was cloned and characterized (20). Strain C6433 is a representative of *B. cepacia* RAPD type 4 strains which have spread among CF patients in Vancouver (19). Strain C1394 was responsible for an outbreak among CF patients attending a treatment center in Manchester, England (19, 27). Strain PC184 was recovered from a pediatric CF patient attending a treatment center in Cleveland, Ohio, and was examined in one of the earliest reports of transmission of *B. cepacia* among patients with CF (17). Genomovar III strain CEP511 was recovered from a CF patient in Sydney, New South Wales, Australia, and is also representative of an epidemic strain which had spread among several patients (19). Strain J415 was not associated with pa-

tient-to-patient spread (8) and does not contain either the BCESM or *cblA* gene (Table 1). This strain was the first reported case of *B. cepacia* syndrome in a CF patient in the United Kingdom; however, it did not transfer to the potentially susceptible CF sibling of the child involved (8). Strain ATCC 17765 was isolated in 1964 from a urinary tract infection of a child in Bristol, England (29).

***B. cepacia* genomovar IV.** Four genomovar IV isolates were included in the panel (Table 1). Strain LMG 14294 was isolated from sputum of a Belgian CF patient (24). A second patient from the same center carried an indistinguishable isolate; the clinical condition of both patients was stable (24). Genomovar IV strain C7322 was recovered from an adult CF patient attending a clinic in Vancouver; no other patients at this center were colonized with the same strain type (19). Strain LMG 14086 was isolated from a respirator in a hospital

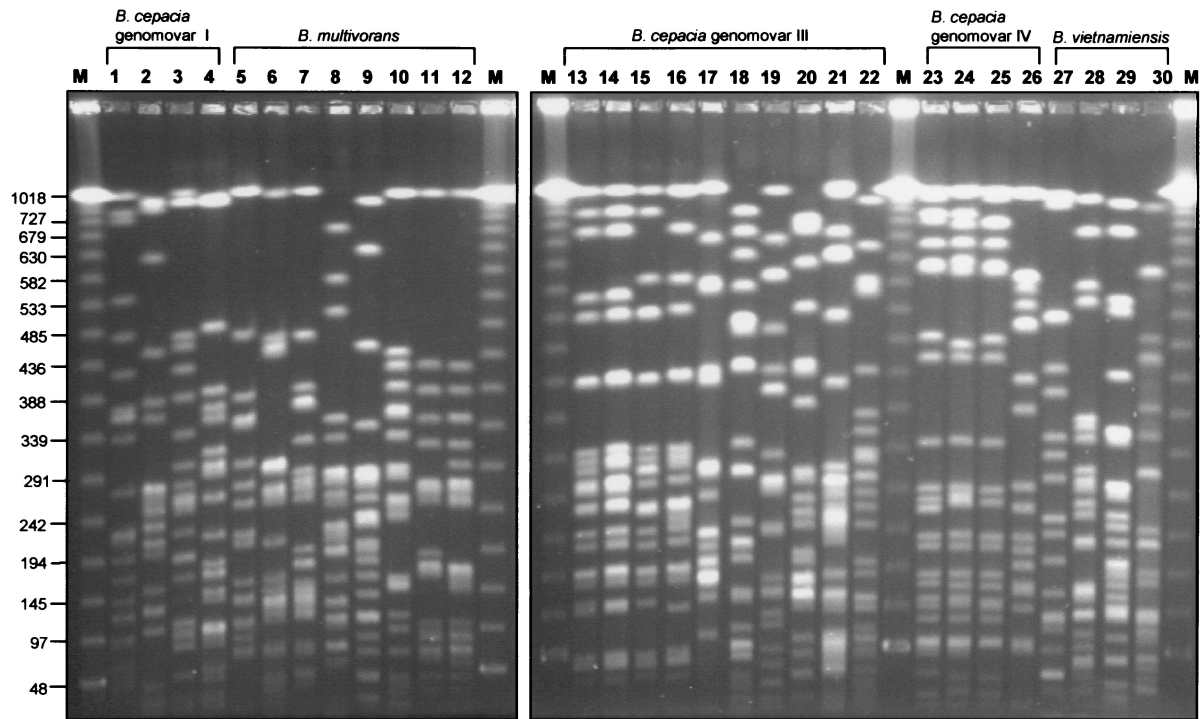


FIG. 1. *Spe*I-generated macrorestriction fragments of the *B. cepacia* complex strain panel separated by PFGE. Digestion and separation of macrorestricted DNA were performed as described in the text, and restriction fragments were visualized after staining with ethidium bromide. Molecular size standards were run in the lanes labelled M, and the sizes of relevant marker bands (in kilobases) are shown on the left. Strains analyzed (see Table 1) in each lane are as follows: 1, ATCC 25416^T; 2, ATCC 17759; 3, CEP509; 4, LMG 17997; 5, C5393; 6, LMG 13010^T; 7, C1576; 8, CP-A1-1; 9, JTC; 10, C1962; 11, ATCC 17616; 12, 249-2; 13, J2315; 14, BC7; 15, K56-2; 16, C5424; 17, C6433; 18, C1394; 19, PC184; 20, CEP511; 21, J415; 22, ATCC 17765; 23, LMG 14294; 24, C7322; 25, LMG 14086; 26, LMG 18888; 27, PC259; 28, LMG 16232; 29, FC441; 30, LMG 10929^T.

in the United Kingdom (4). Strain LMG 18888 is a non-CF isolate involved in an outbreak in a cardiology ward in Belgium (31).

***B. vietnamiensis* (formerly *B. cepacia* genomovar V).** Four *Burkholderia vietnamiensis* strains, three of which were recovered from patients with CF, were included within the panel (Table 1). *B. vietnamiensis* PC259 was recovered from a CF patient attending a treatment center in Seattle, Washington

(15), and subcultures from the same patient have been shown to invade respiratory epithelial cells in culture (2). Strain LMG 16232 was recovered from a CF patient in Sweden. Strain FC441 was recovered from a 9-year-old boy with X-linked recessive CGD who was treated in Vancouver and survived septicemia with multiple-organ involvement (Table 1). Finally, *B. vietnamiensis* LMG 10929 is the type strain for this species (7) and was recovered from rice rhizosphere in Vietnam.

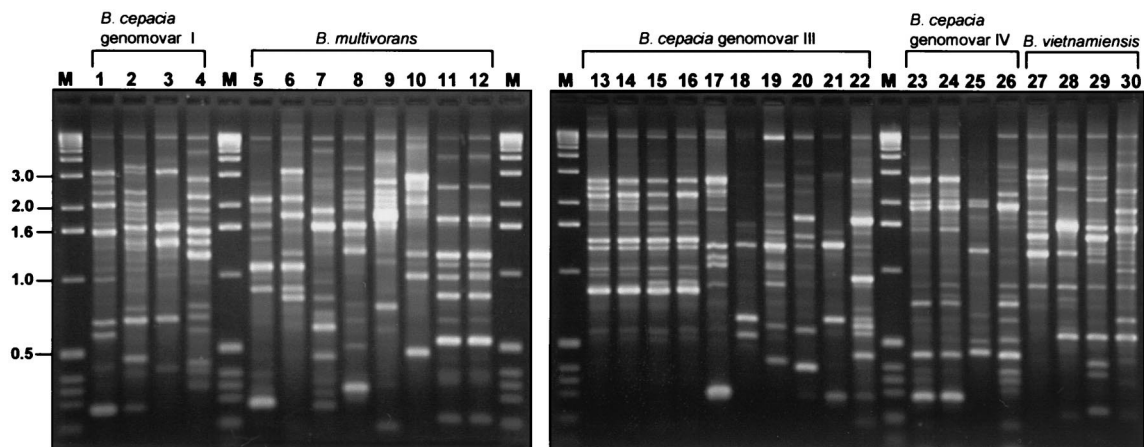


FIG. 2. RAPD fingerprints generated by PCR primer 270 from DNA extracted from strains of the *B. cepacia* complex panel. RAPD analysis of each *B. cepacia* strain from the panel was performed exactly as described previously (19). Molecular size standards were run in the lanes labelled M, and the sizes of relevant marker bands (in kilobases) are indicated to the left. Strains analyzed (Table 1) in each lane are as follows: 1, ATCC 25416^T; 2, ATCC 17759; 3, CEP509; 4, LMG 17997; 5, C5393; 6, LMG 13010^T; 7, C1576; 8, CP-A1-1; 9, JTC; 10, C1962; 11, ATCC 17616; 12, 249-2; 13, J2315; 14, BC7; 15, K56-2; 16, C5424; 17, C6433; 18, C1394; 19, PC184; 20, CEP511; 21, J415; 22, ATCC 17765; 23, LMG 14294; 24, C7322; 25, LMG 18888; 26, LMG 14086; 27, PC259; 28, LMG 16232; 29, FC441; 30, LMG 10929^T.

Genetic heterogeneity. Analysis by RAPD and PFGE fingerprinting demonstrated that the strains selected for the panel were, for the most part, genetically heterogeneous, representing 24 different *B. cepacia* complex strain types (Table 1). Strain types detected by PFGE (Fig. 1) and RAPD (Fig. 2) correlated exactly, and each method was able to type strains from all five genomovars. Three groups of strains were clonal (Table 1): *B. multivorans* strain ATCC 17616 and its laboratory derivative 249-2, the four ET12 strains (J2315, BC7, K56-2, and C5424), and three genomovar IV strains (LMG 14294, C7322, and LMG 14086). The remaining 21 strains within the panel each possessed a unique genetic fingerprint, and each was designated with an individual strain type (Table 1 and Fig. 1 and 2).

Strains suitable for genetic manipulation. Each genomovar possessed a strain which was readily transformable with plasmid DNA encoding a trimethoprim resistance marker, indicating that they may be useful as genetic tools (Table 1). Strain K56-2 (genomovar III) appears to be a particularly useful strain for genetic analysis. It is representative of the major epidemic CF clone (10, 14, 19) and has already proven highly amenable to molecular characterization by a number of different strategies, including transposon mutagenesis, site-directed mutagenesis by allelic exchange, and genetic complementation (16).

In terms of diversity, the panel is representative of the large variety of clinical infections, environments, and geographic locations from which *B. cepacia* complex strains may be recovered; however, the prevalence of each genomovar in both clinical and natural settings remains to be determined by systematic study.

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