

# Rapid identification of microorganisms using MALDI-TOF MS and pattern profiling: The MALDI BioTyper workflow

Rene Krieg<sup>1</sup>, Thomas Maier<sup>2</sup>, Wolfgang Pusch<sup>1</sup>, Guido Mix<sup>1</sup>, Geroald Schwarz<sup>1</sup>, Markus Macht<sup>1</sup>, and Markus Kostrzewa<sup>2</sup>  
Bruker Daltonik GmbH, Bremen<sup>1</sup>, Leipzig<sup>2</sup>, Germany

## Introduction

Accurate and rapid identification as well as taxonomical classification of microorganisms are of main interest in research as well as clinical routine diagnostics, which often bases on biochemical methods. However, results of these tests are obtained after long incubation times, and costs per assay are significant. We report the application of a MS-based technique for the explicit differentiation of bacteria on species as well as on the sub-species level.

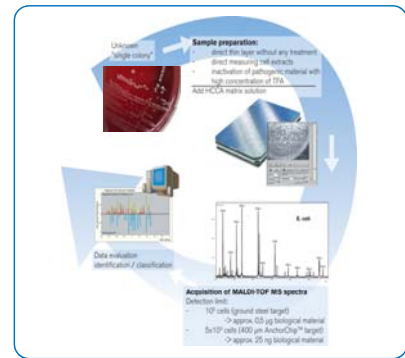


Fig.1: General workflow of microorganism identification with the MALDI BioTyper

## Methods

The workflow is depicted in Fig. 1. Bacteria from a colony were applied to a sample target plate directly or after a short extraction protocol. After air drying and addition of matrix (CHCA), the samples were analyzed using a microflex™ benchtop MALDI-TOF mass spectrometer (Bruker Daltonics). Specimens could be prepared in a few minutes from plate and a spectrum could be acquired within one minute. Profile spectra consisting of a characteristic peak pattern were mainly derived from ribosomal proteins enhancing robustness of the assay. Applying the dedicated MALDI BioTyper™ software solution (Fig. 2) characteristic peaks could be extracted from these spectra which were used for correct identification of unknown microorganisms by comparison to the BioTyper reference database. It has to be pointed out that for successful subspecies identification a consistent standardization of experimental procedures should be applied.

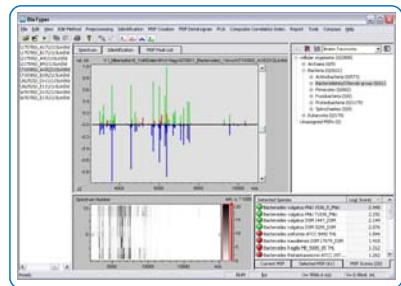


Fig.2: Screenshot of the MALDI BioTyper 2.0 software for microorganism ID

## Results

Starting with a few biological material, peak rich spectra could be obtained for each single isolate.

MALDI profiling combined with a dedicated **pattern matching** algorithm in combination with a library of reference spectra has been found to be excellent for robust identification of bacteria on the genus and mostly on the species level (Fig.3).



Fig.3: MALDI BioTyper identification result for two different Staphylococci species

Based on main spectra calculated by the software, dendrograms could be generated which give insight into phylogenetic relationships at the species and partially also subspecies level (Fig.4).

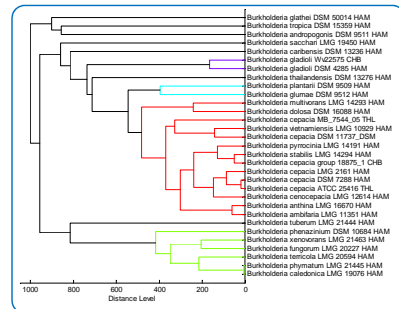


Fig.4: Dendrogram calculated for the genus Burkholderia. The B. cepacia complex forms a common cluster (red)

Applying specific MALDI BioTyper differentiating features in a **weighted pattern matching** algorithm, the identification of closely related species and subspecies could be accomplished.

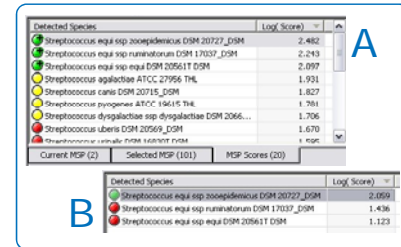


Fig.5: Enhancement of subspecies ID by weighted pattern matching (B) compared to standard pattern matching (A)

Further, **correlation analysis** enables an in-depth analysis of relationships between different species and subtypes. Correlation matrices calculated for groups of bacteria can be used to identify bacteria in an alternative and complementary approach.

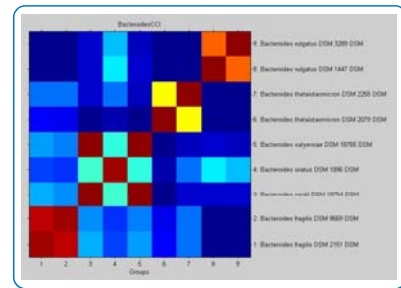


Fig.6: Correlation analysis of Bacteroides species, red color = high correlation

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## Summary

The BioTyper identification outranges traditional biochemically derived IDs by reliability and subspecies capabilities. In addition, this technique allows for high throughput screening at a fraction of the costs as for traditional biochemical tests. Using MALDI-TOF mass spectrometry, powerful mathematical algorithms and a comprehensive reference database of well characterized microorganisms a fast, robust, cheap and reliable identification is possible.

Holland RD, Wilkes JG, Raffi F, Sutherland JB, Persons CC, Voorhees KJ, Lay JO Jr. Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 1996; 10(10): 1227-32.

Fenselau C, Demirev PA. Characterization of intact microorganisms by MALDI-TOF MS. Mass Spectrom Rev. 2001 Jul/Aug; 20(4): 157-71.

Maier T, Kostrzewa M. Fast and reliable MALDI-TOF MS-based microorganism identification. Chemistry Today. 2007 March/April; 25(2): 56-59.

## Conclusions

- Very simple and rapid sample preparation
- Suitable for all kind of microorganisms
- Very fast measurement with high reproducibility
- Secure identification on the species and subspecies level
- No initial assessment like Gram staining or oxidase test is required.
- Minimal costs of sample preparation and analysis