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Application News

No. B112

MALDI-TOF Mass Spectroscopy

# Rapid Identification of Bacillus Spores by MALDImini™-1 Compact MALDI Digital Ion Trap (DIT) Mass Spectrometer

## Introduction

Identification of bacterial species is necessary for the determination of the appropriate antibacterial drugs for pathogenic strains and for identifying the type of bacteria responsible for food spoilage. In recent years, microbial analysis by MALDI-TOF MS has become the technique of choice in microbiology laboratories, as this method enables simple and quick identification of a wide variety of microorganisms. Although powerful, this technique is limited in its ability to identify mixed cultures and spore-based bacteria. Quick identification of powdered anthrax spores as a bio terrorism countermeasure is a prime example. One way to overcome this limitation is to target peptides generated by proteolysis of bacterial proteins. Protein identification using an MS/MS search against available databases enables the determination of the species from which the proteins originated. This workflow can be readily applied to protein extracted from a mixture of microorganisms. Thanks to the precursor selection, specific peaks can be selected from multiple peaks which suggests that microbial identification is possible using a different approach from conventional microbial identification by MALDI. Based on an early approach published by Warscheid, et al. [1], we attempted quick identification of *Bacillus* spores by using a combination of rapid tryptic digestion of proteins on trypsin beads followed by MS/MS fragmentation using the compact MALDI digital ion trap (MALDImini-1) mass spectrometer.

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## MALDImini-1 Compact MALDI-DIT Mass Spectrometer

The MALDImini-1 (Fig. 1) fits in a space the size of a piece of paper, allowing installation in places where mass spectrometers could not previously fit. With this configuration, users can place the MALDImini-1 on a workbench and check MS results right next to the sample preparation area, allowing for a more convenient workflow. The system's digital ion trap uses rectangular wave RF to allow ion trapping up to 70,000 Da. Furthermore, the MS/MS and MS3 functionality of the DIT allows researchers to carry out comprehensive structural analyses, such as direct glycopeptide analysis, post translational modification analysis, and branched glycan structural analysis.

**Fig. 1 MALDImini™-1 Compact MALDI Digital Ion Trap (DIT) Mass Spectrometer**

## Experiment

In this experiment, hay bacillus, *Bacillus subtilis subsp. subtilis* NBRC 13719$^T$, was used as a model sample for spore-forming bacteria. The genus *Bacillus* is a spore-forming gram-positive bacteria which includes species used in food fermentation, such as *B. subtilis natto*, and on the opposite *B. cereus*, which causes food poisoning, and pathogenic *B. anthracis* (anthrax).

In this experiment, spores were formed by culturing *B. subtilis* at 30 °C for a minimum of 1 week in a nutrient agar medium. Spore formation was confirmed by observation with a microscope. Fig. 2 shows the sample preparation workflow. First, the bacterial cells were applied to the MALDI target plate, followed by 1 µL of 10 % trifluoroacetic acid (TFA) for protein extraction. Once spots were dry, 2 µL of trypsin beads (ProteoChem) were added, and tryptic digestion of the spore protein was conducted at 37 °C for 30 min in humid conditions to avoid drying of the trypsin solution. Use of the trypsin beads made it possible to reduce the tryptic digestion time, which normally requires at least several hours. Following tryptic digestion, 1 µL of the matrix (α-cyano-4-hydroxycinnamic acid) solution was overlaid on the sample and dried. MS and MS/MS experiments were subsequently conducted using Shimadzu's MALDImini-1 compact MALDI-DIT mass spectrometer.

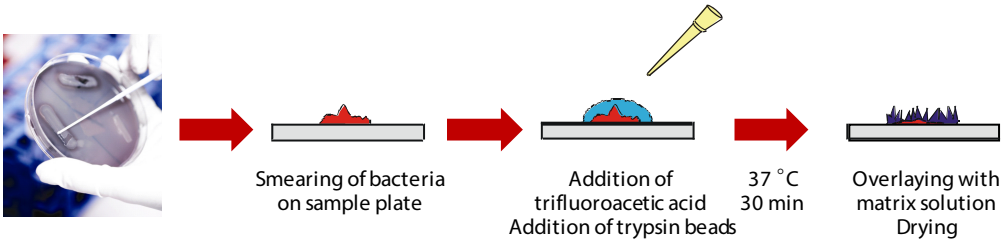


**Fig. 2 Workflow of Sample Preparation**

## Results

Fig. 3 shows the MS spectrum of the "digested" spores. Several proteolytic peptides from spore proteins were detected. The peak detected at *m/z* 1879 was selected and submitted to MS/MS analysis. Fig. 4 shows the resulting spectrum. Data was subsequently submitted to a Mascot (Matrix Science) MS/MS Ion Search resulting in the identification of a *B. subtilis protein*: small, acid-soluble spore protein (SASP) (Fig. 5). The identification was followed by a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the sequence of the identified peptide. The results showed that the peptide has a unique sequence found specifically in *B. subtilis* which was different from the sequences found in *B. anthracis* and *B. cereus* for instance.

This further corroborates the Mascot search results: that the targeted peptide belonged to a *B. subtilis* protein and hence the spotted microorganism was indeed *B. subtilis*. Publication by Warscheid, et al. [1], shows similar studies were conducted for the *B. cereus* and *B. anthracis* group and showed that it is possible to identify the bacterial species using a quick digest followed by MS/MS and Blast searches.

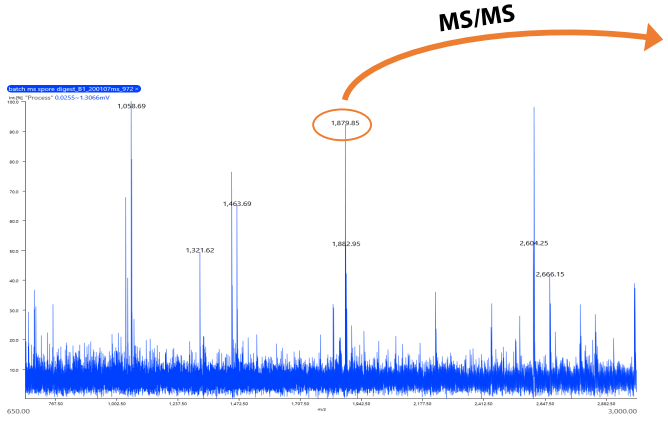


**Fig. 3 Mass Spectrum of Tryptic Digest of Spores**

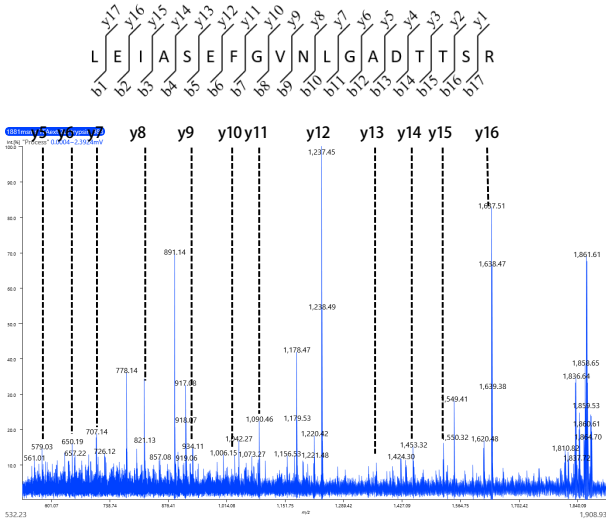


**Fig. 4 Result of MS/MS of *m/z* 1879**

SSPA_BACSU **Mass:** 7066 **Score:** 81 **Matches:** 1(1) **Sequences:** 1(1)
Small, acid-soluble spore protein A OS=Bacillus subtilis (strain 168) GN=sspA PE=3 SV=1
☐ Check to include this hit in error tolerant search or archive report

| Query | Observed | Mr(expt) | Mr(calc) | Delta | Miss | Score | Expect | Rank | Unique | Peptide |
|---|---|---|---|---|---|---|---|---|---|---|
| ☑ 1 | 1879.9400 | 1878.9327 | 1878.9323 | 0.0004 | 0 | 81 | 3.9e-006 | 1 | U | K.LEIASEFGVNLGADTTSR.A |

**Fig. 5 Result of Database Search by MS/MS Ion Search**

## Conclusion

A workflow for rapid identification of spores was presented as an alternative to regular microbial identification by combining on-plate proteolysis followed by MS and MS/MS of resulting peptides. Digestion of spore proteins was conducted by immobilized trypsin which enabled rapid digestion. MALDImini-1 provided a unique combination of size and capability to achieve the goals of the study. Given its compact size, MALDImini-1 will enable performing this workflow in locations that were not available to regular mass spectrometers.

<Reference>

(1) B. Warscheid and C. Fenselau (2003), Characterization of Bacillus Spore Species and Their Mixtures Using Postsource Decay with a Curved-Field Reflectron. *Anal. Chem.* 75:5618-5627.



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