BRUKER DALTONICS®

Fast and reliable MALDI-TOF MS–based microorganism identification

Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) fingerprinting is a fast and reliable method for the classification and identification of microorganisms, with applications in clinical diagnostics, environmental and taxonomical research, or food-processing quality control. The BioTyper™ MALDI-TOF MS fingerprinting system allows researchers to perform this process for the unambiguous identification of bacteria, yeasts and fungi in minutes.

The general workflow of microorganism profiling is a straightforward approach (**Fig. 1**). Starting from a single colony or other biological material, samples can be analyzed within a few minutes. Automated spectra acquisition is completed in a few seconds per sample, and a seamless data transfer to dedicated identification software is possible. This technology is therefore an excellent alternative to classical microbiological identification and classification techniques, requiring only minimal sample preparation efforts and life cycle costs.

MALDI-TOF measurement

Most simple analysis of a sample starts by applying a small amount of biological material directly onto the MALDI target plate. The starting material can be a single colony or a centrifuged portion of a liquid culture. The thin microbial film is overlaid with matrix (α-cyano-4-hydroxycinnamic acid; HCCA).

Mass spectra are acquired using a MALDI-TOF mass spectrometer in linear positive mode at maximum frequency (20–200 Hz depending on the instrument). Measured mass range of spectra is from 2,000 to 20,000 Da. Automated spectrum acquisition can be performed using Bruker's 'autoExecute' software with fuzzy control of laser intensity.

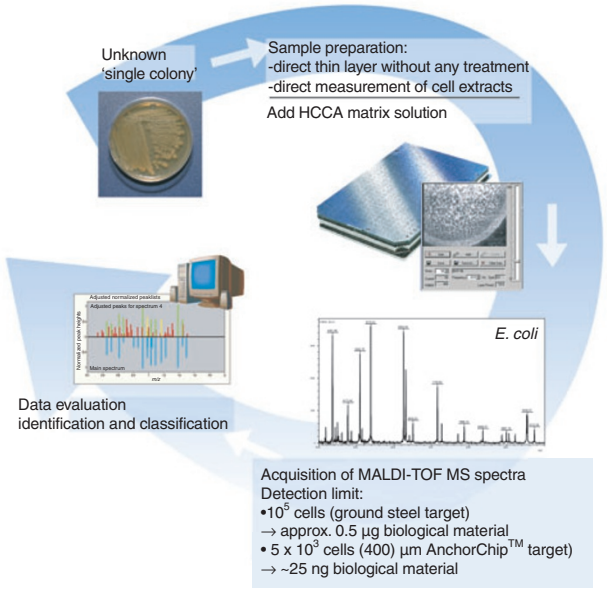

Figure 1 | General workflow for ClinProt BioTyper microorganism identification and classification.

Reproducibility

Method robustness has been demonstrated for a broad range of conditions. Different compositions of growth medium have little effect in the peak pattern distribution; in the range of 4,000 to 12,000 Da, nearly no influence of culture medium is observed. The presence of culture medium adhering to the colonies has no effect on the peak pattern. Also, growth state of the cells has little effect on the peak pattern. Cells in the lag phase of growth show a very similar pattern as cells in log, stationary or early death phase.

Furthermore, given that sample preparation and measurement are performed under standardized conditions, the acquired profile spectra are highly comparable between different MALDI-TOF instruments. Spectra from the same sample target measured in three different instruments are virtually identical. Therefore, spectra from different MALDI-TOF MS instruments can be used to build a substantial and dependable database.

The remarkable reproducibility of the methodology is based on the measurement of constantly expressed high-abundant proteins, such as ribosomal proteins[1]. The observed mass range of spectra is between 2,000 and 20,000 Da where very few metabolites appear. Bacterial spores result in a significantly different peak pattern than living cells, but these 'spore-spectra' are also reproducible.

Thomas Maier, Stefan Klepel, Uwe Renner & Markus Kostrzewa

Bruker Daltonik GmbH, Permoser Strasse 15, D-04318 Leipzig, Germany.
Correspondence should be addressed to M.K. (km@bdal.de).

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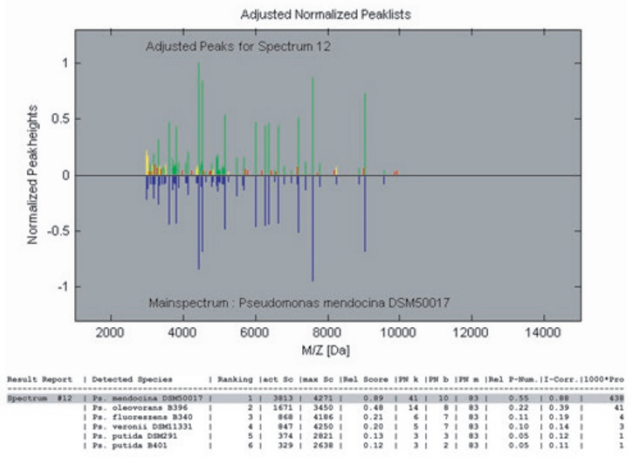

Result Report	Detected Species	Ranking	act Sc	max Sc	Rel Score	PM k	PM b	PM m	Rel P-Mum.	I-Corr.(1000*Pro
Spectrum #12	Ps. mendocina DSM50017	1	3813	4271	0.89	41	10	83	0.55	0.88	438
	Ps. oleovorans B396	2	1671	3450	0.48	14	8	83	0.22	0.39	41
	Ps. fluoreszens B340	3	868	4186	0.21	6	7	83	0.11	0.19	4
	Ps. veronii DSM11331	4	847	4250	0.20	5	7	83	0.10	0.14	3
	Ps. putida DSM291	5	374	2821	0.13	3	3	83	0.05	0.12	1
	Ps. putida B401	6	329	2638	0.12	3	2	83	0.05	0.11	1

Figure 2 | BioTyper identification results. Green lines represent the perfectly matching peaks, yellow marks the peaks matching in broader borders, red peaks did not match within the reference spectrum. Lower table gives an output of the evaluated and ranked results.

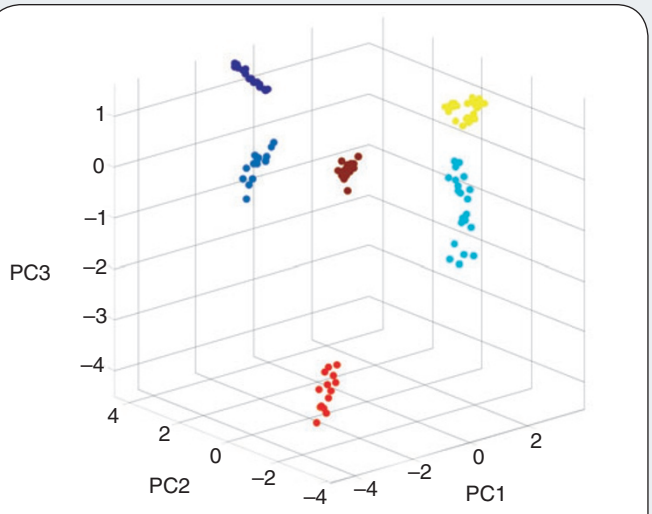

Figure 3 | Three-dimensional (3D) scatter plot of the first three principle component scores for six different species of the genus *Pseudomonas*: *P. veronii* (dark blue), *P. fluoreszens* (light blue), *P. mendocina* (brown), *P. oleovorans* (red), *P. putida* strain B401 (cyan), *P. putida* strain DSM291 (yellow).

The BioTyper software

Our BioTyper analysis software incorporates all functionalities for processing mass spectra as well as for identification and classification. The processing parameters are user definable, and the result is a dedicated peak list.

Pattern matching, for the identification of unknown microorganisms, is accomplished through comparison of the generated peak lists with a library containing the characteristic spectra information of various species. The library spectra are generated by several measurements of known bacterial species and strains under slightly different conditions, and then extracting the specific peak information. A good average is achieved by measuring 20 spectra. The software automatically generates the peak lists from the whole set of spectra and extracts the typical peaks which are present in a certain number of spectra from one species.

Unknown microorganisms are identified by comparing their individual peak lists to the database. A matching score based on identified masses and their intensity correlation is generated and used for ranking of the results (**Fig. 2**). To increase the confidence of database searches, BioTyper is able to correct peak mass deviations through a sophisticated recalibration algorithm. After peak picking it is possible to set an initially accepted error window and a desired adjustment result. The software can adapt the calibration from a new peak list to the known peak list within adjustable limits. Thereby, even spectra with mass deviation of 5,000 p.p.m. can be identified successfully. This functionality makes the BioTyper identification exceptionally robust and accurate.

For dereplication**,** clustering and generation of family trees, the BioTyper offers a variety of functionalities. Based on similarity scores, dendrograms can be constructed. Furthermore, an unsupervised multivariate analysis based on principle component analysis is possible. A variety of clustering algorithms and visualizations are available that use the calculated principle components. **Figure 3** is an example of clustering in the genus *Pseudomonas*.

Applications

The Biotyper offers an excellent alternative to traditional laboratory identification methods for microorganisms in a variety of areas (for example, environmental research, food and water control, and medical diagnostics). The speed, robustness and minimal costs of sample preparation and measurement for this method make it exceptionally well suited for routine and high-throughput use.

This approach is also useful for analysis of taxonomic relationships. MALDI-TOF MS profile analysis results in similar family trees compared to classical methods 16S ribosomal DNA sequencing. Because ribosomal proteins are highly abundant and appear to be very stable, the observed protein pattern allows a direct view to the translated DNA sequence. Therefore, the method comprising the determination of the molecular masses can be assumed to have a value as rough multi-locus sequencing.

Dereplication of complex microorganism communities based on their mass pattern offers new scientific capabilities (for example, in environmental research and investigations of biodiversity). Thousands of microorganisms can easily be analyzed with the BioTyper, and then serve as the basis for further analysis. An initial set of microorganismal reference spectra may be used for comparing them with each other, and redundancies can be removed. Further isolates can then be compared with the initial reference data set and either discarded or added to the library, creating an overview of microorganism diversity.

1. Ryzhov, V. & Fenselau, C. Characterization of the protein subset desorbed by MALDI from whole bacterial cells. *Anal. Chem.* **73**, 746–750 (2001).