

Application News

No. B50

MALDI-TOF Mass Spectrometry

Detection of Secondary Metabolites of Bacteria Using Whole-Cell Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

The microbial identification method using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been gaining attention in recent years as a quick and simple evaluation technique for identifying bacteria. In microbial identification by MALDI-TOF MS, a small amount of a microbial colony is first mixed with a matrix substance which assists in the MALDI ionization process, and the mixture is then measured. The detected group of peaks originating from peptides and proteins is then used as a fingerprint for microorganism identification. In fact, since slight adjustments to the sample preparation method and measurement conditions allow lipids and metabolites of microbial origin to be detected in the low molecular weight region, a quick and simple procedure similar to microbial identification by MALDI-TOF MS can be used

to identify some such products.

Here, we introduce an example in which surfactin, a secondary metabolite of *Bacillus subtilis* which displays antibacterial activity, was easily detected using the *AXIMA Performance*, one of the Shimadzu MALDI-TOF MS instruments in the AXIMA product line.

Fig. 1 shows the flow of this analysis. 1) For this analysis, a single colony of cultured, frozen, and stored *Bacillus subtilis* cells were collected. 2) The collected cells were mixed with the matrix, and approximately 1 μL of this solution was spotted on a sample plate and allowed to dry. 3) The prepared cell sample was analyzed using the *AXIMA Performance*. Sample preparation takes approximately ten minutes to complete followed by one minute MALDI-TOF MS data acquisition.

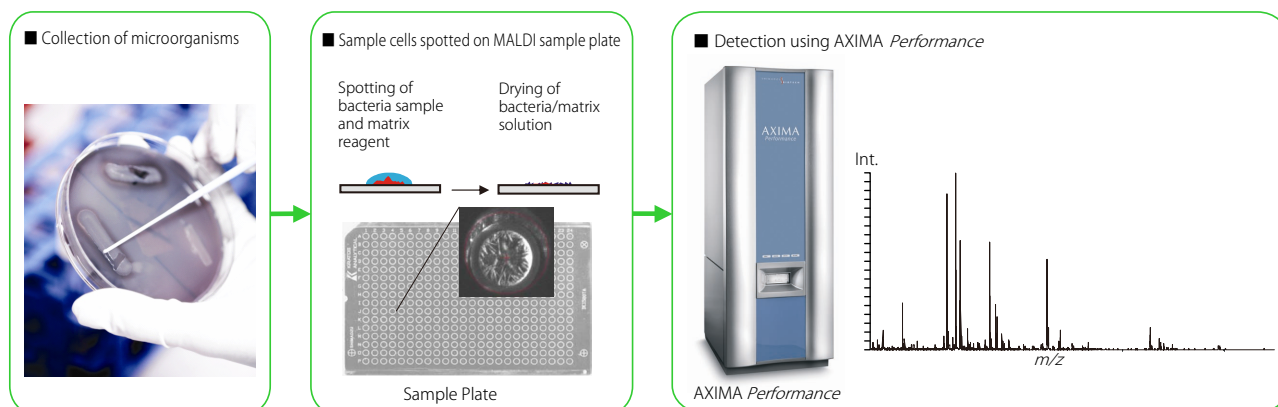


Fig. 1 Schematic Overview of the Detection of Secondary Metabolites of Microorganisms Using MALDI-TOF MS

Fig. 2 shows the mass spectrum of *B. subtilis* acquired using the analytical conditions for identification of microorganisms. Several peaks associated with substances derived from proteins are detected in the range of m/z 4000 to 15000. Microbial identification by MALDI-TOF MS is conducted by comparing the unique spectral fingerprint of a given species of microorganism to a database.

Next, the analytical conditions were changed for *B. subtilis* to obtain the mass spectrum shown in Fig. 3. Several peaks were detected in the range of m/z 700 to 3000. Due to the use of CHCA matrix which favors the detection of peptides, several of the detected peaks are presumed to be non-ribosomal peptides, a class of secondary metabolites¹⁾. Upon submitting m/z 1042 to Norine²⁾, a non-ribosomal peptide database search engine which uses molecular weight as the index, surfactin was returned as one of the likely candidates. The MS/MS results for m/z 1042 precursor also support the search results (Fig. 4).

The above results show that, in addition to proteins, microbial substances including secondary metabolites can also be detected using a simple procedure similar to microbial identification by MALDI-TOF MS.

This technique has been reported to be available for subtyping microorganisms and screening useful biological products, and future applications are expected.^{3), 4)}

[References]

1. Anal. Chem. 2003, 75, 1628-1637.
2. Norine: <http://bioinfo.lifl.fr/norine/>
3. J Mass Spectrom. 2007, 42, 1062-1068.
4. Comb Chem High Throughput Screen. 2003, 6, 557-567.

■ Measurement Conditions

Instrument : AXIMA Performance
 Measurement conditions : Positive/linear mode
 Sample : *Bacillus subtilis* cell suspension
 Matrix : Saturated CHCA solution

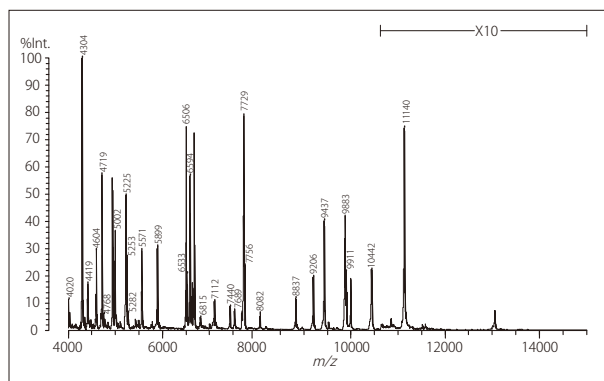


Fig. 2 Typical Mass Spectrum of *B. subtilis* Using Whole-Cell Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

Instrument : AXIMA Performance
 Measurement conditions : Positive/Reflection mode
 Sample : *Bacillus subtilis* cell suspension
 Matrix : Saturated CHCA solution+ lithium salt

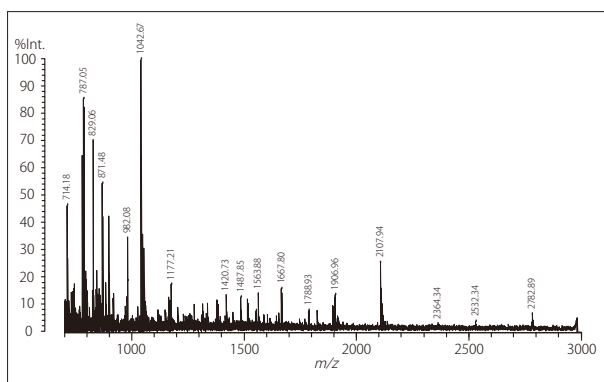


Fig. 3 Detection of Secondary Metabolites of *B. subtilis* Using Whole-Cell MALDI-TOF MS

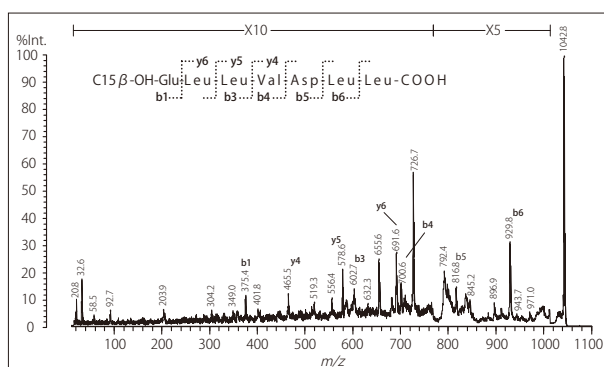


Fig. 4 MS/MS of m/z 1042

Related Products

Some products may be updated to newer models.



> **AXIMA Performance**
AXIMA Performance - a highly flexible
research-gra...

Related Solutions

> Price Inquiry

> Product Inquiry

> Technical Service /
Support Inquiry

> Other Inquiry