Detection and identification of bio-threats using MALDI-TOF-MS

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Abstract

MALDI-TOF-MS emerged as a new diagnostic tool in established clinical laboratories. Advantages compared to conventional techniques are that it is a fast, cost-effective, accurate method, which is suitable for high-throughput identification of bacteria by less skilled laboratory personnel because preliminary identification steps are unnecessary.
We studied whether MALDI-TOF-MS can add a value for the current laboratory detection and identification capabilities of bio-threat agents. Consequently, we showed that by using MALDI-TOF-MS, *Brucella* species can be identified swiftly, while by use of common diagnostic methods this was a laborious and time consuming process. Subsequently, we showed that known genetic relationships between species can help to develop reference libraries for MALDI-TOF-MS. This knowledge helps us to create MALDI-TOF-MS applications for the identification of bio-threat agents. Because these agents are often closely related to less or non-pathogenic species such as. *Y. pestis* with *Y. pseudotuberculosis* or *B. anthracis* with *B. thuringiensis*. Finally, we showed that MALDI-TOF-MS potentially can be used for direct screening of e.g. threat letters containing suspicious powders. What is more, MALDI-TOF-MS applications can improve current detection and identification capabilities for bio-threats.
Keywords: MALDI-TOF-MS, bio-threat, *Bacillus anthracis*, *Brucella*, identification, detection

1 Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a rapid method able to identify bacteria. MALDI-TOF-MS emerged as a new diagnostic tool in established clinical laboratories (1-4). Advantages compared to conventional techniques are that it is a fast, cost-effective, accurate method, which is suitable for high-throughput identification of bacteria by less skilled laboratory personnel because preliminary determination steps are unnecessary (1-4).
MALDI-TOF-MS is a valuable technique in clinical diagnostics and it might be also beneficial in handling biological threat situations. To study whether MALDI-TOF-MS can improve laboratory detection and identification capabilities in bio-threat related situations we conducted three different pilot studies, namely:

1. Determination if the MS-spectra of genetically related species are also highly similar.
2. Examination whether it is possible to use MALDI-TOF-MS for the identification of *Brucella* isolates at the species level.
3. Investigation if MALDI-TOF-MS can be used for fast detection of *Bacillus anthracis* spores.

2 Results

2.1 Relation between genetics of bacteria and their MALDI-TOF-MS spectra

The genus *Burkholderia* contains more than 50 species that are widespread in nature. Those species are found in water, plants, human and animals. Furthermore, many of *Burkholderia* species form a symbiotic relationship with plants, and thus, they are not of clinical or threat relevance and they will not be used in our MALDI-TOF-MS application.
In the literature, hylogenetic and taxonomic studies for *Burkholderia*, *Ralstonia*, *Comamonas* and *Herbaspirillum* determined the genetic relationships between these and other species (5).The genus *Burkholderia* contains potential bio-threat agents such as *Burkholderia mallei* and *Burkholderia pseudomallei* but also medically relevant species of the *Burkholderia cepaciae* complex and non-pathogenic or plant pathogenic species such as *Burkholderia oklahomensis*, *Burkholderia thailandensis, Burkholderia plantarii*, and *Burkholderia gladiolias*.
We determined if knowledge about the genetic relationships between species can be used to improve the construction of MALDI-TOF-MS libraries. Therefore, MS-spectra for 19 *Burkholderia* species, two *Comamonas* and a *Herbaspirillum huttiense* were generated. The MS-spectra were analysed and a dendrogram based on those spectra was constructed to determine the relationships between the above mentioned bacteria. Subsequently, the accuracy of the dendrogram was confirmed with the classification of these bacteria as described by Tayeb *et al*. (5) . Those results showed that by use of MALDI-TOF-MS it is possible to discriminate between the genetically highly related species i.e. *B. mallei*, *B. pseudomallei*, *B. thailandensis*, and *B. oklahomensis*. It was found that the bio-threat agents *B. mallei* and *B. pseudomallei*, can be easily differentiated from non-pathogenic but genetically highly related species i.e. *B. thailandensis*, and *B. oklahomensis*. Therefore, we concluded that MALDI-TOF-MS has the potency as a fast and reliable identification method of genetically related species in order to generate the reference library.

2.2 *Brucella* species identification to the species level

The genus *Brucella* contains highly infectious species that are classified as bio-threat agents (6,7). The timely detection and identification of the microorganism is essential for an effective response either after biological warfare attacks or during natural outbreaks (6-8). Discrepancies on the species and biovar level between the taxonomy and genetic relatedness, complicate the development of precise identification assays (9).
Accurate identification of *Brucella* species using MALDI-TOF-MS was achieved by constructing a *Brucella* reference library based on the multilocus variable-number tandem repeat analysis (or MLVA is a molecular typing technique using the genetic variation in the number of tandem repeated DNA sequences of different loci in genomes from different isolates) data. Previously it was shown that by comparing MALDI-TOF-MS-spectra from *Brucella* species with the custom-made MALDI-TOF-MS reference library, it was possible to rapidly identify *Brucella* species (10). In that way, 99.3% of the 152 isolates tested were identified at the species level, and *B. suis* biovar 1 and 2 were identified at the biovar level (10). This result demonstrates that for *Brucella*, even minimal genomic differences between those serovars translate to specific proteomic differences. As a result we confirm that MALDI-TOF-MS can be developed into a fast and reliable identification method for genetically related *Brucella* species, when potential taxonomic and genetic inconsistencies are taken into consideration during the generation of the reference library.

2.3 Fast detection of *Bacillus anthracis* spores using MALDI-TOF-MS

The first step to control a bio-threat incident is to determine whether a bio-threat is a real threat or a hoax. Fast information about the nature of the incident is crucial to reduce panic in a case of a hoax or take proper countermeasures in case of a real threat. *Bacillus anthracis* in 'powder letters' proved to be a serious threat (11). Currently, detection of *B. anthracis* spores is based on a PCR amplification and/or antibody-based techniques. In order to support these commonly used techniques, a MALDI-TOF-MS test was developed. The aim of our study was to develop a MALDI-TOF-MS based application able to identify swiftly realistic amounts of *B. anthracis* spores (<1 mg). Therefore, three different sample preparation methods were tested with *Bacillus atrophaeus* (*globigii*) and *Bacillus thuringiensis* spores. *B. atrophaeus* (*globigii*) was selected because it is often used as a simulant for *B. anthracis*. *B. thuringiensis* was chosen for this study because this species is genetically highly related to *B. anthracis*. Subsequently, with the preferred method of the three tested sample preparations, MS-spectra were generated from *B. anthracis* spores and five potential hoax materials. From all MS-spectra obtained, a small library was generated containing MS-spectra from *B. atrophaeus* (*globigii*), *B. thuringiensis*, and *B. anthracis*. Next, 12 samples were tested. Interpretation of the MS-spectra was successful because all four *B. anthracis* positive samples were detected. No false positive detections were made in the remaining eight samples containing simulants, hoax materials, or combinations of a simulant and a hoax material.
In conclusion, in spite of that we tested only a few strains, this pilot study showed that the proposed fast MALDI-TOF-MS procedure is an easy method and it is a promising technique to identify *B. anthracis* spores swiftly and accurately. Furthermore, the application can also be extended for other microorganisms and obtained results can also be used to support data obtained by *e.g.* PCR.

3 Conclusions

MALDI-TOF-MS emerges as a new diagnostic tool in established clinical laboratories. The described results of 3 pilot studies indicate that MALDI-TOF-MS application can be successfully used to improve detection and identification capabilities during bio-threat situations. Moreover, we showed that *Brucella* species can be identified swiftly, whereas until now this was a laborious and time consuming process. Next, we showed that known genetic relationships between species can help to develop reference libraries for MALDI-TOF-MS. This knowledge helps us to create MALDI-TOF-MS applications for bio-threat agents, which are often closely related to less or non-pathogenic species like e.g. *Y. pestis* and *Y. pseudotuberculosis* or *B. anthracis* and *B. thuringiensis*. Finally, we demonstrated that MALDI-TOF-MS potentially can be used for direct screening of threat letters or suspicious powders.
In conclusion, MALDI-TOF-MS applications can improve current detection and identification capabilities for bio-threats.

4 References

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