

Matrix Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry of intact cells allows rapid identification of *Burkholderia cepacia* complex (Bcc) bacteria

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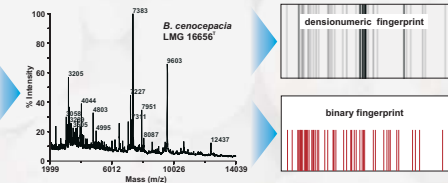
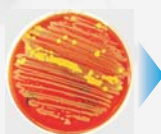
background

The *Burkholderia cepacia* complex (Bcc) 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. The number of infections caused by these bacteria has increased considerably in these susceptible groups. The correct identification of Bcc strains is crucial for a successful treatment but proved to be difficult by routine microbiological approaches [4,5]. We investigated the possibility to identify Bcc bacteria by whole-cell mass spectrometry [6] followed by numerical analysis of spectral data applying two different strategies of data conversion and handling.

methods

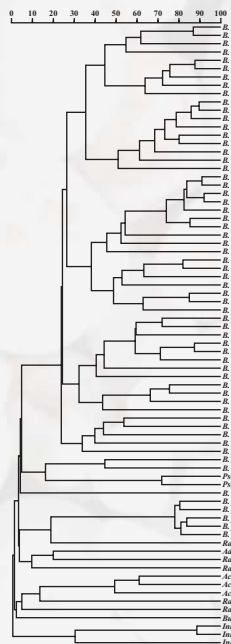
Bcc and related strains grown on agar plates were prepared for MALDI-TOF MS directly on the template by mixing fresh cells with matrix solution. Mass spectra were obtained on a ABI 4700 Proteomics Analyser in a mass range of 2-20 kDa in positive mode [7].



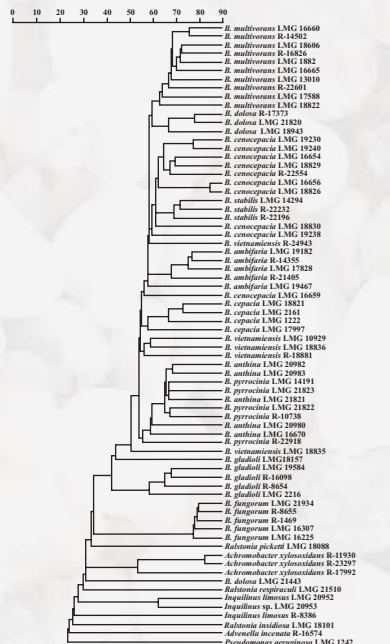
Transformation of spectral data into densitometric fingerprints. Comparison using Pearson's product moment correlation and UPGMA clustering algorithm (BioNumerics 4.5 [8])

Transformation of spectral data into binary peaklists. Comparison using a single link agglomerative clustering algorithm and SuperSpectra (SARAMIS [9])

results



A: Dendrogram based on densitometric fingerprints (BioNumerics)



B: Dendrogram based on binary fingerprints (SARAMIS)



C: Dendrogram based on binary fingerprints including species specific SuperSpectra (SARAMIS)

Bcc strains were mostly grouped in species-specific clusters by both approaches. With the densitometric approach Bcc-strains could not be fully separated from non-Bcc-*Burkholderia* strains while this was the case with the binary approach. However, a number of strains were placed outside their species' clusters or in mixed clusters (*B. anthina* and *B. pyrrocinia*).

With the inclusion of SuperSpectra (consensus spectra of multiple strains of the same species) the species-specific clustering could be improved. The use of SuperSpectra and simple binary mass fingerprints thus is promising with respect to the identification of unknown bacterial samples. This was tested with blinded samples, the majority of which was correctly identified.

sample	strain	identified as	sample	strain	identified as
MS 010	<i>B. cepacia</i> LMG 1222	<i>B. cepacia</i>	MS 041	<i>B. stabilis</i> R-22196	<i>B. stabilis</i>
MS 011	<i>B. cepacia</i> LMG 2161	<i>B. cepacia</i>	MS 042	<i>B. stabilis</i> R-15328	<i>B. stabilis</i>
MS 012	<i>B. cepacia</i> LMG 17997	<i>B. cepacia</i>	MS 049	<i>B. stabilis</i> R-22232	<i>B. stabilis</i>
MS 019	<i>B. cepacia</i>	<i>Burkholderia</i> sp.	MS 050	<i>B. cepacia</i> LMG 10929	<i>B. cepacia/vietnam.</i>
MS 020	<i>B. multivorans</i> LMG 13010	<i>B. multivorans</i>	MS 051	<i>B. vietnamiensis</i> LMG 18835	<i>B. vietnamiensis</i>
MS 021	<i>B. multivorans</i> LMG 16665	<i>B. multivorans</i>	MS 052	<i>B. vietnamiensis</i> R-18881	<i>B. vietnamiensis</i>
MS 022	<i>B. multivorans</i> LMG 18822	<i>B. multivorans</i>	MS 060	<i>B. dolosa</i> LMG 18943	<i>B. dolosa</i>
MS 023	<i>B. multivorans</i> R-14502	<i>B. multivorans</i>	MS 061	<i>B. dolosa</i> LMG 21820	<i>B. dolosa</i>
MS 030	<i>B. cenocepacia</i> LMG 16656	<i>B. cenocepacia</i>	MS 062	<i>B. dolosa</i> LMG 21443	<i>B. dolosa</i>
MS 031	<i>B. cenocepacia</i> LMG 18826	<i>B. cenocepacia</i>	MS 069	<i>B. dolosa</i> R-17373	<i>B. dolosa</i>
MS 032	<i>B. cenocepacia</i> LMG 16659	<i>B. cenocepacia</i>	MS 070	<i>B. ambifaria</i> LMG 19182	<i>B. ambifaria</i>
MS 033	<i>B. cenocepacia</i> LMG 19238	<i>B. cenocepacia</i>	MS 071	<i>B. ambifaria</i> R-14355	<i>B. ambifaria</i>
MS 039	<i>B. cenocepacia</i> R-18022	<i>B. cenocepacia</i>	MS 072	<i>B. ambifaria</i> R-21405	<i>B. ambifaria</i>
MS 040	<i>B. stabilis</i> LMG 14294	<i>B. stabilis</i>	MS 080	<i>B. anthina</i> LMG 20980	<i>B. anthina</i>

sample	strain	identified as
MS 081	<i>B. anthina</i> LMG 20982	<i>B. anthina</i>
MS 082	<i>B. anthina</i> LMG 16670	<i>B. anthina</i>
MS 083	<i>B. anthina</i> LMG 21821	<i>B. anthina</i>
MS 091	<i>B. pyrrocinia</i> LMG 21822	<i>B. pyrrocinia</i>
MS 092	<i>B. pyrrocinia</i> LMG 21823	<i>Burkholderia</i> sp.
MS 093	<i>B. pyrrocinia</i> R-10738	<i>B. pyrrocinia</i>
MS 099	<i>B. stabilis</i>	<i>B. stabilis</i>
MS 100	<i>B. gladioli</i> LMG 2216	<i>B. gladioli</i>
MS 109	<i>B. gladioli</i> LMG 18157	<i>B. gladioli</i>
MS 110	<i>B. fungorum</i> LMG 16225	no identification
MS 120	<i>Alcal. xyloxydans</i> LMG 1863	<i>Achromobacter</i> sp.
MS 149	<i>Parat. pyromorpha</i> LMG 18087	<i>P. pyromorpha</i>
MS 151	<i>Ral. mannitolilytica</i> LMG 18104	no identification
MS 160	<i>Inqui. limosus</i> LMG 20952	no identification

Summary of analyses of blinded samples. Mass spectra were obtained on an AXIMA MALDI-TOF MS (Shimadzu) and analysed with SARAMIS. **blue**: identification with 90-99% confidence; **green**: identification with 70-90% confidence. The SARAMIS database holds spectral data of most clinically relevant bacterial and fungal species. Non-*Burkholderia* species in the table were presently not contained.

conclusions

For species identification of Bcc-strains based on mass spectral data, a binary fingerprint listing only the presence of mass signals is sufficient. Compared to densitometric data, this has the advantage of a strongly reduced size of individual data files - to less than one percent of the original data - thus facilitating the data handling and reducing computing time enormously.

Densitometric data could be, nonetheless, of importance for taxonomic studies below the species level as well as for physiological studies where the intensity of mass signals - presumably representing proteins - may be of importance. A mass spectral identification of Bcc bacteria by SARAMIS is applicable to high-throughput, largely automated microbiological routine analysis.

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