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Application News

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

Identification of *Acinetobacter* Species Using MicrobialTrack™ and Benchtop MALDI-TOF MS

Kanae Teramoto, Takashi Nishikaze

User Benefits

- *Acinetobacter* species, previously difficult to distinguish, can now be identified with high accuracy.
- The MALDI-8030 benchtop MALDI-TOF MS system enables rapid and simple acquisition of MALDI mass spectra from microorganisms.
- Detected proteins are analyzed using theoretical masses predicted from their amino acid sequences.

■ Introduction

Acinetobacter species are Gram-negative bacteria widely distributed in natural environments, including soil and river water. While some strains perform industrially useful functions, such as degrading environmental pollutants and crude oil, others, such as *A. baumannii* and *A. haemolyticus,* are pathogenic and multidrug-resistant. In addition, some of these species are difficult to identify using the MALDI-TOF MS systems currently used for microbial testing.

This application news describes the identification of *Acinetobacter* species using MALDI-TOF MS with the MicrobialTrack software, focusing on type strains such as *A. baumannii, A. haemolyticus,* and *A. lwoffii,* which are typically challenging to identify.

■ Experimental

MALDI-TOF MS Measurements

Sixteen strains of the genus *Acinetobacter* were obtained from the National Institute of Technology and Evaluation (NITE) Biotechnology Center (NBRC), the RIKEN BioResource Center (BRC) Microbial Materials Development Office (JCM) and used in this study. Each strain was cultured on agar media recommended by the culture collection center at 30 °C for 24 hours.

For MALDI-TOF MS analysis, α-cyano-4-hydroxycinnamic acid (CHCA) was used as the MALDI matrix reagent. A small amount of cultured bacterial cells was smeared onto the MALDI-MS sample plate. One microliter of CHCA solution was spotted onto the smeared bacterial cells and allowed to air-dry . MALDI mass spectra were acquired over an *m/z* range of 3,500-20,000 using a MALDI-8020 benchtop linear MALDI-TOF mass spectrometer in positive-ion mode.

Analysis of MALDI Mass Spectra

The acquired MALDI mass spectra were exported in ASCII format and analyzed using MicrobialTrack, which identifies microorganisms using a database comprising approximately 400,000 genomes from 85,000 prokaryotic species, including *Bacteria* and *Archaea* (Fig. 1).

Identification results rated "High" or "Very High" were accepted. If the results were not "High" or "Very High," or if the identification results for the same strain were inconclusive, samples prepared using on-plate 25 % formic acid extraction were analyzed. If that failed, samples prepared using the in-tube formic acid/acetonitrile extraction method were analyzed.

■ Results and Discussion

MALDI Mass Spectra

Fig. 2 shows the MALDI mass spectrum of *A. baumannii* NBRC 109757^T at *m/z* 4,000 to 18,500. MicrobialTrack assigned 20 peaks to ribosomal proteins and 41 to other proteins. Among the assigned peaks, ribosomal protein peaks that are easily detected by MALDI-TOF MS and are known to be useful as phylogenetic markers are shown with an asterisk.

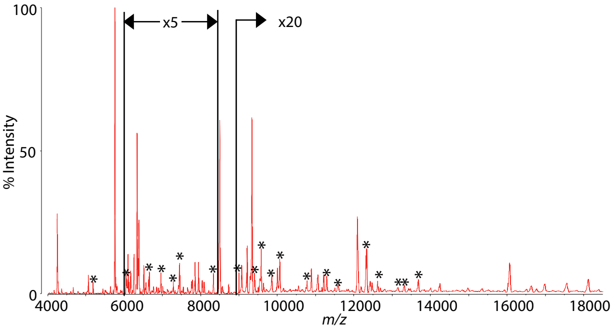


Fig. 2 MALDI Mass Spectrum from *A. baumannii* NBRC 109757^T

Ribosomal proteins are reported to be detected as major peaks in microorganisms. However, analysis of MALDI mass spectra based on theoretical protein masses revealed the presence of numerous on-ribosomal proteins. These non-ribosomal proteins appeared as major peaks in the mass spectra shown here.

Among the proteins detected in addition to ribosomal proteins, the following are considered to be of particular functional importance: glutaredoxin, bacterioferritin-associated ferredoxin, beta-lactamase hydrolase-like protein, lactoylglutathione lyase, major cold shock protein CspA, DNA-binding protein Fis, translational regulator CsrA, potassium-binding protein Kbp, trans-acting regulatory protein HvrA, and sec-independent protein translocase protein TatB.

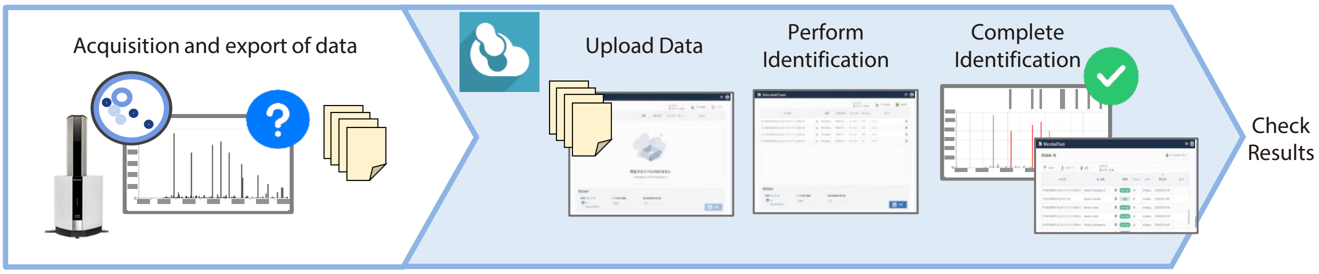


Fig. 1 Flow for Microbial Identification Using MicrobialTrack

Table 1 Identification Results of 16 Acinetobacter Strains by MicrobialTrack

Sample strain		Top candidate	Reliability	Resampling	
Acinetobacter baumannii	NBRC 109757^{T}	*Acinetobacter baumannii*	**Very High**	*Acinetobacter baumannii*	**100 %**
Acinetobacter baylyi	NBRC 112578^{T}	*Acinetobacter baylyi*	**Very High**	*Acinetobacter baylyi*	**100 %**
Acinetobacter beijerinckii	NBRC 112579^{T}	*Acinetobacter beijerinckii*	**Very High**	*Acinetobacter beijerinckii*	**100 %**
Acinetobacter bouvetii	JCM 18991^{T}	*Acinetobacter bouvetii*	**Very High**	*Acinetobacter bouvetii*	**100 %**
Acinetobacter calcoaceticus	NBRC 12552	*Acinetobacter calcoaceticus*	**Very High**	*Acinetobacter calcoaceticus*	**100 %**
Acinetobacter gyllenbergii	NBRC 112581^{T}	*Acinetobacter gyllenbergii*	**Very High**	*Acinetobacter gyllenbergii*	**100 %**
Acinetobacter haemolyticus	NBRC 109758^{T}	*Acinetobacter haemolyticus*	**Very High**	*Acinetobacter haemolyticus*	**100 %**
Acinetobacter johnsonii	NBRC 102197	*Acinetobacter johnsonii*	**Very High**	*Acinetobacter johnsonii*	**100 %**
Acinetobacter junii	NBRC 109759^{T}	*Acinetobacter junii*	**Very High**	*Acinetobacter junii*	**100 %**
Acinetobacter lwoffii	NBRC 109760^{T}	*Acinetobacter lwoffii*	**Very High**	*Acinetobacter lwoffii*	**100 %**
Acinetobacter nosocomialis	NBRC 110498	*Acinetobacter nosocomialis*	**Very High**	*Acinetobacter nosocomialis*	**100 %**
Acinetobacter parvus	NBRC 112582^{T}	*Acinetobacter parvus*	**Very High**	*Acinetobacter parvus*	**100 %**
Acinetobacter pittii	NBRC 110505	*Acinetobacter pittii*	**Very High**	*Acinetobacter pittii/lactucae*	**93 %**
Acinetobacter radioresistens	NBRC 102413^{T}	*Acinetobacter radioresistens*	**Very High**	*Acinetobacter radioresistens*	**100 %**
Acinetobacter schindleri	NBRC 112583^{T}	*Acinetobacter schindleri*	**Very High**	*Acinetobacter schindleri*	**100 %**
Acinetobacter venetianus	NBRC 112585^{T}	*Acinetobacter venetianus*	**Very High**	*Acinetobacter venetianus*	**100 %**

In addition to species identification, MicrobialTrack can also analyze observed peaks based on theoretical mass. For unmodified proteins and proteins with predicted N-terminal methionine truncations, the corresponding amino acid sequences are displayed. Utilizing these amino acid sequences enables the application of biomarker methods that focus on subtle variations in the amino acid sequences of specific proteins, enabling sub-species or strain-level classification (typing).

Note: MicrobialTrack assigns a peak when the observed mass matches the theoretical mass within acceptable error limits. This does not definitively confirm the actual presence of the assigned protein.

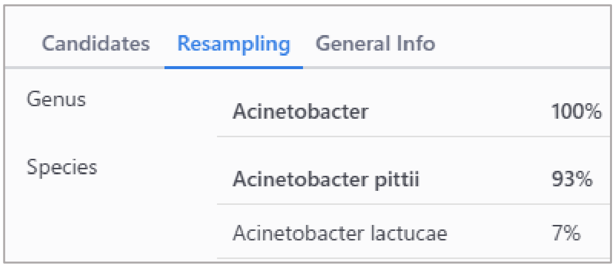
Identification Results

Table 1 shows the identification results for 16 *Acinetobacter* strains using MicrobialTrack. It also shows the resampling results (the resampling function evaluates identification result reliability by randomly removing detected peaks and re-analyzing the remaining peaks). Of the 16 *Acinetobacter* strains, 15 were identified by their NITE NBRC or RIKEN JCM names using prepared microbial samples and CHCA solutions (direct smear method). For *A. pittii,* the reliability scores of the direct smear method and the on-plate 25 % formic acid extraction method were both Low to Middle, and two microbial species were the most likely candidates. Therefore, the reliability of *A. pittii* identified by the in vitro formic acid/acetonitrile extraction method was considered the final result. However, *A. lactucae* was considered to be in complex with *A. pittii* (a different species with over 80 % identity in theoretical ribosomal protein).

Results of Polyphasic Analysis by Resampling

Using MicrobialTrack's proprietary resampling algorithm, which randomly selects portions of observation peaks for repeat identification, it was found that all the sample bacteria, excluding *A. pittii,* were identified as top candidate species. This supports the reliability of these identifications. For *A. pittii,* resampling estimated that 93 % of the strains were *A. pittii,* while 3 % were identified as *A. lactucae,* a related species. This strongly supports the identification of *A. pittii* in the sample strains, despite their close protein-level relationship (Table 2).

Table 2 Resampling Results for *A. pittii* NBRC 110505^{T}



Genus	Acinetobacter	100%
Species	Acinetobacter pittii	93%
	Acinetobacter lactucae	7%

■ Conclusion

In this study, MALDI-8030 and MicrobialTrack were used to analyze *Acinetobacter* species, which are widely found in natural environments and have a variety of functions. This tool combination successfully identified the *Acinetobacter baumannii* complex, which is typically difficult to distinguish. Resampling analysis confirmed that *A. pittii* was correctly identified, despite the existence of a closely related species with over 80 % similarity at the ribosomal protein level.

<Related Applications>

1) MALDI-MS Proteotyping of *Cutibacterium Acnes*, Application News No. eB114.
2) Identification of *Acinetobacter* Species Using MicrobialTrack, Application News No. 01-00954-EN.

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01-01040-EN First Edition: Mar. 2026

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