



Application Note

● Using the MALDI Biotyper® for microbial identification at the China Center of Industrial Culture Collection (CICC)

Introduction

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the MALDI Biotyper® (MBT) is used at the China Center of Industrial Culture Collection (CICC) to identify strains. The MBT technology plays a key role in the identification process, helping CICC achieve rapid results from test samples from a range of industries including food, pharmaceutical, cosmetics, feed, and environmental protection.

About CICC

CICC was established in 1953 and is a member of both the World Federation for Culture Collections and the China Committee for Culture Collection of Microorganisms, as well as a core agency of National Infrastructure of Microbial Resources.

CICC has approximately 50 employees and one of its main functions is to provide microbial identifications and strain preservation for its customers. Working in collaboration with Bruker,

CICC holds a database of more than 12,000 industrial microbial strains and over 300,000 microorganism copies including bacteria, yeasts, molds, large filamentous fungi, phages and plasmids. The MBT is one of the identification technologies used primarily by CICC for rapid identification in industrial microbiology. The MBT was chosen due to its ability to quickly and easily expand the local library with previously unidentified microorganisms.

Keywords:
Industrial microbiology
Identification
Quality and safety
MALDI-TOF MS
Reference Library
Biodiversity
Fast Moving Consumer Goods
Food
Beverage
Cosmetic

Instrumentation and Software:
MALDI Biotyper
MALDI Biotyper smart
microflex LT/SH
microflex LT/SH smart
MBT Compass Reference Library
MBT Compass Software
MBT Explorer Module

This database is used to assist the rapid identification of strains and is constantly expanding to meet growing demand.

Using the MALDI Biotyper for rapid and simple strain identification

Customers contact CICC directly and send samples to its facility in Beijing using proper packaging and delivery conditions for microbial hazards. While it usually took 24 hours or more to identify a strain, the MBT enables identification within minutes. Therefore, the MBT is firstly used to analyse the delivered microorganisms. This saves time and money while delivering highly accurate results.

One of the main advantages of the MBT is the simple workflow (Figure 1). There is no requirement to know the type of microorganism prior to analysis, as bacteria and fungi samples are all analysed together using the same workflow. Typically, no more than one isolated single colony on selective or non-selective media is required. The isolates are transferred onto a MALDI target and covered with a matrix. Mass spectra are acquired and analysed with the MBT, using MALDI-TOF MS principles combined with the MBT Compass software and Reference Library. Isolates are identified by comparing the generated spectra to the Reference Library, which is updated annually. Matching hints are displayed in the Reference Library and MBT report, giving a clear result in instances when it is difficult to discriminate between different species or when the identification is done at the genus level only. In addition, reference spectra can be created by the users to develop customized libraries.

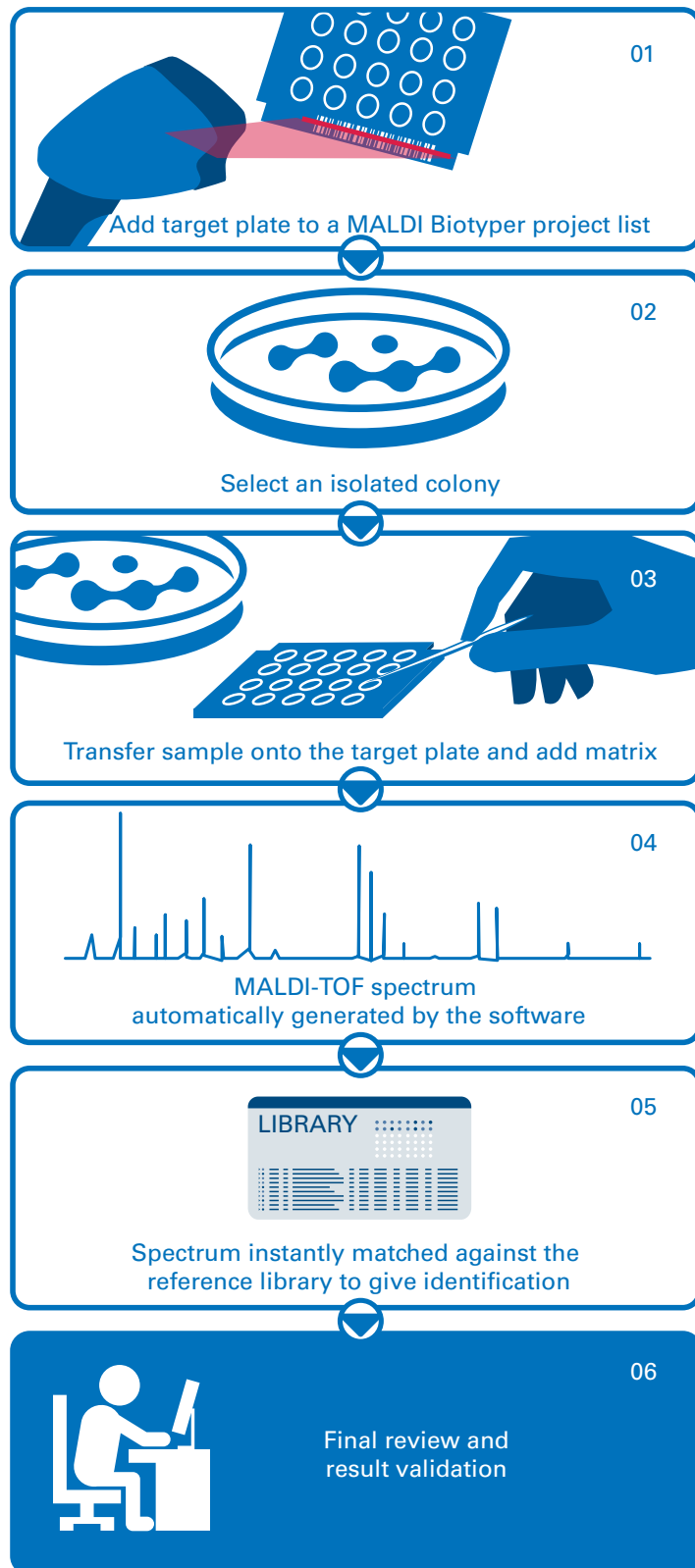


Figure 1: Workflow for the identification of unknown samples with the MALDI Biotyper

The type of microorganism varies depending on its origin, such as food, cosmetics, water or environmental. When it is difficult to reliably identify a strain, a polyphasic approach, combining various technologies and principles, is used. Some strains might not be identified with the MBT when corresponding reference spectra are missing in the MBT Compass Reference Library. Laboratories such as CICC can easily create their own libraries to complement Bruker's to improve the accuracy of the identification results.

The MBT Compass Reference Library, combined with the CICC's extensive library containing hundreds of reference spectra, allows fast and accurate measurements [1].

Range	Interpretation	Symbols	Color
2.000 - 3.000	High Confidence Identification	(+++)	green
1.700 - 1.999	Low Confidence Identification	(+)	yellow
0.000 - 1.699	No Organism Identification Possible	(-)	red

Figure 2: Log scoring structure

Expanding the CICC library using an advanced polyphasic identification scheme

When isolates are not identified using the MBT, biochemical and other conventional tests can be run as an alternative. rDNA and other relevant housekeeping genes such as *atpD*, *gyrA*, *gyrB*, *rpoB* and *tuf* are usually sequenced to further characterize the strains.

This technique was used at CICC to identify strains in Fast Moving Consumer Goods (FMCG) markets, such as packaged foods, beverages, cosmetics and other consumables. Hundreds of strains were analysed using the MBT. Most of the samples were successfully identified at the species level, with score values higher or equal to 2.0 (Figure 2). Of the hundreds of unknown samples received at CICC, 74 isolates were identified at the genus level with scores values between 1.7 and 1.99, with 78 isolates not identified at all.

Strain No.	Genus (Strain number)	16S rRNA gene sequencing		MALDI-TOF MS		Remark
		Species ID	Level of ID	Species ID	Score	
CICC 23998	<i>Bacillus</i> (7)	<i>Bacillus circulans</i>	Species	-	<1.700	
CICC 23977		<i>Bacillus infantis</i>	Species	-	<1.700	
CICC 23972		<i>Bacillus licheniformis</i>	Species	-	<1.700	Housekeeping gene (<i>gyrA</i>) analysis
CICC 23974		<i>Bacillus licheniformis</i>	Species	-	<1.700	Housekeeping gene (<i>gyrA</i>) analysis
CICC 24016		<i>Bacillus endophyticus</i>	Species	-	<1.700	
CICC 24017		<i>Bacillus subtilis</i>	Species	-	<1.700	
CICC 24034		<i>Bacillus subtilis</i>	Species	-	<1.700	
CICC 23966	<i>Brevibacterium</i> (1)	<i>Brevibacterium sanguinis</i> or <i>Brevibacterium celere</i>	Two different species possible	-	<1.700	
CICC 24026	<i>Curtobacterium</i> (1)	<i>Curtobacterium</i> sp.	Genus	-	<1.700	
CICC 24018	<i>Dermacoccus</i> (1)	<i>Dermacoccus</i> sp.	Genus	-	<1.700	
CICC 24060	<i>Exiguobacterium</i> (3)	<i>Exiguobacterium</i> sp.	Genus	-	<1.700	
CICC 24040		<i>Exiguobacterium</i> sp.	Genus	-	<1.700	
CICC 24022		<i>Exiguobacterium</i> sp.	Genus	-	<1.700	
CICC 23952	<i>Kocuria</i> (2)	<i>Kocuria carniphila</i>	Species	-	<1.700	Physiological and biochemical analysis
CICC 23997		<i>Kocuria rosea</i> or <i>Kocuria polaris</i> or <i>Kocuria himachalensis</i>	Three different species possible	-	<1.700	
		<i>Curtobacterium</i> sp.	Species	-	<1.700	
CICC 23990	<i>Micrococcus</i> (2)	<i>Micrococcus lylae</i>	Species	-	<1.700	
CICC 23993		<i>Micrococcus lylae</i>	Species	-	<1.700	
CICC 24020	<i>Moraxella</i> (1)	<i>Moraxella osloensis</i>	Species	-	<1.700	
CICC 24043	<i>Paenibacillus</i> (1)	<i>Paenibacillus lactis</i>	Species	-	<1.700	
CICC 23976	<i>Staphylococcus</i> (2)	<i>Staphylococcus hominis</i>	Species	-	<1.700	Housekeeping gene (<i>tuf</i>) analysis
CICC 23992		<i>Staphylococcus warneri</i>	Species	-	<1.700	Housekeeping gene (<i>tuf</i>) analysis

Table 1: Illustrations of the data acquisition with the polyphasic approach

These non-identified isolates were submitted to rDNA sequencing. In some cases, other housekeeping genes were also analysed.

55 of the isolates were successfully identified at the species level. 16 isolates were identified at the genus level only, and two at the family level. Table 1 illustrates the data acquisition using the developed polyphasic approach to expand the CICC library.

The CICC library was expanded with the isolates identified at the species level using the polyphasic approach, in order to improve the MBT identification rate. The isolates were recovered on TSA plates and single

colonies were picked. The colonies were prepared according to the Bruker Extraction Procedure (EX) instructions. 24 mass spectra were generated for each isolate to create a reference mass spectrum (MSP) and update the CICC library. Examples of mass spectra generated with CICC strains are shown in Figure 3.

For some of these isolates, the species were already present in the MBT Compass Library. This includes 27 isolates identified with low confidence scores (scores between 1.7 and 1.99) and 21 isolates showing no reliable identification results (scores below 1.7). For those cases, the number of MSPs did not fully cover the micro-

bial biodiversity encountered in the analysed FMCG samples, which are usually products which are high in moisture and contain various additives. In some cases, the absence of reliable identification results might be due to the physiological state of the analysed isolates. The MSPs not entirely covering the biodiversity of the analysed FMCG samples result in matching score levels which are lower than they would be for the same strain isolated from other samples.

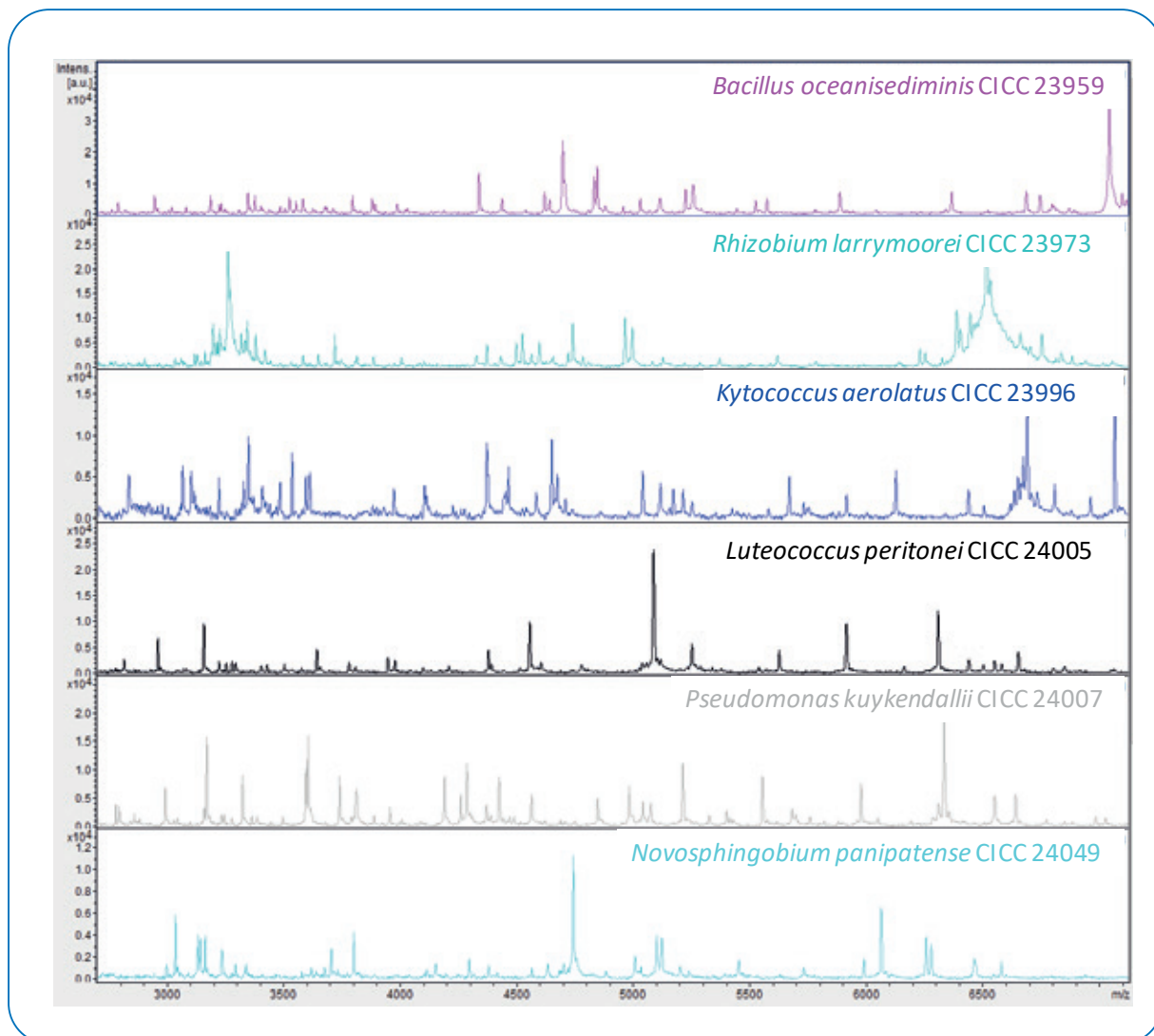


Figure 3: Mass spectra generated with CICC strains to create the CICC internal library

Conclusion

The MBT is one of the quickest solutions offered by CICC to identify unknown microbial isolates. However, for the typical samples dealt with by CICC, the MBT Compass Reference Library does not always include sufficient biodiversity to ensure highly reliable identification results. When combined with the polyphasic approach developed by CICC, more isolates were identified accurately, by creating new MSPs and adding them to the library. The expanded library ensured that the isolates previously showing no reliable identification results became reliably identified with log scores above 2.

The last version of the MBT Compass Reference Library that was released in April 2019 contains 2,969 species and 8,468 MPSs. Several

species of interest in industrial microbiology have been added including heat resistant bacteria that were identified in the CICC study, such as *Bacillus atrophaeus*, *Bacillus pumilus* or *Paenibacillus humicus*, and environmental microorganisms such as *Kocuria marina* and *Pseudomonas oryzihabitans*.

The current CICC library includes 30 species and 40 MSPs and this in-house library is continually growing.

Working in collaboration with CICC, the MBT Compass Reference Library is continually expanding with relevant species and strains for industrial microbiology. Analysts are able to carry out rapid microbial identification on an even wider range of typical culture media, using the MBT to generate rapid and reliable results.



Learn More

For more information about Bruker's MALDI Biotyper systems visit the website

<https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html>

or take a look at the brochures

https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1866135_MALDI_Biotyper_RUO_brochure_01-2019_eBook.pdf

https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1862840_MBT_Food_brochure_01-2019_ebook.pdf

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[1] The application of MALDI-TOF MS for identification of bacteria from consumer goods industries. Journal of Pure and Applied Microbiology, v.10, no.3, 2016 Sept, p.1701(14) (ISSN: 0973-7510)

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