Evaluation of MALDI Biotyper Mycobacteria Library v3.0 for Identification of Nontuberculous Mycobacteria

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has demonstrated its ability to promptly identify nontuberculous mycobacteria using the Mycobacteria Library v2.0. However, some species are particularly difficult to identify reliably using this database, providing a low log(score). In this study, the identification power of an updated Mycobacteria Library (v3.0) has been evaluated. Overall, 109 NTM isolates were analyzed with both databases. The v3.0 database allowed a high-level confidence in the identification [log(score) value, ≥ 1.8] of 91.7% of the isolates versus 83.5% with the v2.0 version ($P < 0.01$).

Rapid identification of nontuberculous mycobacteria (NTM) with matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) has outperformed molecular techniques, such as GenoType (Hain Lifescience GmbH, Nehren, Germany), and provides accurate identification that correlates well with 16S rRNA gene sequencing when applied to the most common species of NTM (1–3). The Mycobacteria Library database (Bruker Daltonik GmbH, Bremen, Germany) available so far (v2.0) provided low scores, particularly for NTM belonging to the slow-growing groups (3–5). In the present study, we assessed the power of a new database to identify NTM (i.e., Mycobacteria Library v3.0) using 109 isolates from 26 NTM species (Table 1) and compared the identification scores of version v2.0 and version v3.0.

Ninety-nine nonselected NTM isolates from clinical samples and 10 reference strains (Table 1) were collected in the clinical microbiology laboratory from the Hospital Gregorio Marañón (Madrid, Spain) between January 2011 and May 2015. These isolates were routinely identified by 16S rRNA *hsp65* sequencing and, in parallel, by MALDI-TOF MS using a Microflex LT benchtop mass spectrometer (Bruker Daltonik) and the Mycobacteria Library v2.0, containing 313 *Mycobacterium* isolates from 131 species (Bruker Daltonik) (4). Sample preparation was described elsewhere (6). Briefly, colonies of NTM isolates grown on Lowenstein-Jensen medium were harvested into a 1.5-ml Eppendorf tube with 300 μ l of deionized water and inactivated for 30 min at 95°C under biosafety level 3 conditions. Then, they were centrifuged at maximal speed and subsequently resuspended in 300 μ l of water and 900 μ l of absolute ethanol and centrifuged again at 13,000 rpm. The supernatant was discarded, and the pellet was taken to biosafety level 2 conditions in order to disrupt the mycobacteria cell aggregates with silica bead vortexing and extract the bacterial proteins using formic acid and acetonitrile. In the end, 1 μ l of supernatant was placed onto a steel plate for MALDI-TOF MS analysis. Samples were analyzed in duplicates; the species identification, using the Mycobacteria Library v2.0, and the higher log(score) value result were recorded. For comparison reasons, all of the protein spectra from the 109 NTM isolates were reanalyzed using the new Mycobacteria Library v3.0, containing 853 references from 149 *Mycobacterium* species. Log(score) value differ-

ences of 0.1 or greater between the databases were considered significant (Table 2).

Log(score) values of 1.6 and 1.8 were proposed as new mycobacteria thresholds for low- and high-confidence identification levels, respectively (7). Sensitivity values obtained with both Mycobacteria Library databases were compared using the McNemar test for paired samples, with two tails. SPSS software package 18.0 (IBM, Chicago, IL, USA) was used for the data analysis.

All of the NTM isolates included in this study obtained a correct MALDI-TOF MS identification using the Mycobacteria Library v2.0, although 18 isolates were identified with scores below 1.8 and 4 of these had scores below 1.6 (Table 1). The isolates with low scores belonged mainly to *Mycobacterium avium*, *Mycobacterium gordonae*, and *Mycobacterium kansasii* species. However, the Mycobacteria Library v3.0 improved the log(score) value of 45 (41.3%) NTM isolates: mass spectra exceeded 1.6 for 4 of the isolates (2 *M. avium*, 1 *M. gordonae*, and 1 *Mycobacterium xenopi*) and reached or exceeded 1.8 for 5 isolates (2 *M. avium*, 2 *Mycobacterium fortuitum*, and 1 *Mycobacterium intracellulare*); the log(score) for the other 36 isolates was ≥ 1.8 with both database versions, although these score increased when they were identified with the Mycobacteria Library v3.0 (Table 2). Also, the log(score) values of 4 *M. avium*, 3 *M. gordonae*, and 2 *M. kansasii* isolates remained below 1.8 despite the increase. On the other hand, the log(score) value for 1 additional isolate of *M. gordonae* reached ≥ 1.8 with the updated library, although it did not meet the criteria established to be considered an increased log(score). In

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TABLE 1 MALDI-TOF MS identification of NTM isolates grouped according the Runyon classification

	Identification score with Mycobacteria Library v2.0:			Identification score with Mycobacteria Library v3.0:			No. of isolates
Mycobacterium group	3.000–1.800	1.799–1.600	≤1.599	3.000–1.800	1.799–1.600	≤1.599	
Rapid growers							
<i>M. abscessus</i>	13			13			13
<i>M. arupense</i>	1			1			1
<i>M. chelonae</i>	6			6			6
<i>M. fortuitum</i>	16	2		18			18
<i>M. insubricum</i>	1			1			1
<i>M. mageritense</i>	3			3			3
<i>M. mucogenicum</i>	2			2			2
<i>M. peregrinum</i>	2			2			2
<i>M. porcinum</i>	1			1			1
<i>M. smegmatis</i>	1			1			1
No. of rapid growers (% of total organisms)	46 (95.8)	2 (4.2)	0 (0.0)	48 (100)	0 (0.0)	0 (0.0)	48
Slow-growing nonchromogens							
<i>M. avium</i>	11	5	2	14	4		18
<i>M. haemophilum</i>	1			1			1
<i>M. intracellulare</i>	5	1		6			6
<i>M. malmoense</i>	1			1			1
<i>M. palustre</i>	1			1			1
<i>M. shimoidei</i>	1			1			1
<i>M. triplex</i>	1			1			1
No. of slow-growing nonchromogens (% of total organisms)	21 (72.4)	6 (20.7)	2 (6.9)	25 (86.2)	4 (13.8)	0 (0.0)	29
Slow-growing scotochromogens							
<i>M. bohemicum</i>	1			1			1
<i>M. europaeum</i>	1			1			1
<i>M. gordonae</i>	5	3	1	6	3		9
<i>M. lentiflavum</i>	5			5			5
<i>M. szulgai</i>	1			1			1
<i>M. xenopi</i>	4		1	5			5
No. of slow-growing scotochromogens (% of total)	17 (77.3)	3 (13.6)	2 (9.1)	19 (86.4)	3 (13.6)	0 (0.0)	22
Slow-growing photochromogens							
<i>M. kansasii</i>	5	3		6	2		8
<i>M. marinum</i>	1			1			1
<i>M. simiae</i>	1			1			1
No. of slow-growing photochromogens (% of total)	7 (70.0)	3 (30)	0 (0.0)	8 (80.0)	2 (20)	0 (0.0)	10
Total no. (%)	91 (83.5)	14 (12.8)	4 (3.7)	100 (91.7)	9 (8.3)	0 (0.0)	109

all the cases, the improvement in the identification quality was due to higher homology with mycobacteria strains added to the updated library. The impact of the update on the identification of different NTM species is detailed in Table 3.

None of the log(score) values decreased with the Mycobacteria Library v3.0 according to the criteria established in this study. The identification obtained with both databases matched in all cases.

Using the older version of the database, only 91 isolates (83.5%) met the criteria for the high-confidence identification level [log(score), ≥ 1.8] (Table 1). However, the new database allowed the identification of 100 isolates (91.7%; $P < 0.01$) with a log(score) value of ≥ 1.8 and also improved the rate of NTM isolates identified at a low-confidence level [log(score), ≥ 1.6], since none of the analyzed isolates was identified with a log(score) below 1.636.

NTM isolates were grouped following the Runyon classification, and the identification results obtained with each database were compared. Using the Mycobacteria Library v3.0, a higher number of isolates belonging to all of the mycobacteria groups was correctly identified with a log(score) value of ≥ 1.8 . Although the number of rapid growing isolates reaching high scores was similar with both databases (46 [95.8%] in v2.0 versus 48 [100%] in v3.0), the number of slow-growing mycobacteria isolates identified at a high-confidence level was higher when the Mycobacteria Library v3.0 was used (Table 1). Differences were mainly due to the increased number of *M. avium*, *M. gordonae*, *M. kansasii*, and *M. xenopi* isolates correctly identified at a high-confidence level. Previous studies have already shown that the identification of *M. avium* and *M. gordonae* with a log(score) value of ≥ 1.8 was sometimes difficult to reach using older databases (3–5). However, the

TABLE 2 Isolates with an improved score when identified with the Mycobacteria Library v3.0

Isolate number	Mycobacteria Library v2.0		Mycobacteria Library v3.0		Consistency Category
	Identification	Score	Identification	Score	
4	<i>M. abscessus</i> subsp. <i>abscessus</i> DSM 44567 DSM b	1,815	<i>M. abscessus</i> 545_11 FZB b	2,012	A
14	<i>M. arupense</i> DSM 44942T DSM b	1,918	<i>Mycobacterium arupense</i> CCUG 52359 CCUG b	2,067	A
17	<i>M. avium</i> subsp. <i>avium</i> DSM 44157 DSM b	1,889	<i>M. avium</i> subsp. <i>hominissuis</i> 276_04 FZB b M	2,026	A
18	<i>M. avium</i> subsp. <i>avium</i> CCUG 28067 CCUG b	1,636	<i>M. avium</i> subsp. <i>hominissuis</i> 252_11 HLG b	1,742	B
20	<i>M. avium</i> subsp. <i>avium</i> DSM 44158 DSM b	1,711	<i>M. avium</i> subsp. <i>hominissuis</i> 1244_09 FZB b	1,917^a	B
22	<i>M. avium</i> subsp. <i>avium</i> DSM 44158 DSM b	1,589	<i>M. avium</i> subsp. <i>hominissuis</i> 1244_09 FZB b	1,875	B
23	<i>M. avium</i> subsp. <i>avium</i> DSM 44156T DSM b	1,587	<i>M. avium</i> subsp. <i>hominissuis</i> 9469_10 FZB b	1,745	B
24	<i>M. avium</i> subsp. <i>avium</i> CCUG 28067 CCUG b	1,608	<i>M. avium</i> 22_027242 MML b	1,788	B
25	<i>M. avium</i> subsp. <i>avium</i> DSM 44157 DSM b	1,924	<i>M. avium</i> subsp. <i>hominissuis</i> 1840_09 FZB b	2,117	A
26	<i>M. avium</i> subsp. <i>silvaticum</i> DSM 44175T DSM b	1,705	<i>M. avium</i> subsp. <i>hominissuis</i> 10756_10 FZB b M	1,820	B
32	<i>M. bohemicum</i> DSM 44408 DSM b	1,903	<i>M. bohemicum</i> DSM 44277T DSM b	2,048	A
40	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	1,985	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,092	A
41	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 46622 DSM b	1,953	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,193	A
43	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	1,963	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,126	A
45	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 46622 DSM b b	2,056	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,161	A
46	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 46622 DSM b b	2,109	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,265	A
47	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	1,797	<i>M. fortuitum</i> 121126_11 UOK b	1,950	B
48	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 46622 DSM b	1,888	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,032	A
53	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	1,950	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,056	A
54	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	1,648	<i>M. fortuitum</i> 7655_05 FZB b M	1,806	B
56	<i>M. fortuitum</i> subsp. <i>fortuitum</i> DSM 43477 DSM b	2,220	<i>M. fortuitum</i> subsp. <i>fortuitum</i> CCUG 46694 CCUG b	2,390	A
NC10394	<i>M. fortuitum</i> subsp. <i>acetamidolyticum</i> DSM 44220T DSM b	1,912	<i>M. fortuitum</i> 5879_96 FZB b	2,097	A
58	<i>M. gordonae</i> NO1493 LIG b	2,123	<i>M. gordonae</i> CCUG 21812 CCUG b L	2,226	A
59	<i>M. gordonae</i> NO1493 LIG b	1,909	<i>M. gordonae</i> 21_006209 MML b	2,012	A
61	<i>M. gordonae</i> NO1493 LIG b	1,543	<i>M. gordonae</i> 177_12 FZB b M	1,737	B
65	<i>M. gordonae</i> NO1493 LIG b	1,843	<i>M. gordonae</i> CCUG 21812 CCUG b L	2,098	A
DSM 44634	<i>M. haemophilum</i> DSM 44634T DSM b	1,891	<i>M. haemophilum</i> 7958_11 FZB b	2,058	A
67	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. intracellulare</i> DSM 44365 DSM b)	1,705	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. intracellulare</i> 5878_96 FZB b)	1,939	B
68	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. chimaera</i> 12030617 MVD b)	1,974	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. chimaera</i> 3720_10 FZB b)	2,170	A
69	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. intracellulare</i> DSM 44365 DSM b)	1,896	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. intracellulare</i> 9643_11 FZB b)	2,167	A
72	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. chimaera</i> 12030617 MVD b)	2,061	<i>M. chimaera</i> - <i>M. intracellulare</i> group (<i>M. chimaera</i> 3720_10 FZB b)	2,165	A
80	<i>M. kansasii</i> DSM 44162T DSM b	1,857	<i>M. kansasii</i> NLA001001128 UMR b	2,207	A
82	<i>M. lentiflavum</i> DSM 44421 DSM b	2,071	<i>M. lentiflavum</i> DSM 44420 DSM b	2,308	A
83	<i>M. lentiflavum</i> DSM 44421 DSM b	2,074	<i>M. lentiflavum</i> DSM 44420 DSM b	2,186	A
85	<i>M. lentiflavum</i> DSM 44419 DSM b	2,014	<i>M. lentiflavum</i> DSM 44420 DSM b	2,115	A
87	<i>M. mageritense</i> DSM 44476T DSM b	2,140	<i>M. mageritense</i> 22_043151 MML b	2,310	A
88	<i>M. mageritense</i> DSM 44476T DSM b	2,091	<i>M. mageritense</i> 2105743 IIUB b	2,222	A
90	<i>M. mucogenicum</i> - <i>M. phocaicum</i> group (<i>M. mucogenicum</i> CCUG 47451T CCUG b)	2,218	<i>M. mucogenicum</i> - <i>M. phocaicum</i> group (<i>M. mucogenicum</i> 1323_12 FZB b)	2,362	A
93	<i>M. peregrinum</i> DSM 43271T DSM b	2,155	<i>M. peregrinum</i> 130506271201 HPL b	2,299	A
94	<i>M. porcinum</i> DSM 44242T DSM b	2,150	<i>M. porcinum</i> WC14_0103 NYDH b	2,361	A
DSM 44626	<i>M. triplex</i> DSM 44626T DSM b	1,933	<i>M. triplex</i> 19_017398 MML b	2,049	A
96	<i>M. xenopi</i> DSM 43995T DSM b	1,943	<i>M. xenopi</i> 3238_11 FZB b	2,237	A
97	<i>M. xenopi</i> DSM 44169 DSM b	1,485	<i>M. xenopi</i> 11720_11 FZB b	2,033	A
99	<i>M. xenopi</i> DSM 43995T DSM b	1,827	<i>M. xenopi</i> 11163_11 FZB b	1,947	B
DSM 43995	<i>M. xenopi</i> DSM 43995T DSM b	1,905	<i>M. xenopi</i> AFB047 MCW b	2,125	A

^a When the score obtained with the v3.0 database allowed an upgrade within the low- and high-confidence level categories established in this study, the score is shown in bold.

updated Mycobacteria Library v3.0 overcame this drawback and allowed a high-confidence identification of 14 of 18 *M. avium* and 6 of 9 *M. gordonae* isolates, while the remaining 4 *M. avium* and 3 *M. gordonae* isolates were reliably and unequivocally identified with log(score) values of ≥ 1.7 .

In conclusion, the use of the improved Mycobacteria Library

v3.0 has increased the number of NTM isolates that attain a high-confidence identification level. The NTM species reportedly identified with low-confidence log(score) values using the previous, v2.0, database (*M. avium* and *M. gordonae* isolates in particular) (1, 3) are now reliably identified with log(score) values of ≥ 1.7 . This is very important for clinical microbiology laboratories,

TABLE 3 Impact of database update on identification of clinical isolates

Mycobacterium species	No. of isolates	No. of references in:		Mean (range) log(score) value(s) with:	
		v2.0	v3.0	v2.0	v3.0
<i>M. abscessus</i>	13	11	24	2.036 (1.815–2.183)	2.059 (1.830–2.209)
<i>M. arupense</i>	1	1	8	1.918	2.067
<i>M. avium</i>	17	10	39	1.872 (1.587–2.222)	1.963 (1.738–2.298)
<i>M. bohemicum</i>	1	3	10	1.903	2.048
<i>M. chelonae</i>	6	10	19	2.058 (1.989–2.208)	2.084 (2.017–2.208)
<i>M. europaeum</i>	1	1	2	1.847	1.847
<i>M. fortuitum</i>	17	9	20	2.052 (1.648–2.393)	2.160 (1.806–2.393)
<i>M. gordonae</i>	8	10	23	1.824 (1.543–2.123)	1.904 (1.737–2.226)
<i>M. haemophilum</i>	1	1	6	1.891	2.058
<i>M. insubricum</i>	1	3	3	2.018	2.018
<i>M. chimaera-M. intracellulare</i> group	6	9	32	1.971 (1.705–2.137)	2.105 (1.939–2.170)
<i>M. kansasii</i>	8	14	26	1.927 (1.636–2.243)	1.992 (1.636–2.243)
<i>M. lentiflavum</i>	5	3	13	2.063 (2.014–2.132)	2.175 (2.043–2.308)
<i>M. mageritense</i>	2	2	4	2.146 (2.091–2.207)	2.249 (2.214–2.310)
<i>M. malmoense</i>	1	5	14	2.233	2.233
<i>M. marinum</i>	1	8	18	2.345	2.345
<i>M. mucogenicum-M. phocaicum</i> group	2	3	21	2.218/2.101 ^a	2.362/2.127 ^a
<i>M. palustre</i>	1	2	2	2.086	2.086
<i>M. peregrinum</i>	2	1	15	2.349/2.155 ^b	2.349/2.299 ^b
<i>M. porcinum</i>	1	2	8	2.150	2.361
<i>M. shimoidei</i>	1	1	6	2.225	2.225
<i>M. simiae</i>	1	3	13	2.166	2.166
<i>M. smegmatis</i>	1	5	9	2.034	2.034
<i>M. szulgai</i>	1	5	15	2.125	2.199
<i>M. triplex</i>	1	1	2	1.933	2.019
<i>M. xenopi</i>	4	5	18	1.852 (1.485–2.099)	2.093 (1.947–2.237)
Total	104	128	370		

^a Score values for the two representative *M. mucogenicum-M. phocaicum* group strains.

^b Score values for the two representative *M. peregrinum* strains.

where the achievement of a rapid identification is as crucial as the robustness of the identification itself. Finally, the high rate of high-confidence NTM identifications obtained with the Mycobacteria Library v3.0 underlines the importance of implementing MALDI-TOF MS technology, coupled with updated databases containing several strains per NTM species, for the rapid and accurate identification of these microorganisms.

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