Enfermedades Infecciosas y Microbiología Clínica 40 (2022) 385–387

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Brief report

A rapid proteomic system (MALDI-TOF) for nontuberculous-mycobacteria identification☆

Ramiro López Medrano[a,*], Isabel Burgos Asurmendi[b], Octavio Miguel Rivero Lezcano[c]

[a] Servicio de Microbiología, Complejo Asistencial Universitario de León, León, Spain
[b] Servicio de Anestesiología y Reanimación, Complejo Asistencial Universitario de León, León, Spain
[c] Unidad de Investigación, Complejo Asistencial Universitario de León, León, Spain

ARTICLE INFO

Article history:
Received 23 April 2021
Accepted 3 June 2021
Available online 7 May 2022

Keywords:
MALDI-TOF
Nontuberculous mycobacteria
identification
Proteomics

ABSTRACT

Proteomic techniques relaying upon mass spectrometry (MALDI_TOF) applied to nontuberculous mycobacteria (NTM) identification, constitute a difficult goal. Cell wall structure features complicates the protein extraction procedure. A total of 106 isolates belonging to a variety of MNTs species isolated from clinical samples taken at the Complejo Asistencial Universitario de León for a two years period (2019–20) were identified following a simplified method (MALDI-TOF Biotyper Bruker®) developped in our laboratory. The resultant identification was compared to a parallel one ruled on the Centro de Referencia de Majadahonda. A total of 22different MNTs species were tested, obtaining an agreement of 92,45%. Only 8 minor discrepancies between species belonging to same taxonomic group of MNTs were detected. The score obtained in the 67.92% of the cases was higher than 1.8. A time-saving of 24 min compared to the manufacturer's proceeding was achieved.

Identificación rápida de micobacterias no tuberculosas mediante técnicas proteómicas (MALDI-TOF)

Palabras clave:
MALDI-TOF
Identificación micobacterias no
tuberculosas
Proteómica

RESUMEN

La identificación proteómica de micobacterias no tuberculosas (MNTs) mediante MALDI-TOF presenta una mayor complejidad debido a la especial composición de su pared celular, que complica la extracción de proteínas. Un total de 106 aislamientos pertenecientes a diferentes especies de MNTs procedentes de muestras clínicas del Complejo Asistencial Universitario de León recogidas durante los años 2019 y 2020 se han identificado por un método proteómico abreviado (MALDI-TOF Biotyper Bruker®) desarrollado en nuestro laboratorio. La identificación se ha comparado con la realizada en paralelo en el Centro de Referencia de Majadahonda. Se analizaron un total de 22 especies diferentes de MNTs obteniendo una concordancia del 92,45%. Las 8 discrepancias detectadas se dieron entre especies pertenecientes al mismo grupo taxonómico. En el 67.92% de las identificaciones el score fue superior a 1.8. En el tiempo de procesamiento se obtuvo un ahorro aproximado de 24 minutos con respecto al recomendado por el fabricante.

Introduction

For some years now, identification of non-tuberculous mycobacteria (NTM) has been based on molecular methods. The use of proteomic techniques such as mass spectrometry (MALDI-TOF) has become established and has revolutionised microbiology laboratories. However, this technology encounters special difficul-

DOI of original article: https://doi.org/10.1016/j.eimc.2021.06.007
☆ Please cite this article as: López Medrano R, Burgos Asurmendi I, Rivero Lezcano OM. Identificación rápida de micobacterias no tuberculosas mediante técnicas proteómicas (MALDI-TOF). Enferm Infecc Microbiol Clin. 2022;40:385–387.
* Corresponding author.
E-mail addresses: ramirozlm@gmail.com, ramirozlm@yahoo.es
(R. López Medrano).

Table 1
Correlation between the NTM species identified by proteomic techniques (MALDI-TOF) and the genomic identification by the Reference Centre (PRA HSP-65).

NTM species, N = 22	N = 106	<1.59	1.6–1.79	>1.80 (%)	Mean MALDI score	No. of discrepancies	Reference Centre ID
SLOW GROWING NTM, N = 72					1.84		
M. avium	17	3	3	11 (64.7)	1.838	1	*M. intracellulare II*
M. chimaera IC	15	2	3	10 (66.6)	1.961	2	*M. intracellulare I*
M. lentiflavum	7	1	1	5 (71.4)	1.861	1	*M. interjectum*
M. gordonae	14	2		12 (85.7)	1.855		
M. celatum	2			2 (100)	2.210		
M. paragordonae	7	2	1	4 (57.1)	1.922	2	*M. gordonae III* (both)
M. kumamotonense	1			1 (100)	2.262		
M. malmoense	2	1		1 (50)	1.545		
M. xenopi	1	1			1.410		
M. kansasii	1			1 (100)	1.980		
M. marinum	1			1 (100)	2.090		
M. paraffinicum	1		1		1.600		
M. vulneris	1				1.408	1	*M. intracellulare II*
FAST GROWING NTM, N = 34					1.94		
M. chelonae	8	1	3	4 (50)	1.743		
M. abscesus	7		2	5 (71.4)	1.905		
M. mageritense	6			6 (100)	2,198		
M. peregrinum	4		2	2 (50)	2.021		
M. elephantis	4			4 (100)	2.028		
M. porcinum	1			1 (100)	2.410		
M. septicum	1			1 (100)	1.877	1	*M. peregrinum III*
M. salmoniphilum	2		1	1 (50)	1.844	1	*M. chelonae I*
M. holsaticum	1	1	1		1.460		

NTM: non-tuberculous mycobacteria.

ties in the field of mycobacteriology. The special complexity of the mycobacterial cell wall and its complex proteome[1] makes protein extraction a difficult task, and less efficient than in other bacteria. Up to 35 different proteins have recently been described in the mycobacterial ribosome, many of them larger than their equivalents in *E. coli* and some of them specific to mycobacteria[2]. This specificity allows MALDI-TOF to distinguish the proteomic profiles of *M. chimaera* and *M. intracellulare*, both species of the same taxonomic group[3].

Another added problem is that the databases of the commercially available MALDI systems do not have a complete catalogue that covers all the species of NTM or that the most common isolates of our geographical area are not sufficiently represented. As shown by Alcaide et al. in their collaborative review, at least 17 rare species of NTM were not found in version 4.0 of the Bruker database and at least 12 in the following version 5.0[4].

The extraction systems recommended by the manufacturer (Bruker®) are laborious and with variable results[5]. Meanwhile, some publications show that identification based on mass spectrometry is equal to or superior to some commercial reverse hybridisation systems such as GenoType[6]. In our laboratory, we tried to simplify the protein extraction system so as to shorten this tedious extraction and preparation process. Our objective was to compare the identification of NTM by the abbreviated proteomic method (Bruker MALDI Biotyper®) with the identification carried out at the Reference Centre.

Methods

Data were collected on 106 isolates belonging to different species of NTM from clinical samples from the Complejo Asistencial Universitario de León [University Care Complex of Leon] taken during 2019 and 2020. For identification, colonies of preferably young cultures with good growth were started, isolated on commercial Lowenstein-Jensen medium and on fresh Middlebrook 7H11 agar (non-commercial). They were resuspended in 1 mL of water and inactivated in a thermoblock at 95 °C for 20 min. They were centrifuged, the supernatant was decanted, and they were resuspended in 20 μL of ethanol and vortexed. After a brief centrifugation, the ethanol was removed and the pellet was left to dry in a thermoblock at 45 °C. The pellet was then placed directly on the well of the plate with the help of a toothpick. Next, 1 μL of 100% formic acid was added and, once dry, 1 or 2 μL (depending on the pellet) of matrix was added and allowed to dry. Finally, reading was carried out automatically using the Bruker MALDI *Biotyper* ® "standard" method and then in manual mode. For identification, the Bruker mycobacteria database (versions v5.0 and v6.0) was used. At the same time, the same strains were sent to the Mycobacteria Laboratory of the CNM [Centro Nacional de Microbiología] (National Centre for Microbiology) Reference Centre in Majadahonda, where the final identification was carried out by molecular methods (PRA HSP-65).

Results

A total of 22 different species of NTM were identified. Of the 106 NTM isolates studied, 17 were identified as *M. avium*, 15 as *M. chimaera-intracellulare*, 14 as *M. gordonae*, eight as *M. chelonae*, seven as *M. lentiflavum*, seven as *M. abscessus*, seven as *M. paragordonae*, six as *M. mageritense*, four as *M. peregrinum*, four as *M. elephantis*, two as *M. celatum*, two as *M. salmoniphilum*, two as *M. malmoense* and a single isolate from the rest of the species, as indicated in Table 1. When comparing the proteomic identification with that carried out at the Reference Centre, 98 of the 106 mycobacteria were correctly identified at the species level, which represents a concordance rate of 91.5%. The nine discrepancies detected all occurred in those belonging to the same taxonomic group: four discrepancies between those of the *M. avium complex* group, one between those of the *M. fortuitum complex* group, one between those of the *M. chelonae* group, two between *M. gordonae* and *M. paragordonae*, and one between M. lentiflavum and M. interjectum. With regard to the score obtained in the MALDI identification system, in 72 of the 106 strains studied (67.92%), the mean score was greater than 1.8, being slightly higher in those with rapid growth (1.94) than in those with slow growth (1.84). Regarding the extraction method, the approximate processing time was reduced from 83 min (Bruker® MycoEx procedure) to 59 min with the abbreviated procedure (Table 2).

Table 2
Comparison of the extraction and processing times of non-tuberculous mycobacteria (NTM) isolates according to the method recommended by the manufacturer (Bruker® MycoEx rev 3) and according to the abbreviated method.

Process	Abbreviated procedure (min)	Manufacturer's procedure (min)
Inactivation 90 °C	20	30
Centrifugation	5	5
Water	–	2
Absolute ethanol	2	2
Centrifugation	2	4 (2 × 2)
Pellet drying	10	10
Acetonitrile zirconia	–	4
Formic acid	10[a]	4
Centrifugation	–	2
Placing and drying of sample	-[a]	10
Placing and drying of matrix	10	10
Total time	59 min	83 min

[a] Includes placement in well.

Discussion

Version 5.0 of the MALDI Biotyper mycobacteria library contains 912 spectra corresponding to 159 mycobacterial species, while version 2.12 of the VITEK MS RUO library contains 1286 spectra that represent the 45 most frequent mycobacterial species in the clinical setting[7]. The latest version of MALDI Biotyper (v6.0) contains 1038 spectra corresponding to 177 species of mycobacteria. Despite the notable increase in the number of species and isolates in the Bruker database, when the number of strains of the same species in the database is small, there is a possibility that the software will take us to the closest taxon with an insufficient score. The great geographic variability of NTM poses specific problems in the identification of those species that are rare, but with a local predominance.

The extraction protocol recommended by the Sociedad Española de Microbiología y Enfermedades Infecciosas [Spanish Society of Microbiology and Infectious Diseases] in most cases[3,6,7] includes the use of acetonitrile together with zirconia beads. In some publications, the acetonitrile step has been suppressed, obtaining similar scores[8], so we tried eliminating this step and placing the pellet directly on the well of the plate (not the supernatant) and then adding the matrix (50% composed of acetonitrile). A good part of the ribosomal proteins would be in this pellet mass, as occurs with conventional bacteria, which would explain the score obtained. As established in some publications, the inactivation protocols used for DNA extraction in *Mycobacterium tuberculosis*[9,10] recommend an inactivation time of 20 min at 95 °C, which, applied to NTM, represents a reduction of the overall processing time.

In the identification of mycobacteria, a score greater than or equal to 1.8 is considered highly reliable. In our case, the final score obtained could be comparable to that of other authors[11] based on the liquid medium pellet, except for the disparity in the proportion of species studied. In our experience, the use of colonies grown on fresh Middlebrook 7H11 medium (non-commercial) increases the score and improves the probability of identification with this abbreviated extraction system, avoiding having to repeat the process, being especially notable in those with rapid growth. Unexpected identifications of *M. chelonae* are quite often made from abscesses that have undergone the same processing as *E. coli*, obtaining scores higher than 2. This suggests that in fast-growing colonies conventional protein extraction could suffice, or at least one that is not so demanding. In our experience, when starting from liquid media, the scores obtained with the standard or the abbreviated method are lower or do not identify species the first time, which prolongs the processing time. The advantage of starting from isolated colonies in solid media is to avoid possible mixtures of colonies/species in liquid media, thereby avoiding mixed and erroneous proteomic profiles, leading to false identifications. To perfect this abbreviated extraction system, it would be necessary to process a greater number of species and isolates of NTM that would contribute to improving its performance.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Jungblut PR, Schaible UE, Mollenkopf HJ, Zimny-Arndt U, Raupach B, Mattow J, et al. Comparative proteome analysis of Mycobacterium tuberculosis and Mycobacterium bovis BCG strains: towards functional genomics of microbial pathogens. Mol Microbiol. 1999;33:1103–17, http://dx.doi.org/10.1046/j.1365-2958.1999.01549.x.
2. Kushwahaa AK, Bhushana S. Unique structural features of the Mycobacterium ribosome. Prog Biophys Mol Biol. 2020;152:15–24, http://dx.doi.org/10.1016/j.pbiomolbio.2019.12.001.
3. Pranada AB, Witt E, Bienia M, Kostrzewa M, Timke M. Accurate differentiation of Mycobacterium chimaera from Mycobacterium intracellulare by MALDI-TOF MS analysis. J Med Microbiol. 2017;66:670–7, http://dx.doi.org/10.1099/jmm.0.000469.
4. Alcaide F, Amlerov J, Bou G, Ceyssens PJ, Coll P, Corcoran D, et al. How to identify non-tuberculous Mycobacterium species using MALDI-TOF mass spectrometry. Clin Microbiol Infect. 2018;24:599–603, http://dx.doi.org/10.1016/j.cmi.2017.11.012.
5. Procedimiento MicoEx de Brucker: rev. 3. Brucker Daltonics. 2020.
6. Costa-Alcalde JJ, Barbeito-Castiñeiras G, González-Alba JM, Aguilera A, Galán JC, Pérez-del-Molino ML. Comparative evaluation of the identification of rapidly growing non-tuberculous mycobacteria by mass spectrometry (MALDI-TOF MS), GenoType Mycobacterium CM/AS assay and partial sequencing of the rpo gene with phylogenetic analysis as a reference method. Enferm Infecc Microbiol Clín (Engl Ed). 2019;37:160–6, http://dx.doi.org/10.1016/j.eimc.2018.04.012.
7. Oviaño García M, Rodríguez Sánchez B, Caballero Pérez J, Muñoz Bellido JL. Aplicaciones de la Espectrometría de masas MALDI-TOF en Microbiología Clínica. 2019. SEIMC. 2019:20.
8. Alcolea-Medina A, Cabezas Fernandez MT, Montiel N, Luzón García MP, Delamo Sevilla C, North N, et al. An improved simple method for the identification of Mycobacteria by MALDI-TOF MS. Sci Rep. 2019:9, http://dx.doi.org/10.1038/s41598-019-56604-7.
9. Doig C, Seagar AL, Watt B, Forbes KJ. The efficacy of the heat killing of Mycobacterium tuberculosis. J Clin Pathol. 2002;55:778–9.
10. Bemer-Melchior P, Drugeon HB. Inactivation of *Mycobacterium tuberculosis* for DNATyping analysis. J Clin Microbiol. 1999:2350–1.
11. Fernández-Esgueva M, Fernández-Simona R, Monforte-Ciraca ML, López-Calleja AI, Fortuño B, Viñuelas-Bayon J. Use of MALDI-TOF MS (Bruker Daltonics) for identification of Mycobacterium species isolated directly from liquid medium. Enferm Infecc Microbiol Clin (Engl Ed). 2021;39:241–3, http://dx.doi.org/10.1016/j.eimc.2020.05.011.