

## Application News

MALDI-TOF Mass Spectrometer  
MALDI-TOF MS Microbial Identification Software

### Identification of *Acinetobacter* Species Using MicrobialTrack

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#### User Benefits

- ◆ Microbial clusters can be identified with high accuracy, which is difficult using conventional MALDI-TOFMS microbial identification technology.
- ◆ Theoretical protein information predicted from genomic data enables detailed analysis of mass spectra.
- ◆ MicrobialTrack is a cloud-based service, so there is no need for a dedicated PC or to install software.

#### ■ Introduction

MALDI-TOFMS microbial identification has attracted much attention in the clinical and food fields due to its low cost, speed and ease of use. However, some clinically important microorganisms have been difficult to identify using conventional MALDI-TOFMS microbial identification technology. One example is the *A. calcoaceticus*-*baumannii* (ACB) complex, which consists of six species, including *A. baumannii* and *A. calcoaceticus*. In particular, *A. baumannii*, the most well-known bacteria of the ACB complex, has high antimicrobial resistance and is regarded as an important cause of nosocomial pneumonia.

This Application News introduces the results of identifying the species within the ACB complex using the MicrobialTrack microbial identification software.

#### ■ Experiment

We targeted four species in the ACB complex, *A. baumannii*, *A. calcoaceticus*, *A. nosocomialis* and *A. pittii*. The total number of strains was 33, including the reference strains for each species. Table 1 is a partial list of the strains.

The test microbial strains were cultured on Heart Infusion Agar medium at 30 °C for 20 hours. The cultured cells were then treated with formic acid and mixed with a matrix solution (CHCA) to prepare samples. The MALDI-TOFMS AXIMA Performance™ was used to measure the mass spectrum in linear positive mode over the *m/z* range of 2,000-20,000.

Table 1 Partial List of Target Strains

Species	Strain
<i>A. baumannii</i>	NBRC 109757 <sup>T</sup>
	GTC 03322
<i>A. calcoaceticus</i>	KCTC 2357 <sup>T</sup>
	NBRC 12552
<i>A. nosocomialis</i>	LMG 10619 <sup>T</sup>
	NBRC 110500
<i>A. pittii</i>	DSM 25618 <sup>T</sup>
	NBRC 110505

#### ■ Analysis Method

MALDI mass spectra were analyzed using MicrobialTrack. Two databases (All and Representatives; Reps) were used (Fig. 1), and the minimum peak threshold (the denoising parameter) was set to the default value (0.0002). Fig. 2 shows the analysis flow using MicrobialTrack.

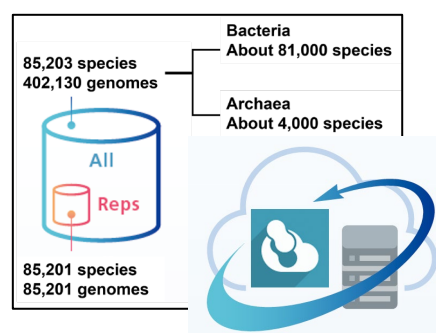


Fig. 1 Two Types of MicrobialTrack Database  
"Reps" contains only representative genomes selected from "All."  
These databases are updated regularly.

#### ■ Results

##### Analysis of MALDI mass spectra

MicrobialTrack compares the measured mass with the theoretical mass predicted from genomic information to verify whether they match within a permissible threshold. If a match is found, the peak is displayed in red, along with the protein name. Relevant information, such as the amino acid sequence, is also displayed in a table.

As an example, Fig. 3 shows the mass spectrum of *A. baumannii*. Most of the major peaks matched the theoretical mass, and ribosomal proteins (30S/50S ribosomal proteins) accounted for more than half (13 in total). Ribosomal proteins are one of the indicators that serve as markers for phylogenetic taxonomy. The detection of a large number of ribosomal proteins that characterize the microbial species is considered a key factor in the correct identification.

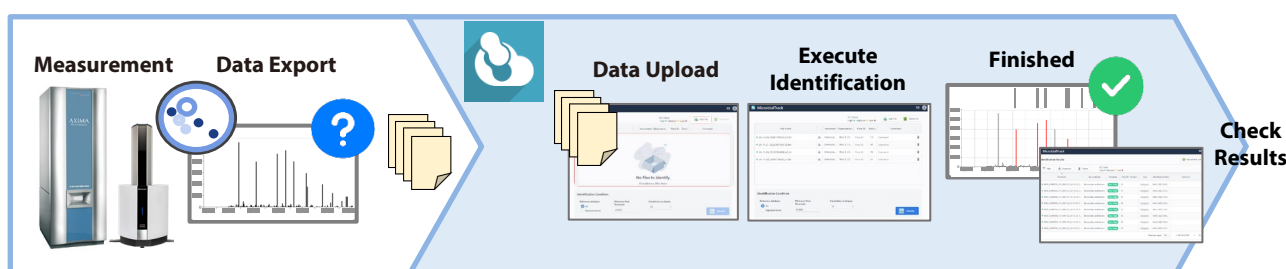


Fig. 2 Analysis Flow Using MicrobialTrack

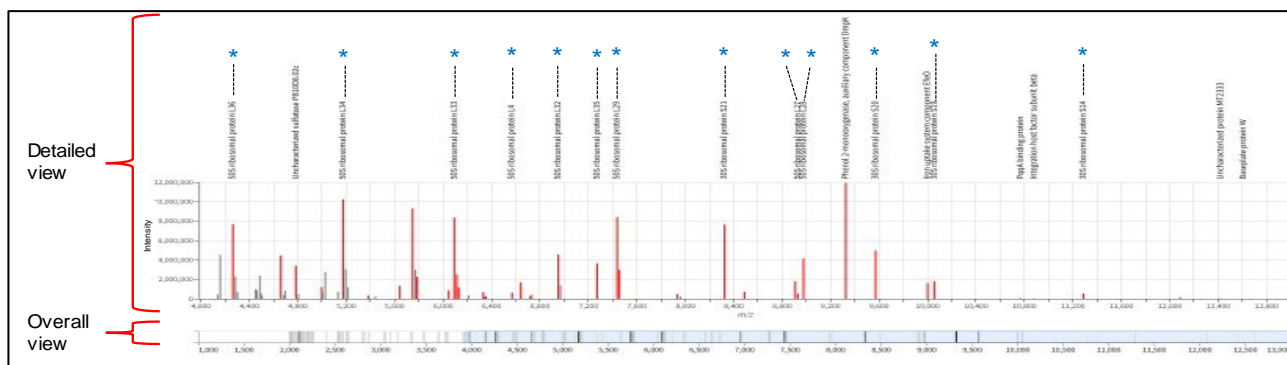


Fig. 3 MicrobialTrack - Identification Result Details Screen (Peak Graph) -

Zooming in or out with the mouse enables the view to be enlarged or reduced. In this figure, the focus is on  $m/z$  4,000 and above. Peaks that match the theoretical mass of the protein within the permissible tolerance are in red, along with the protein name. Of these, ribosomal proteins are marked \*.

## Identification

Reliability represents the degree of confidence in the microorganism candidate. It is categorized into four levels.

- Very High : The target is almost certain to be the identified microorganism species.
- High : The target is likely to be the identified microorganism species.
- Middle : The target may not be the identified microorganism species.
- Low : The target is unlikely to be the identified microorganism species.

[illegible]

Fig. 4 MicrobialTrack – Identification Results Screen –

If the reliability is Middle or lower, check whether the major peaks are attributed on the identification result details screen.

As a result of analyzing 159 data points using the All and Reps DBs, the reliability of 156 of the data were identified as being "Very High," 0 as "High" or "Middle," and 3 as "Low" (Fig. 4).

The 156 data points identified as having “Very High” reliability matched the scientific names listed in culture collections provided by strain providers at a rate of 98.7 % (Fig. 5).

On the other hand, the 3 data points classified as "Low" could not be identified. It is believed that the small number of peaks was the reason why species identification was not possible.

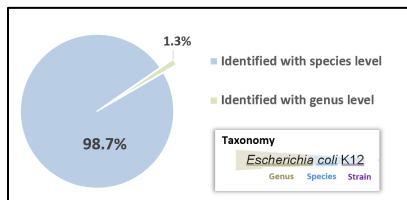


Fig. 5 Identification Result of "Top Candidate" of 156 Data with "Very High"

## ■ Conclusion

In this Application News, mass spectra of four bacterial species (*A. baumannii*, *A. calcoaceticus*, *A. nosocomialis* and *A. pittii*) included in the ACB complex, which have been difficult to identify using conventional MALDI-TOFMS microbial identification technology, were analyzed using MicrobialTrack. As a result, the measured masses of the major peaks detected in each sample matched the theoretical mass of the protein predicted from the genome information. Among these were many ribosomal proteins, which are known to be effective indicators of phylogenetic taxonomy. In addition, the identification results from the two databases were concordant, with approximately 99 % of data identified to species level with “Very High” accuracy.

In mass spectra analysis using MicrobialTrack, the masses measured by MALDI-TOFMS were compared with the theoretical masses of proteins characterizing the microbial species predicted from genomic information. This enabled the identification of microorganisms that had previously been difficult to identify using conventional MALDI-TOFMS alone.

## <References>

- 1) Nithichanon A, Kewcharoenwong C, Da-oh H, Surajinda S, Khongmee A, Koosakunwat S, Wren BW, Stabler RA, Brown JS, Lertmemongkolchai G. *Acinetobacter nosocomialis* causes as severe disease as *Acinetobacter baumannii* in Northeast Thailand: Underestimated role of *A. nosocomialis* in Infection. Microbiol Spectr 10(6):e02836-22 (2022).
- 2) Sekiguchi Y, Teramoto K, Tourlousse D.M. et al. A large-scale genomically predicted protein mass database enables rapid and broad-spectrum identification of bacterial and archaeal isolates by mass spectrometry. Genome Biol 24, 257 (2023).

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Application News No. B114

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MALDI-TOFMS Microbial Identification Software



### › AXIMA Performance

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