Medical/Scientific

Identification of *Burkholderia cepacia* complex bacteria

Peter Vandamme, PhD
Professor
Laboratory of Microbiology, Faculty of Sciences
Ghent University
Belgium

The *Burkholderia cepacia* complex (Bcc) is a versatile group of bacteria that occupies diverse ecological niches such as soil, water, animals, plants and humans. Although originally known as plant pathogens, Bcc bacteria are also recognized for improving plant health and for their use for the degradation of environmental pollutants. Bcc bacteria are typically not pathogenic for healthy humans. However, the lungs of cystic fibrosis (CF) patients are unusual in many ways and this includes also the repertoire of organisms that is able to cause infections therein. Although not very commonly isolated Bcc bacteria emerged as notorious opportunistic pathogens in CF patients. They are particularly feared for their potential patient-to-patient spread, their inherent resistance to antimicrobial therapy, the risk of 'cepacia syndrome' (a rapidly proceeding pneumonia, sometimes accompanied by septicaemia) and the marked reduction in overall life expectancy (Govan et al., 2007). For these reasons, strict cross-infection control guidelines have generally been issued.

Essential for cross-infection control is the rapid and accurate identification of potential Bcc bacteria when isolated from CF specimens. This has been hampered by the recognition of a growing number of species belonging to this group of closely related bacteria, their limited role as pathogens for the general public and their absence in databases of commercial identification systems. The substantial interest in Bcc bacteria triggered a long term study of the natural biodiversity of Bcc-like bacteria which started in the early 1990s and which is still ongoing. A first study, published in 1997, revealed that *B. cepacia* like isolates, cultured from clinical or environmental specimens, belonged to at least five distinct genomic species, referred to collectively as the *B. cepacia* complex (Vandamme et al., 1997). Subsequent studies performed by members of the International *Burkholderia cepacia* Working Group (established in 1996 as "… a forum for clinicians and scientists interested in advancing knowledge of *B. cepacia* infection in persons with cystic fibrosis through the collegial exchange of information and promotion of coordinated approaches to research"; http://go.to/cepacia) revealed an even higher diversity and gradually new Bcc species were described. Currently 17 Bcc species have been reported and formally named. Table 1 presents an overview of these species and their isolation sources. Nearly all of these Bcc bacteria have been isolated from CF and environmental sources. However, taken together, two of them account for the large majority of Bcc bacteria isolated from CF patients worldwide: *Burkholderia multivorans* and *Burkholderia cenocepacia*. Whereas the latter is best known to be capable of patient-to-patient spread, the former seems to become more common among recently infected CF patients both in European and North American CF centers (Govan et al., 2007; LiPuma, 2007)

Table 1. Overview of *B. cepacia* complex species and their sources of isolation (CF, cystic fibrosis).

Name	Habitat	Reference
B. cepacia	Human (CF and non-CF), soil, rhizosphere soil, plant, water	Vandamme et al., 1997
B. multivorans	Human (CF and non-CF), soil, rhizosphere soil, plant material, water, industrial contaminant	Vandamme et al., 1997
B. cenocepacia	Human (CF and non-CF), animals, soil, rhizosphere soil, plant, water, industrial contaminant	Vandamme et al., 2003
B. stabilis	Human (CF and non-CF), rhizosphere soil, hospital	Coenye et al., 2001

	equipment	
B. vietnamiensis	Human (CF and non-CF), Soil, rhizosphere soil, plant material, animal	Coenye et al., 2001
B. dolosa	Human (CF), plant material, rhizosphere soil	Vermis et al., 2004
B. ambifaria	Human (CF), soil, rhizosphere soil	Coenye et al., 2001
B. anthina	Human (CF), animals, soil, rhizosphere soil, river water	Vandamme et al. 2002
B. pyrrocinia	Human (CF and non-CF), soil, rhizosphere soil, water	Vandamme et al. 2002
B. ubonensis	Human (non CF), soil	Vanlaere et al., in press
B. latens	Human (CF)	Vanlaere et al. 2008
B. diffusa	Human (CF and non-CF), soil, hospital equipment	Vanlaere et al. 2008
B. arboris	Human (CF and non-CF), soil, rhizosphere soil, water, industrial contaminant	Vanlaere et al. 2008
B. seminalis	Human (CF and non-CF), Plant material, rhizosphere soil	Vanlaere et al. 2008
B. metallica	Human (CF)	Vanlaere et al. 2008
B. contaminans	Human (CF and non CF), soil, animal, hospital equipment	Vanlaere et al., in press
B. lata	Human (CF and non CF), soil, plant material, water	Vanlaere et al., in press

The ongoing diversity studies also revealed a growing list of species that are regularly misidentified as Bcc bacteria. These include well-known bacteria such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans,* but also several previously unknown organisms including *Pandoraea, Cupriavidus* and *Inquilinus* species. Although most of these species are quite uncommon and are not considered true pathogens, it is of prime importance that they are not misidentified as Bcc bacteria.

Identification of *B. cepacia* complex bacteria

Methods for the identification of Bcc organisms must distinguish Bcc organisms from a variety of gram-negative bacteria and if possible, allow discrimination between Bcc species, in particular the predominant species in CF infections *B. multivorans* and *B. cenocepacia*. The current Bcc infection control policy is not species dependent. However, species level identification of Bcc is essential for epidemiological surveillance and for enabling the clinical significance of less common species to be established (Govan et al., 2007). In addition, identification methods should be relatively quick and easy to perform, given the clinical relevance of these organisms and the relatively large number of isolates involved (Coenye et al., 2001b; Vandamme et al., 2007).

In diagnostic clinical laboratories identification of putative Bcc isolates is performed using a combination of selective media, conventional biochemical analysis, commercial test systems and PCR-based assays if available. The use of selective media is very important when culturing CF specimens that regularly contain a mixed flora which, unless inhibited, may overgrow Bcc organisms. Several selective media for Bcc bacteria have been described but none of these is truly fully selective. Although growth of many other bacteria is inhibited, a significant number of CF microbes are able to grow on all of them. Among these selective media, BCSA and Mast *B. cepacia* medium supported growth of Bcc isolates most efficiently whereas BCSA was reported superior for the specificity and rapidity to recover Bcc organisms from CF sputum samples (Vandamme et al., 2007). The complexity of the Bcc diagnostic problem is further enhanced when searching for environmental sources of infection as these environmental sources naturally comprise a much larger diversity of bacteria and, not surprisingly, many of them are able to grow on these media which should more appropriately be referred to as semi-selective.

Following the isolation of pure cultures, putative Bcc bacteria will typically be examined by traditional biochemical tests or by using commercial identification systems. As mentioned above identification results obtained this way should be carefully considered as Bcc bacteria and most other uncommonly isolated bacteria from CF specimens are not or not adequately represented in the commercial identification systems and misidentification occurs very frequently. Especially the first isolation of such bacteria from a CF individual requires molecular tests for confirmation of the identity of the bacterium involved. If these molecular tests are not available the isolates should be sent to a Bcc reference laboratory for confirmation (**http://go.to/cepacia**). The molecular tests include a range of species specific PCR tests, and sequence or restriction profile analysis of individual or multiple genes. More recently, new analytical tools such as MALDI-TOF (matrix assisted laser desorption ionisation time of flight) mass spectrometry or FT-IRS (fourier transform infrared spectroscopy) have been used successfully for the identification of putative Bcc bacteria.

Many of these methods are complex or require expensive equipment and are therefore restricted to use in reference laboratories. Among these, species-specific PCR tests are technically the most simple. A range of primers has been designed for Bcc bacteria and bacteria commonly misidentified as such. It should be noted that several PCR based tests were developed prior to our present knowledge of the taxonomic complexity of Bcc bacteria and their specificity and sensitivity often needs evaluation. Among the most evaluated and best performing tests are a *recA* based test to identify *B. multivorans* and a *recA* based test which identifies all Bcc bacteria in one assay as members of the Bcc complex (Mahenthiralingam et al., 2000). The identification of *B. cenocepacia* by *recA* based tests requires multiple assays which are not fully specific; however, very recently a *repA* based PCR test with high specificity and sensitivity has been reported (Drevinek et al., 2008). In addition, specific PCR tests for those species which are most commonly misidentified as Bcc species, such as *Cupriavidus*, *Ralstonia*, *Burkholderia gladioli, Pandoraea* and *Stenotrophomonas* are available (Coenye et al., 2001a, 2001b, 2005; da Silva Filho et al., 2004; Liu et al., 2002; Spilker et al., 2004; Whitby et al., 2000).

Peter Vandamme, PhD

Amplification of 16S rRNA or *recA* genes, followed by restriction profile analysis has been described for the identification of Bcc bacteria. Whereas the former generated profiles that were not sufficiently discriminatory (Vermis et al., 2002), the latter yielded multiple restriction profiles for each of the Bcc species and several restriction profiles were shared among different Bcc species (Vanlaere et al., in press). Direct sequence analysis of 16S rRNA genes proved again not sufficiently discriminatory to distinguish between different Bcc species. *recA* sequence analysis, in contrast, is a highly specific identification approach which mostly offers a clear differentiation between Bcc species. More recently a multi-gene sequence based identification approach, known as MLST (multi locus sequence typing), was described (Baldwin *et al.*, 2005). The method has the important advantage to allow simultaneous species level identification but also individual strain characterisation and is therefore considered superior by many.

Finally, basic scientific research is providing researchers with a number of new sophisticated analytical tools such as MALDI-TOF mass spectrometry (Vanlaere et al., 2008) and FT-IRS (Bosch et al., 2008). Application of these technologies requires expensive equipment and considerable scientific and technical expertise. However, the first studies demonstrated that these novel approaches allow to identify most Bcc species and bacteria that are commonly misidentified as such with high precision, at low cost and very rapidly. Especially in the context of a reference laboratory with dedicated staff such technologies may prove very useful in the future.

In summary, the care and concern for CF patients prompted researchers to thoroughly examine bacteria present in CF respiratory specimens which revealed an unexpected high microbial diversity. Although surveillance studies revealed that only few of these have true clinical relevance, the rapidly evolving insights in the natural diversity of the bacteria in the CF lungs pose a considerable challenge to diagnostic laboratories and to the optimal management of CF patients for whom misidentification may have serious psychosocial, prognostic and health care implications.

References

Baldwin, A., E. Mahenthiralingam, K. M. Thickett, D. Honeybourne, M. C. J. Maiden, J. R. W. Govan, D. P. Speert, J. J. LiPuma, P. Vandamme and C. G. Dowson. 2005. Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. J. Clin. Microbiol. 43:4665-4673.

Bosch, A., A. Miñán, C. Vescina, J. Degrossi, B. Gatti, P. Montanaro, M. Messina, M. Franco, C. Vay, J. Schmitt, D. Naumann and O. Yantorno. 2008. Fourier transform infrared spectroscopy for rapid identification of nonfermenting gram-negative bacteria isolated from sputum samples from cystic fibrosis patients. J. Clin Microbiol. 46:2535-2546.

Coenye, T., L. Liu, P. Vandamme and J. J. LiPuma. 2001a. Identification of *Pandoraea* species by 16S ribosomal DNA-based PCR assays. J. Clin. Microbiol. 39:4452-4455.

Coenye, T., T. Spilker, R. Reik, P. Vandamme, and J. J. LiPuma. 2005. Use of PCR analyses to define the distribution of *Ralstonia* species recovered from patients with cystic fibrosis. J. Clin. Microbiol. 43:3463-3466.

Coenye, T., P. Vandamme, J. R. W. Govan, and J. J. LiPuma. 2001b. Taxonomy and identification of the *Burkholderia cepacia* complex. J. Clin. Microbiol. 39:3427-3436.

da Silva Filho, L.V., A.F. Tateno, L. de F.Velloso, J. E. Levi, S. Fernandes, C. N. Bento, J. C. Rodrigues, and S. R. Ramos. 2004. Identification of *Pseudomonas aeruginosa, Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* in respiratory samples from cystic fibrosis patients using multiplex PCR. Pediatr Pulmonol. *37,* 537-547.

Drevinek, P., A. Baldwin, C. G. Dowson, and E. Mahenthiralingam. 2008. Diversity of the parB and repA genes of the *Burkholderia cepacia* complex and their utility for rapid identification of *Burkholderia cenocepacia*. BMC Microbiol. 7:44.

Govan, J. R., A. R. Brown, and A. M. Jones. 2007. Evolving epidemiology of *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex in cystic fibrosis lung infection. *Future Microbiol* 2, 153-164

LiPuma, J. J. 2007. Update on *Burkholderia* nomenclature and resistance. Clin. Microbiol. Newsletter 29:65-69.

Liu, L., T. Coenye, J. L. Burns, P. Whitby, T. L. Stull, and J. J. LiPuma. 2002. Ribosomal DNA-directed PCR for identification of *Achromobacter (Alcaligenes)* xylosoxidans recovered from sputum samples from cystic fibrosis patients. J. Clin. Microbiol. *40*, 1210-1213.

Mahenthiralingam, E., J. Bischof, S.K. Byrne, C. Radomski, J.E. Davies, Y. Av-Gay, and P. Vandamme. 2000. DNA-based diagnostic approaches for the identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. J. Clin. Microbiol. 38:3165-3173.

Spilker, T., T. Coenye, P. Vandamme, and J. J. LiPuma. 2004. PCR-Based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. J. Clin. Microbiol. 42:2074-2079

Vandamme, P. , J. R. W. Govan and J. J. LiPuma. 2007. Diversity and role of *Burkholderia* spp., p. 1-28. *In* T. Coenye and P. A. R. Vandamme (eds.). *Burkholderia* genomics Horizon Scientific Press, Norwich, United Kingdom.

Vandamme, P., D. Henry, T. Coenye, S. Nzula, M. Vancanneyt, J. J. LiPuma, D. P. Speert, J. R. W. Govan, and E. Mahenthiralingam. 2002. *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia,* two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. FEMS Immunol. Med. Microbiol. 33:143-149.

Vandamme, P., B. Holmes, T. Coenye, J. Goris, E. Mahenthiralingam, J. J. LiPuma, and J. R. W. Govan. 2003. *Burkholderia cenocepacia* sp. nov. - a new twist to an old story. Res. Microbiol. 154:91-96

Vandamme, P., B. Holmes, M. Vancanneyt, T. Coenye, B. Hoste, R. Coopman, H. Revets, S. Lauwers, M. Gillis, K. Kersters, and J.R.W. Govan. 1997. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int. J. Syst. Bact. 47:1188-1200.

Vanlaere, E., J. J. LiPuma, A. Baldwin, D. Henry, E. De Brandt, E. Mahenthiralingam, D. Speert, C. Dowson and P. Vandamme. 2008. *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. Int. J. Syst. Evol. Microbiol. 58:1580-1590.

Vanlaere, E., K. Sergeant, P. Dawyndt, W. Kallow, M. Erhard, H. Sutton, D. Dare, B. Devreese, B. Samyn and P. Vandamme. 2008. Matrix-assisted laser desorption-ionisation-time of flight mass spectrometry of intact cells allows rapid identification of *Burkholderia cepacia* complex. J. Microbiol. Meth. 75:279-286

Vanlaere, E., A. Baldwin, D. Gevers, D. Henry, E. De Brandt, J. J. LiPuma, E. Mahenthiralingam, D. Speert, C. Dowson and P. Vandamme. Taxon K, a complex within the *Burkholderia cepacia* complex comprises at least two novel species: *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int. J. Syst. Evol. Microbiol. (in press)

Vermis, K., T. Coenye, J. J. LiPuma, E. Mahenthiralingam, H. J. Nelis, and P. Vandamme. 2004. Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int. J. Syst. Evol. Microbiol. 54:689-691.

Vermis, K., C. Vandekerckhove, H. J. Nelis and P. Vandamme. 2002. Evaluation of restriction fragment length polymorphism analysis of 16S rDNA as a tool for genomovar characterisation within the *Burkholderia cepacia* complex. FEMS Microbiol. Lett. 214:1-5.

Whitby, P.W., L. C. Pope, K.B. Carter, J. J. LiPuma, and T. L. Stull. 2000. Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. J. Clin. Microbiol. *38*, 282-285.