



## Identification and characterisation of *Burkholderia cepacia* complex

*Burkholderia cepacia* complex bacteria were first described in the 1940s as a disease of onion bulbs and were considered to be a single species, named *Pseudomonas cepacia*. Over the following decades, as molecular taxonomic approaches developed in sophistication, it became clear that *Pseudomonas cepacia* required a genus of its own and from this, the genus *Burkholderia* was created. To date, there are 60 validly named *Burkholderia* species and a number of candidate species.

*Burkholderia* species are characterised by their large genomes and an unusually diverse array of variable phenotypes, including the ability to degrade complex hydrocarbons, and a well-documented association with plant rhizospheres. These properties have made them of particular interest in biocontrol, bioremediation and plant growth promotion but their role in human infection has hindered attempts to utilise these properties. The versatility that makes these organisms so interesting poses a significant challenge when attempting to identify them.

*Burkholderia cepacia* infection was identified as an increasing problem in North American cystic fibrosis centres during the 1970s and 1980s and was associated with increased morbidity and mortality in infected individuals (CF Trust, 2004). As well as generally decreasing life expectancy, *Burkholderia cepacia* infection may cause a fatal pneumonia, sometimes associated with septicaemia, and this is a significant cause for anxiety in colonised individuals.

In 1997, Vandamme *et al* performed a polyphasic taxonomic study analysing a number of *B. cepacia* isolates from cystic fibrosis patients (Vandamme *et al* 1997). As strains of *B. cepacia* are so phenotypically diverse, the identification tests available at the time lacked sensitivity, specificity or sometimes both. This made infection difficult to diagnose accurately.

The Vandamme study indicated that *B. cepacia* was, in fact, composed of multiple distinct subgroups. Five genomovars were initially identified (these could not be reliably differentiated by 16S rDNA analysis) and *B. cepacia* was renamed *B. cepacia* complex. There are currently at least 17 validly named *Burkholderia cepacia* complex species, with several other subgroups that have not yet been formally assigned species status (Vandamme and Dawyndt, 2011). The species within the *B. cepacia* complex that are most commonly identified in cystic fibrosis infections are *B. cenocepacia*, *B. cepacia* and *B. multivorans* although their distribution varies between geographic regions.

The relative virulence of species within the *B. cepacia* complex has not been exhaustively established. However, there is some evidence that infection with *Burkholderia cenocepacia* may be associated with a particularly poor prognosis (CF Trust, 2004). Infection with *B. cepacia* complex can be considered to be a

contraindication for lung transplantation due to increased mortality but the increase in risk appears to vary significantly from species to species (Murray *et al* 2008).

Culture-based microbiological techniques applied to sputum are sufficient to diagnose infection and, in the case of some pathogens (such as *Pseudomonas aeruginosa*), the infecting organism is readily identifiable. Due to the relative difficulty in identifying and speciating *B. cepacia* complex using biochemical testing, molecular methods such as diagnostic PCR are of use in better characterising infection.

There is a large body of evidence describing the transmission of *B. cepacia* complex infection between individuals with CF. This has led to widespread segregation of patients attending CF clinics according to their infection status. Epidemiological surveillance to identify and characterise infecting organisms is an effective tool to detect potential instances of patient-to-patient spread.

At Micropathology Ltd, we use PCR amplification and sequencing of the *recA* gene to speciate members of the *B. cepacia* complex. *recA* is a gene involved in DNA replication that exhibits greater levels of sequence heterogeneity than 16S rDNA, yet is still highly conserved. Typically, members of the same *B. cepacia* complex species show 98-99% sequence similarity across this locus whilst sequence similarity between species drops to 94-95% (Vandamme and Dawyndt, 2011). This means that most species within the complex may be unambiguously speciated using this method although the less well defined taxonomic groups may be problematic to identify.

*recA* typing is appropriate when a patient is culture positive for a Gram-negative organism but automated identification systems such as BD Phoenix/VITEK/API 2ONE give ambiguous results. If infection with a non *B. cepacia* complex organism is possible, we may perform 16S rDNA amplification and sequencing to provide a preliminary complex ID prior to typing. The preferred sample type for this test is a cultured isolate but respiratory specimens (including sputum) are also acceptable although testing may be less sensitive.

Where *recA* typing gives an ambiguous result or more detailed strain typing is required, we are also able to perform multilocus sequence typing on confirmed isolates of *B. cepacia* complex. This technique involves the amplification and sequencing of 7 conserved genes and permits better strain-to-strain differentiation for the purposes of epidemiological surveillance (Baldwin *et al* 2005) but this testing is considerably more expensive than *recA* typing.

#### References

Baldwin, A *et al* 'Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex.' J Clin Microbiol 43:9(2005):4665-73

Burkholder, WH 'Sour skin, a bacterial rot of onion bulbs' Phytopathology, 40(1950) p115

CF Trust 'The *Burkholderia cepacia* complex: Suggestions for Prevention and Infection Control' (2004) Report of the UK Cystic Fibrosis Trust Infection Control Group.

Murray, S *et al* 'Impact of *Burkholderia* infection on lung transplantation in cystic fibrosis' (2008) Am J Resp Crit Care Med 178, 4:363-71

Vandamme, P *et al* 'Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov.' Int J Syst Bacteriol, 47 (1997), 1188-1200

Vandamme, P and Dawyndt, P 'Classification and identification of the *Burkholderia cepacia* complex: Past, present and future' Systematic and Applied Microbiology 34(2)(2011):87-95