

Performance Evaluation of Microbial Identification Using a Benchtop MALDI-TOF MS

Yumi Unno, Tomonori Oshikawa, Kanae Teramoto

User Benefits

- ◆ Microbial species can be identified with high accuracy using a database containing over 80,000 registered entries.
- ◆ Post-acquisition data are processed by a cloud-based identification algorithm, enabling simple and rapid result output.
- ◆ By using the MALDI-8020 / 8030 EasyCare with standardized measurement procedures, highly reproducible data acquisition is achieved.

Introduction

Microbial identification is a fundamental technology supporting research and development, quality control, and risk management across diverse fields, including human health, food, and environmental sciences. In recent years, MALDI-TOF MS has been increasingly adopted for on-site microbial testing because it enables rapid identification with simple sample preparation. However, conventional fingerprint-based methods relying on MALDI mass spectral pattern matching face challenges when reference spectra are unavailable, often requiring the construction of in-house spectral libraries.

MicrobialTrack, a MALDI-TOF MS-based microbial identification software, employs a proteomics-based approach^{*1} derived from genetic information of more than 80,000 taxonomically classified prokaryotic microorganisms. This method enables identification of prokaryotic microorganisms with higher discriminatory resolution than 16S rRNA gene sequence analysis, without the need to build in-house spectral libraries.

In this application news, the identification performance of MicrobialTrack combined with the benchtop MALDI-TOF MS system MALDI-8030 EasyCare (Fig. 1) was evaluated using 50 bacterial strains with high analytical demand in research, food, and industrial applications.



Fig. 1 MALDI-8030 EasyCare (Left) and MicrobialTrack™ (Right)

*1 The proteomics-based approach refers to a class of methods in which theoretical protein mass patterns predicted from genomic information are compiled into a database and compared with experimentally acquired mass spectra of microbial proteins for microbial identification. In MicrobialTrack, a theoretical MS spectral database is constructed based on public nucleotide sequence databases, and microbial nomenclature and taxonomy follow the Genome Taxonomy Database (GTDB). Because new species registration and updates to taxonomic classification and nomenclature occur frequently, the database is regularly updated to reflect these changes.

Experiment

1. Microbial strains and reagents

The microbial strains listed in Table 1 were obtained from the National Institute of Technology and Evaluation, Biotechnology Center (NBRC), and the Microbial Resource Development Section, RIKEN BioResource Research Center (JCM). Each strain was cultured on standard agar plates at 30°C for 24 h.

α -Cyano-4-hydroxycinnamic acid (CHCA) was used as the matrix reagent. The matrix solution was prepared by dissolving CHCA at a concentration of 10 mg/mL in an aqueous solution containing 1% trifluoroacetic acid, 15% ethanol, and acetonitrile.

Table 1 Fifty microbial strains used for microbial identification

| Sample Strain | |
|---|--------------------------|
| <i>Acinetobacter baumannii</i> | NBRC 109757 ^T |
| <i>Acetobacter orientalis</i> | NBRC 16606 ^T |
| <i>Acinetobacter lwoffii</i> | NBRC 109760 ^T |
| <i>Aerococcus viridans</i> | NBRC 12219 ^T |
| <i>Aeromonas caviae</i> | JCM 1060 ^T |
| <i>Bacillus amyloliquefaciens</i> | NBRC 3007 |
| <i>Bacillus atrophaeus</i> | NBRC 15539 ^T |
| <i>Bacillus mycoides</i> | NBRC 101228 ^T |
| <i>Bacillus subtilis</i> subsp. <i>subtilis</i> | NBRC 13719 ^T |
| <i>Bacillus thuringiensis</i> | NBRC 101235 ^T |
| <i>Bacteroides thetaiotaomicron</i> | JCM 5827 ^T |
| <i>Brevundimonas vesicularis</i> | NBRC 12165 ^T |
| <i>Citrobacter werkmanii</i> | NBRC 105721 ^T |
| <i>Clostridium beijerinckii</i> | NBRC 109359 ^T |
| <i>Cronobacter sakazakii</i> | NBRC 102416 ^T |
| <i>Cutibacterium acnes</i> subsp. <i>acnes</i> | JCM 6425 ^T |
| <i>Deinococcus radiodurans</i> | NBRC 15346 ^T |
| <i>Enterobacter hormaechei</i> | NBRC 105718 ^T |
| <i>Escherichia coli</i> | NBRC 3301 |
| <i>Geobacillus stearothermophilus</i> | NBRC 12550 ^T |
| <i>Lactocaseibacillus casei</i> | NBRC 15883 ^T |
| <i>Lactocaseibacillus paracasei</i> subsp. <i>paracasei</i> | JCM 8130 ^T |
| <i>Lactiplantibacillus pentosus</i> | NBRC 106467 ^T |
| <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> | NBRC 15891 ^T |
| <i>Lactobacillus gasserii</i> | JCM 1131 ^T |
| <i>Lactobacillus johnsonii</i> | JCM 2012 ^T |
| <i>Lapidilactobacillus dextrinicus</i> | JCM 5887 ^T |
| <i>Latilactobacillus curvatus</i> | NBRC 15884 ^T |
| <i>Latilactobacillus sakei</i> subsp. <i>sakei</i> | NBRC 15893 ^T |
| <i>Lentilactobacillus hilgardii</i> | NBRC 15886 ^T |
| <i>Levilactobacillus brevis</i> | NBRC 107147 ^T |
| <i>Limosilactobacillus reuteri</i> | NBRC 15892 ^T |
| <i>Listeria innocua</i> | JCM 32814 ^T |
| <i>Micrococcus luteus</i> | NBRC 3333 ^T |
| <i>Micrococcus lylae</i> | NBRC 15355 ^T |
| <i>Morganella morganii</i> subsp. <i>morganii</i> | NBRC 3848 ^T |
| <i>Oligella urethralis</i> | NBRC 14589 ^T |
| <i>Paenibacillus polymyxa</i> | NBRC 15309 ^T |
| <i>Pediococcus acidilactici</i> | NBRC 109619 ^T |
| <i>Pseudomonas oryzae</i> subsp. <i>oryzae</i> | NBRC 102199 ^T |
| <i>Ralstonia pickettii</i> | NBRC 102503 ^T |
| <i>Rhodococcus corynebacterioides</i> | NBRC 14404 ^T |
| <i>Shouchella lehensis</i> | JCM 13820 ^T |
| <i>Staphylococcus capitis</i> subsp. <i>capitis</i> | JCM 2420 ^T |
| <i>Staphylococcus cohnii</i> subsp. <i>cohnii</i> | NBRC 109713 ^T |
| <i>Staphylococcus epidermidis</i> | NBRC 100911 ^T |
| <i>Staphylococcus hominis</i> subsp. <i>hominis</i> | NBRC 110726 ^T |
| <i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i> | NBRC 102446 ^T |
| <i>Staphylococcus warneri</i> | NBRC 109769 ^T |
| <i>Weizmannia coagulans</i> | NBRC 12583 ^T |

2. Sample preparation of microbial strains

In this study, three commonly used sample preparation methods were evaluated: the direct smear method (DS), the on-plate formic acid extraction method (FA), and the in-tube ethanol washing followed by formic acid/acetonitrile extraction method (EtOH).

All samples were initially prepared using the DS method. Samples yielding identification confidence rated as Middle or Low were re-prepared and reanalyzed using the FA or EtOH method. An overview of each method is described below.

3. Direct smear method (DS)

The DS method is the simplest sample preparation approach and is suitable for rapid screening. A single colony grown on an agar plate was picked using a toothpick and directly smeared onto a MALDI target plate. Subsequently, 1 μ L of matrix solution was applied to the smear and allowed to dry at room temperature.

4. On-plate formic acid extraction method (FA)

The FA method involves the addition of formic acid to disrupt the cell wall and extract intracellular ribosomal proteins and is applied when sufficient identification results are not obtained with the DS method. A small amount of a colony was picked from an agar plate using a toothpick and smeared onto a MALDI sample slide.

Then, 0.5 μ L of 25% aqueous formic acid was added to the smeared well and dried. After drying, 1 μ L of matrix solution was applied and allowed to dry again.

5. In-tube ethanol washing followed by formic acid/acetonitrile extraction method (EtOH)

The EtOH method is effective for the identification of Gram-positive bacteria with thick cell walls. Bacterial cells were collected into a 0.5 mL tube, ethanol was added to a final concentration of 80%, and the cells were washed by centrifugation. The resulting pellet was resuspended in an appropriate volume of 70% formic acid, followed by the addition of an equal volume of 100% acetonitrile and thorough mixing. After centrifugation, 1 μ L of the supernatant was spotted onto a MALDI sample slide and dried. Subsequently, 1 μ L of matrix solution was applied and allowed to dry again.

6. MALDI-TOF MS measurement and microbial identification

MALDI-TOF MS spectra were acquired using the benchtop MALDI-TOF MS system MALDI-8030 EasyCare. Measurements were performed in linear positive ion mode over a mass range of m/z 3,500–20,000. *Escherichia coli* DH5 α Electro-Cells (Takara Bio) were used for mass calibration. The acquired MALDI-TOF MS spectra were exported in ASCII format and analyzed for microbial identification with MicrobialTrack.

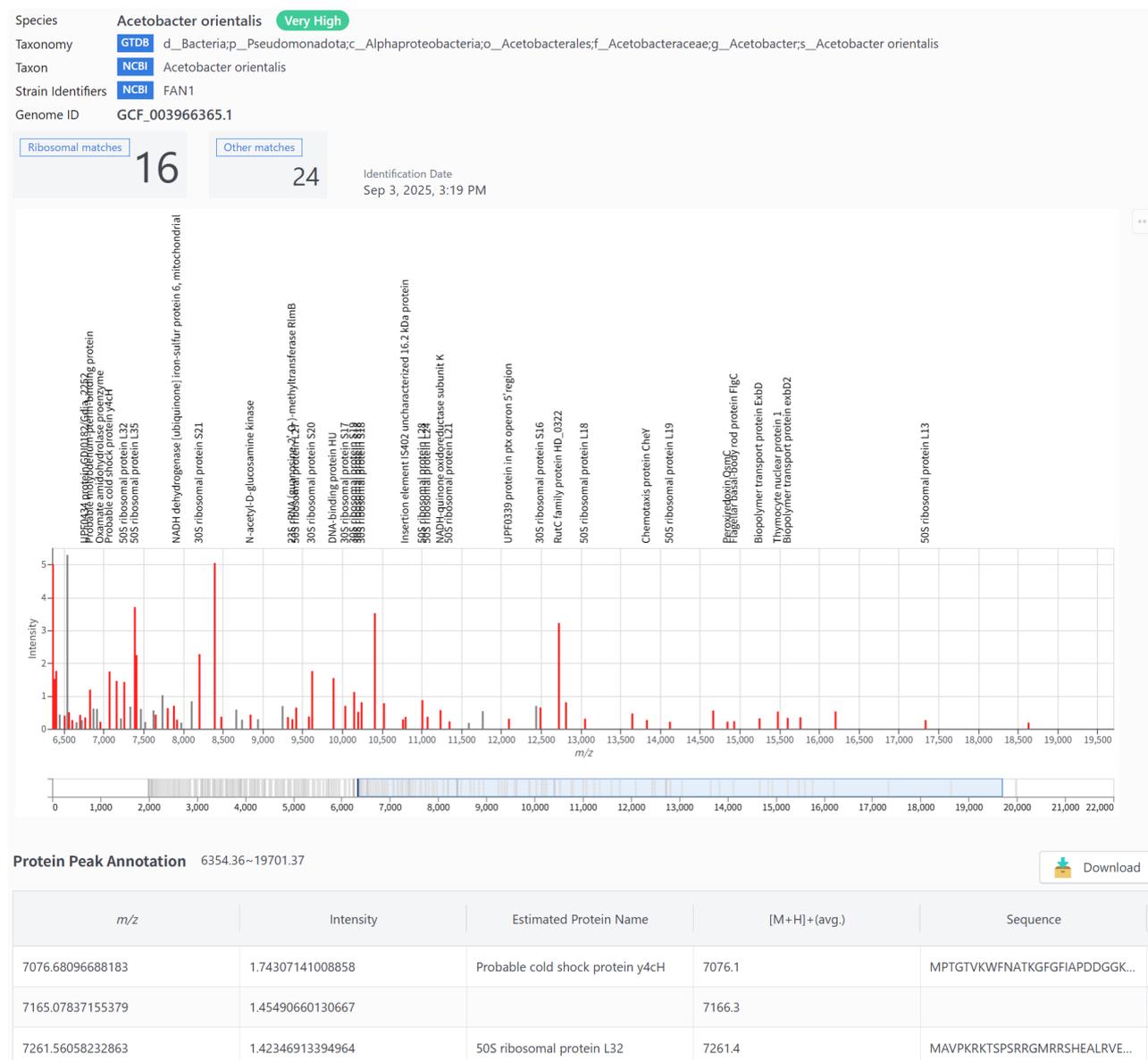


Fig. 2 Example of microbial identification using MicrobialTrack

Peaks for which the observed masses match the theoretical protein masses are highlighted in red. For proteins with assigned names, the corresponding protein names are displayed within the spectrum. Protein peak annotations are summarized in a table showing the observed mass, peak intensity, estimated protein name, theoretical mass, and amino acid sequence for each detected peak.

■ Results and Discussion

The analysis results obtained using MicrobialTrack display the species name, taxonomic information, genome ID, number of protein hits, and protein annotation information (Fig. 2).

1. Microbial identification results using the DS method

The analysis results for the 50 strains evaluated in this study are summarized in Table 2. Using the DS method, identification confidence rated as *High* or *Very High* was achieved for 47 of the 50 strains (94%), and agreement with the reference species name was confirmed for 46 strains.

2. Improvement of identification confidence by changing the sample preparation method

Four microbial strains that yielded *Middle* or *Low* identification confidence with the DS method were re-prepared and reanalyzed using the FA and EtOH methods. As a result, identification confidence improved to *Very High* for three strains—*Lentilactobacillus hilgardii*, *Levilactobacillus brevis*, and *Micrococcus luteus*—and agreement with the reference species names was confirmed. These results demonstrate that optimization of sample preparation methods can enhance identification performance.

3. Analysis of *Bacillus amyloliquefaciens*

Bacillus amyloliquefaciens is closely related to *Bacillus velezensis*, which was presented as the top candidate in this analysis, with an Average Nucleotide Identity (ANI) of approximately 93–94% or higher¹. These species share more than 80% concordance in the theoretical masses of ribosomal proteins and belong to a microbial taxonomic group referred to as a “complex”, in which discrimination based solely on MALDI-TOF MS patterns is challenging.

ANI is a metric for species delineation based on overall genomic similarity, encompassing both protein-coding and non-coding regions. In contrast, MALDI-TOF MS-based identification primarily targets ribosomal proteins, which are commonly used as phylogenetic markers. Therefore, in taxonomic groups with high ribosomal protein similarity, unambiguous species-level discrimination may be difficult.

In this study, analysis of *B. amyloliquefaciens* NBRC 3007 detected nine ribosomal proteins derived from the type strain of *B. amyloliquefaciens*, all of which were identical to the corresponding ribosomal proteins of the *B. velezensis* type strain. These results indicate that the two species exhibit extremely high relatedness at the protein level.

Table 2 Results of microbial identification

| Sample Strain | | Top candidate | Reliability |
|---|--------------------------|---|-------------|
| <i>Acinetobacter baumannii</i> | NBRC 109757 ^T | <i>Acinetobacter baumannii</i> | Very High |
| <i>Acetobacter orientalis</i> | NBRC 16606 ^T | <i>Acetobacter orientalis</i> | Very High |
| <i>Acinetobacter lwoffii</i> | NBRC 109760 ^T | <i>Acinetobacter lwoffii</i> | Very High |
| <i>Aerococcus viridans</i> | NBRC 12219 ^T | <i>Aerococcus viridans</i> | Very High |
| <i>Aeromonas caviae</i> | JCM 1060 ^T | <i>Aeromonas caviae</i> | Very High |
| <i>Bacillus amyloliquefaciens</i> | NBRC 3007 | <i>Bacillus velezensis</i> | Very High |
| <i>Bacillus atrophaeus</i> | NBRC 15539 ^T | <i>Bacillus atrophaeus</i> | Very High |
| <i>Bacillus mycoides</i> | NBRC 101228 ^T | <i>Bacillus_A mycoides</i> | Very High |
| <i>Bacillus subtilis</i> subsp. <i>subtilis</i> | NBRC 13719 ^T | <i>Bacillus subtilis</i> | Very High |
| <i>Bacillus thuringiensis</i> | NBRC 101235 ^T | <i>Bacillus_A thuringiensis_S</i> | Very High |
| <i>Bacteroides thetaiotaomicron</i> | JCM 5827 ^T | <i>Bacteroides thetaiotaomicron</i> | Very High |
| <i>Brevundimonas vesicularis</i> | NBRC 12165 ^T | <i>Brevundimonas vesicularis</i> | High |
| <i>Citrobacter werkmanii</i> | NBRC 105721 ^T | <i>Citrobacter werkmanii</i> | Very High |
| <i>Clostridium beijerinckii</i> | NBRC 109359 ^T | <i>Clostridium beijerinckii</i> | Very High |
| <i>Cronobacter sakazakii</i> | NBRC 102416 ^T | <i>Cronobacter sakazakii</i> | Very High |
| <i>Cutibacterium acnes</i> subsp. <i>acnes</i> | JCM 6425 ^T | <i>Cutibacterium acnes</i> | Very High |
| <i>Deinococcus radiodurans</i> | NBRC 15346 ^T | <i>Deinococcus radiodurans</i> | Very High |
| <i>Enterobacter hormaechei</i> | NBRC 105718 ^T | <i>Enterobacter hormaechei_A</i> | Very High |
| <i>Escherichia coli</i> | NBRC 3301 | <i>Escherichia coli</i> | Very High |
| <i>Geobacillus stearothermophilus</i> | NBRC 12550 ^T | <i>Geobacillus stearothermophilus</i> | Very High |
| <i>Lactocaseibacillus casei</i> | NBRC 15883 ^T | <i>Lactocaseibacillus casei</i> | Very High |
| <i>Lactocaseibacillus paracasei</i> subsp. <i>paracasei</i> | JCM 8130 ^T | <i>Lactocaseibacillus paracasei</i> | Very High |
| <i>Lactiplantibacillus pentosus</i> | NBRC 106467 ^T | <i>Lactiplantibacillus pentosus</i> | Very High |
| <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> | NBRC 15891 ^T | <i>Lactiplantibacillus plantarum</i> | Very High |
| <i>Lactobacillus gasserii</i> | JCM 1131 ^T | <i>Lactobacillus gasserii</i> | Very High |
| <i>Lactobacillus johnsonii</i> | JCM 2012 ^T | <i>Lactobacillus johnsonii</i> | Very High |
| <i>Lapidilactobacillus dextrinicus</i> | JCM 5887 ^T | <i>Lapidilactobacillus dextrinicus</i> | Very High |
| <i>Latilactobacillus curvatus</i> | NBRC 15884 ^T | <i>Latilactobacillus curvatus</i> | Very High |
| <i>Latilactobacillus sakei</i> subsp. <i>sakei</i> | NBRC 15893 ^T | <i>Latilactobacillus sakei</i> | Very High |
| <i>Lentilactobacillus hilgardii</i> | NBRC 15886 ^T | <i>Lentilactobacillus hilgardii</i> ^{*2} | Very High |
| <i>Levilactobacillus brevis</i> | NBRC 107147 ^T | <i>Levilactobacillus brevis</i> ^{*2} | Very High |
| <i>Limosilactobacillus reuteri</i> | NBRC 15892 ^T | <i>Limosilactobacillus reuteri</i> | Very High |
| <i>Listeria innocua</i> | JCM 32814 ^T | <i>Listeria innocua</i> | Very High |
| <i>Micrococcus luteus</i> | NBRC 3333 ^T | <i>Micrococcus luteus</i> ^{*2} | Very High |
| <i>Micrococcus lylae</i> | NBRC 15355 ^T | <i>Micrococcus lylae</i> | Very High |
| <i>Morganella morganii</i> subsp. <i>morganii</i> | NBRC 3848 ^T | <i>Morganella morganii</i> | Very High |
| <i>Oligella urethralis</i> | NBRC 14589 ^T | <i>Oligella urethralis</i> | Very High |
| <i>Paenibacillus polymyxa</i> | NBRC 15309 ^T | <i>Paenibacillus polymyxa</i> | Very High |
| <i>Pediococcus acidilactici</i> | NBRC 109619 ^T | <i>Pediococcus acidilactici</i> | Very High |
| <i>Pseudomonas oryzae</i> subsp. <i>oryzae</i> | NBRC 102199 ^T | <i>Pseudomonas_B oryzae</i> | Very High |
| <i>Ralstonia pickettii</i> | NBRC 102503 ^T | <i>Ralstonia pickettii</i> | Very High |
| <i>Rhodococcus corynebacterioides</i> | NBRC 14404 ^T | <i>Rhodococcus corynebacterioides</i> | Very High |
| <i>Shouchella lehensis</i> | JCM 13820 ^T | <i>Bacillus_H oshimensis</i> ^{*3} | Very High |
| <i>Staphylococcus capitis</i> subsp. <i>capitis</i> | JCM 2420 ^T | <i>Staphylococcus capitis</i> | Very High |
| <i>Staphylococcus cohnii</i> subsp. <i>cohnii</i> | NBRC 109713 ^T | <i>Staphylococcus cohnii</i> | Very High |
| <i>Staphylococcus epidermidis</i> | NBRC 100911 ^T | <i>Staphylococcus epidermidis</i> | Very High |
| <i>Staphylococcus hominis</i> subsp. <i>hominis</i> | NBRC 110726 ^T | <i>Staphylococcus hominis</i> | Very High |
| <i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i> | NBRC 102446 ^T | <i>Staphylococcus saprophyticus</i> | Very High |
| <i>Staphylococcus warneri</i> | NBRC 109769 ^T | <i>Staphylococcus warneri</i> | Very High |
| <i>Weizmannia coagulans</i> | NBRC 12583 ^T | <i>Weizmannia coagulans</i> | Very High |

Results for which agreement between the reference species name and the top candidate was not confirmed are indicated by red cells. Identification confidence is color-coded according to its level.

*2 For *Lentilactobacillus hilgardii*, *Levilactobacillus brevis*, and *Micrococcus luteus*, identification confidence was low using the DS method; therefore, the results shown are based on reanalysis after sample preparation using the FA and EtOH methods.

*3 *Shouchella lehensis* and *Bacillus hoshimensis* represent the same species with different names resulting from taxonomic reclassification.

Accordingly, accurate identification of taxonomic groups containing genetically closely related microbial species requires genetic approaches such as whole-genome analysis or analysis of specific genes capable of discriminating between species within a complex.

■ Conclusion

In this study, microbial identification performance was evaluated using the MALDI-8030 EasyCare in combination with MicrobialTrack for 50 bacterial strains with high analytical demand in research, food, and industrial fields. Using the DS method, agreement with the reference species name at an identification confidence of *High* or above was confirmed for 46 of the 50 strains. For three of the four strains that were difficult to identify using the DS method, switching the sample preparation method to the FA or EtOH method resulted in agreement with the reference species names, demonstrating that optimization of sample preparation methods is effective for improving identification performance.

MicrobialTrack is based on a predictive database comprising more than 80,000 entries and rapidly provides identification results by analyzing acquired MALDI-TOF MS cloud-based system. In cases where phylogenetically closely related species exist, MicrobialTrack does not present a potentially misleading single species name; instead, it reports candidates as a species complex, thereby providing practical identification results while maintaining taxonomic validity. MicrobialTrack is a useful microbial identification tool across a wide range of applications, from research to quality control and routine testing.

<Reference>

- 1) B. Fan, J. Blom, H. Klenk, R. Borriss, "*Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an "Operational Group *B. amyloliquefaciens*" within the *B. subtilis* Species Complex", *Frontiers in Microbiology*, 2017 Jan 20;8:22.

<Related Applications>

1. Identification of *Acinetobacter* Species Using MicrobialTrack
[Application News 01-00954-EN](#)

MicrobialTrack is a trademark of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.



Shimadzu Corporation

www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. See <https://www.shimadzu.com/about/trademarks/index.html> for details.

Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

› Please fill out the survey

Related Products

Some products may be updated to newer models.



› MALDI-8030

Dual-Polarity Benchtop Linear MALDI-TOF Mass Spect...



› MALDI EasyCare

Upgrade for the MALDI-8000 series



› MicrobialTrack

MALDI-TOFMS Microbial Identification Software

Related Solutions

› Life Science

› Microorganisms

› Food and Beverages

› Food Contamination

› Price Inquiry

› Product Inquiry

› Technical Service /
Support Inquiry

› Other Inquiry