

# Analysis of MALDI-TOF mass spectra of intact cells of the *Burkholderia cepacia* complex using SARAMIS

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## abstract

We evaluated the identification of *Burkholderia cepacia* complex (Bcc)-like bacteria using Matrix Assisted Laser Desorption/Ionisation Time Of Flight mass spectrometry (MALDI-TOF MS) of intact cells. 33 Bcc strains and 10 non-Bcc strains representing 19 species potentially identified as Bcc were examined. Analyses were performed directly from intact cells without further extraction steps. Bacterial mass spectra were obtained in the *m/z* range 3-20 kDa. All spectra were automatically processed with the Shimadzu software and resulting peak lists were exported into the SARAMIS software for further analysis. All Bcc species clustered together, separated from non-Bcc strains. Within the Bcc, all strains clustered into distinct species except for two strains of *B. pyrrocinia* and *B. cenocepacia*. Thus, the rapid identification of Bcc strains with SARAMIS software is possible using superspectra, consensus spectra calculated for each species from multiple mass spectra. In order to improve the discriminatory power, mass signals shared by all *Burkholderia* species can be excluded from the numerical analysis to enhance the resolving power. The species-specific clustering suggests that individual species produce mass signals that can be used as biomarkers to distinguish between species or even strains. The data show that MALDI-TOF MS of intact cells combined with the SARAMIS identification software is a powerful approach for the rapid identification of Bcc bacteria.

## background

The *Burkholderia cepacia* complex is a group of Gram-negative, non-spore-forming bacilli that comprises at least nine species [1]. The new genus *Burkholderia* was established in 1992 [2] and in 1997 *B. cepacia* was divided into five genomovars based on a polyphasic approach [3]. To date, nine species/genomovars have been described. Bcc species have emerged as problematic opportunistic human pathogens in the past 20 years. Affected are primarily patients with cystic fibrosis and immunocompromised individuals [4]. The number of infections caused by these bacteria has increased in these susceptible groups. The correct identification of Bcc strains is crucial for a successful treatment but proved to be difficult in routine microbiological analysis.

### Phenotypic identification

With commercial phenotypic assays the identification of Bcc strains is not straightforward and misidentification of genomovars has occurred [5]. Although selective agars have improved the isolation of Bcc strains from human samples, the ability of non-Bcc species (*B. gladioli*) and

others (*Ralstonia* sp., *Pandoraea* sp.) hamper the routine identification.

### Molecular identification

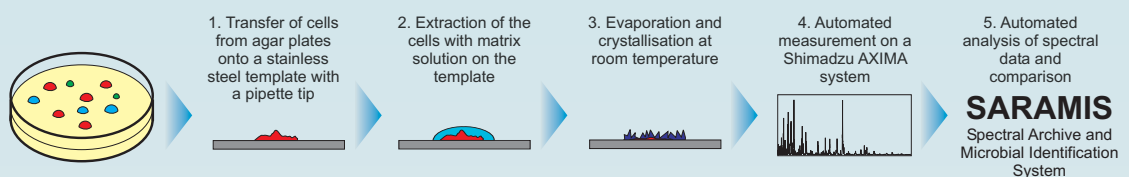
Among Bcc species sequence diversity of the 16S rRNA gene is not sufficient for a discrimination. Other genes, like *recA*, proved to be more variable and were used to discriminate genomovars [6].

### Mass spectral identification

Intact (whole) cell mass spectrometry has been shown to be a very rapid and reliable technique to identify bacterial species or sub-specific taxonomic units [7]. Mass spectra in a range of 2-20 kDa primarily show peaks of abundant proteins such as ribosomal proteins or DNA-binding proteins [8]. The low-molecular weight proteome has been shown to be stable for individual strains but variable enough to be used as a taxonomic tool [9]. MALDI-TOF MS combined with SARAMIS has been successfully tested in the routine identification of clinical samples [10].

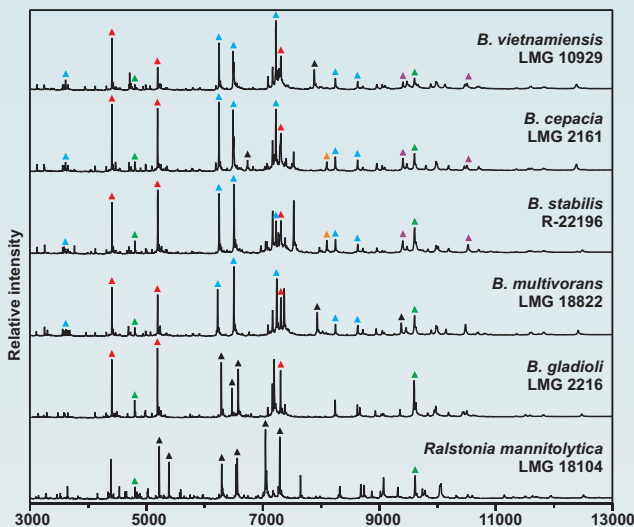
## methods

All tested strains were held at the culture collection at the Laboratorium voor Microbiologie at the Universiteit Gent (LMG). Bacteria were received as blinded samples and prepared following the simple standard protocol developed by Anagnostec [11]:



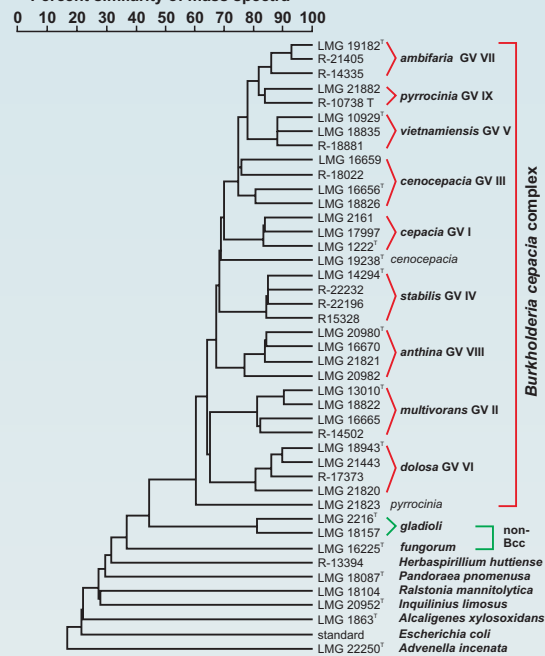
## results

All strains produced mass spectra of good quality with some 100 automatically detected peaks. The simple sample preparation protocol proved thus to be also applicable to *Burkholderia* sp. A number of high-intensity mass signals were recorded for all *Burkholderia* strains, but a number of mass signals was recorded exclusively for Bcc strains. Differences between species were generally found for medium- to low-intensity mass signals, several of which were found species-specific. In a dendrogram calculated with SARAMIS, Bcc and non-Bcc strains were clearly separated. Strains of particular Bcc genomovars exhibited similarities of about 80% shared mass signals, while Bcc and non-Bcc strains shared only some 40%.



Exemplary mass spectra of Bcc and non-Bcc strains. Triangles above mass signals indicate their specificity: green; present in all six strains; red; in all *Burkholderia* strains; blue; in Bcc strains; violet; in three Bcc strains; orange; in two Bcc strains, and black; strain-specific. For clarity, only a small fraction of preferentially intense mass signals were marked.

### Percent similarity of mass spectra



Dendrogram based on similarity of mass spectra of individual strains calculated with SARAMIS using a single-link agglomerative clustering algorithm. Strains other than *Burkholderia* were mostly from species that also grow on respective selective agars.

## conclusions

- > MALDI-TOF MS of intact cells followed by spectral analysis with SARAMIS is a very fast and reliable tool to differentiate Bcc strains.
- > Mass spectra of Bcc species are sufficiently variable to allow an automated discrimination by numerical analysis
- > The rapid identification of clinical samples directly from primary agar plates without laborious sample preparation is feasible

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