Identification of bacteria and spores suitable as biological warfare agents by the MALDI Biotyper

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MALDI-TOF MS fingerprinting of bacteria, spores and white powders combined with an analysis of spectra by the MALDI Biotyper software was shown to be an excellent method for identification and differentiation of common pathogens, bacteria based biological warfare agents (BWA) including *Bacillus anthracis* spores as well as for discrimination of fakes/hoaxes from real biological threats.

**Keywords:** BWA, Bacteria, Spores, MALDI Biotyper,

1 **Introduction**

Worldwide, there is an evident danger for the use of biological hazardous agents as weapons of mass destruction. The potential spectrum of bioterrorism ranges from hoaxes to classic biological warfare agents, which can produce mass casualties. Therefore, the reliable and fast identification of bacteria and spores as well as “white powders” is a challenging task. MALDI-TOF mass spectrometry fingerprinting has been shown to be well suitable for the identification of microorganisms without prior assessment [1, 2].

2 **Sample preparation**

Generally, starting point for the analysis is a single colony from an agar plate, few microliters of a liquid culture or a small amount of “white powder” (Fig. 1). In the simplest case, non-pathogenic biological material could be transferred directly onto the MALDI target plate and overlaid with the MALDI matrix. However, in case of an unknown sample or white powder the biological material should be inactivated before analysis. Several extraction/inactivation protocols using ethanol/formic acid and/or trifluoroacetic acid (TFA) can be applied prior to target preparation. Especially, spores of *Bacillus* sp. have to be inactivated and disrupted by 80 % TFA.

Although generally pure cultures are being used for analysis mixed cultures can be analysed if the ratio is not less than 1:10. The less represented microorganism may get lost in analysis of a mixture but it will not lead to a wrong classification.

3 **Sample analysis by MALDI-TOF mass spectrometry**

For analysis by MALDI-TOF mass spectrometry the prepared sample is deposited onto a MALDI target plate. After drying, the same sample position on the target is covered with matrix solution (alpha-cyano-4-hydroxycinnamic acid). Sample is now ready for introduction into MALDI-TOF mass spectrometer. Mass spectra are acquired between 2,000 and 20,000Da, automatically controlled via dedicated easy-to-use software interface.
Figure 1: General workflow for microorganism identification using the MALDI Biotyper.

The automated workflow of the MALDI Biotyper enables optimal sample acquisition (accumulation of typically 300 to 500 shots of high quality from different optimal spot positions), raw data processing and final identification in a few simple steps. Different compositions of growth media have nearly no effect in the peak pattern distribution. The remarkable reproducibility of the methodology is based on the measurement of constantly expressed high-abundant proteins, i.e. ribosomal proteins [3].

4 BWA identification using pattern matching and reference databases

Microorganisms are identified by comparison of their individual peak list (MS fingerprint spectrum) via pattern matching with reference library entries. They cannot be differentiated by only single peaks. Pattern matching is accomplished through calculation of a matching score. This score is calculated using a dedicated, proprietary algorithm on basis of matched number of peaks and the correlation of the overall intensity profile of the spectra. Identification results with high reliability are based on significant matching scores which are clearly separated from worse matches. Correct matches with highly probable species identification are displayed green-coloured, whereas missing matches are shown red-coloured (Fig. 2). The influence of peak intensities is reduced in the applied algorithm and identification mainly based on exact mass determination of the marker peaks. Therefore, the effect of instrument parameter settings is significantly reduced. This functionality makes the identification exceptionally robust and accurate and enables inter-laboratory comparability of results and the creation of common databases.

The regular database contains more than 3200 entries of microorganisms which are relevant in clinical and veterinary diagnostics, environmental analysis or food safety control. Moreover, a second security-related database containing more than 100 entries enables the identification of microorganisms suitable as biological warfare agents (e.g. B. anthracis, Brucella melitensis, Francisella tularensis). But any time the database can be adjusted to new threats.
Figure 2: Result table for identification of F. tularensis by the MALDI Biotyper.

5 Discrimination of B. anthracis spores

The identification of B. anthracis spores - the causative agent of anthrax - is of particular interest. Discrimination of B. anthracis from closely related Bacillus cereus, Bacillus thuringiensis and Bacillus mycoides (B. cereus group) is a challenging task because of their very close phylogenetic relationship, reflected also by DNA homology. Small, acid-soluble proteins (SASP) were found to be biomarkers for spore differentiation/identification by MS [4]. SASPs could be extracted from spores after treatment with 80% TFA and detected by MALDI-TOF MS. Spore spectra were run against the regular Bruker MALDI Biotyper database (> 3200 different entries), but no matches with common microorganisms exhibiting a reliable score could be observed. This indicates that the pattern of spore spectra are completely different compared to spectra of corresponding vegetative cells.
Furthermore, some “white powders” (e.g. flour,) which have been reported to be potentially used as hoaxes/fakes in a terrorist attack were analyzed in the same way. The analysis of these spectra using the MALDI Biotyper revealed no matches with bacteria or spore spectra of the reference database.

References