JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4481–4486 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.01397-10

Rapid Identification of Mycobacterial Whole Cells in Solid and Liquid Culture Media by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry▽

Aurélie Lotz,[1,2] Agnès Ferroni,[1]* Jean-Luc Beretti,[1] Brunhilde Dauphin,[3] Etienne Carbonnelle,[4] Hélène Guet-Revillet,[1,2] Nicolas Veziris,[5] Béate Heym,[6] Vincent Jarlier,[5] Jean-Louis Gaillard,[6] Catherine Pierre-Audigier,[7] Eric Frapy,[2] Patrick Berche,[1,2] Xavier Nassif,[1,2] and Emmanuelle Bille[1,2]

Assistance Publique-Hôpitaux de Paris, Laboratoire de Microbiologie, Hôpital Necker-Enfants Malades, Paris, France[1]; Université Paris 5 Descartes, Faculté de Médecine, Site Necker, Paris, France[2]; Andromas SAS, 156 rue de Vaugirard, Paris, France[3]; Assistance Publique-Hôpitaux de Paris, Laboratoire de Microbiologie, Hôpital Européen Georges Pompidou, Paris, France[4]; Assistance Publique-Hôpitaux de Paris, National Reference Center for Mycobacteria, Hôpital Pitié-Salpêtrière, Paris, France[5]; Assistance Publique-Hôpitaux de Paris, Laboratoire de Microbiologie, Hôpital Ambroise Paré, Boulogne-Billancourt, France[6]; and Assistance Publique-Hôpitaux de Paris, Laboratoire de Microbiologie, Hôpital Bichat-Claude Bernard, Paris, France[7]

Received 8 July 2010/Returned for modification 9 September 2010/Accepted 1 October 2010

Mycobacterial identification is based on several methods: conventional biochemical tests that require several weeks for accurate identification, and molecular tools that are now routinely used. However, these techniques are expensive and time-consuming. In this study, an alternative method was developed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). This approach allows a characteristic mass spectral fingerprint to be obtained from whole inactivated mycobacterial cells. We engineered a strategy based on specific profiles in order to identify the most clinically relevant species of mycobacteria. To validate the mycobacterial database, a total of 311 strains belonging to 31 distinct species and 4 species complexes grown in Löwenstein-Jensen (LJ) and liquid (mycobacterium growth indicator tube [MGIT]) media were analyzed. No extraction step was required. Correct identifications were obtained for 97% of strains from LJ and 77% from MGIT media. No misidentification was noted. Our results, based on a very simple protocol, suggest that this system may represent a serious alternative for clinical laboratories to identify mycobacterial species.

The genus *Mycobacterium* encompasses over 100 species. The most important mycobacterial diseases are predominantly caused by the *Mycobacterium tuberculosis* complex. The incidence of other mycobacterial diseases due to nontuberculous mycobacteria (NTM) appears to be increasing in recent years due to the increasing number of immunocompromised individuals (1). Because the treatment of these infections differs depending on the isolated species, the correct and rapid identification of causative organisms is essential.

Conventional methods for the identification of mycobacteria were classically based on biochemical tests. They required several weeks for adequate growth, and sometimes accurate identification was not possible. Difficulties, such as the lack of adequate reproducibility, the variability of phenotypes, and the fact that phenotype information is limited to common species, may lead to ambiguous or erroneous results (29). New strategies have been developed in the last decades using molecular biology tools (6, 10, 16, 24). The techniques based on DNA hybridization are sensitive, fast, and simple, but the available commercial assays (AccuProbe; Gen-Probe, San Diego, CA) are able to identify only four species and two complexes of mycobacteria (10). Techniques requiring amplification followed by a hybridization step on a solid support are more complete than probes, but commercially available kits are limited to 5 (GenoType MTBC; Hain Lifescience GmbH, Nehren, Germany), 16 (Inno-LiPa Mycobacteria v2; Innogenetics, Gent, Belgium), or 30 (GenoType Mycobacterium; Hain Lifescience GmbH, Germany) species (19, 22, 30). Systems based on sequencing or enzymatic restriction targeting the *hsp65*, 16S rRNA, *sod*, and *rpoB* genes allow good identification of all mycobacteria at the species level but remain limited to specialized laboratories (14, 17, 23, 31, 36, 37). In addition, they are expensive and time-consuming and require qualified operators (20). Recently, alternatives based on the analysis of mycolic acid by high-performance liquid chromatography (HPLC) (2) or electrospray ionization-tandem mass spectrometry analysis (28) have been proposed. However, these methods are still labor-intensive.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allows rapid identification of the most frequently isolated bacteria grown on solid medium by the identification of species-specific profiles obtained from isolated colonies (3–5). This technique is now routinely used in a few laboratories (26, 34). Some authors have used MALDI-TOF MS for rapid identification of *Mycobacterium* species (12, 18, 21). However, the techniques used require several steps, such as 16S rRNA gene-based techniques

* Corresponding author. Mailing address: Laboratoire de Microbiologie, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France. Phone: 33 1 44 49 49 62. Fax: 33 1 44 49 49 60. E-mail: agnes.ferroni@nck.aphp.fr.

▽ Published ahead of print on 13 October 2010.

(18), cell extractions (12), or a statistical analysis (12, 21). In addition, the number of tested strains in these studies is less than 40, encompassing a maximum of 13 species. It was first reported by Hettick et al. that the analysis of mycobacterial whole cells by MALDI-TOF MS could be used for identification. However, the limited number of strains prevented the engineering of a useful database (11).

Recently, we engineered a strategy to identify bacteria using MALDI-TOF MS, based on the choice of a limited number of species-specific profiles (3, 5). The aim of the present work is to extend this strategy to the identification of mycobacterial strains without cell extraction. This method will allow us to have a rapid, accurate, and inexpensive identification tool in routine laboratories. The first step was to build a complete database for mycobacterial species isolated in human pathology. This database was then validated by using clinical strains cultivated in solid and liquid media.

MATERIALS AND METHODS

Bacterial strains. Fifty-three different species of mycobacteria identified by molecular techniques were used to generate the database. Thirty-eight are reference strains, and 15 are isolates from different clinical microbiology departments (Table 1). Three hundred eleven other clinical and reference strains identified by molecular techniques were used to validate the database (Table 2). Three molecular techniques were used by the different laboratories: *hsp65* gene sequencing (all mycobacteria except *M. tuberculosis*), *rpob* or *sod* gene sequencing (*M. abscessus* complex), and the exact tandem repeat D (ETR-D) for the species of the *M. tuberculosis* complex (7, 14, 23, 37).

Growth conditions. The strains used to engineer and validate the database were grown on Löwenstein-Jensen (LJ) medium (Bio-Rad, Hercules, CA). Among the clinical strains used to validate the database, 82 were also cultivated in a mycobacterium growth indicator tube (MGIT) (Becton-Dickinson Microbiology Systems, Cockeysville, MD) with enrichment supplement (oleic acid-albumin-dextrose-citric acid [OADC]) and antimicrobial supplement (polymyxin B, nalidixic acid, trimethoprim, and azlocillin [PANTA]). The LJ media were incubated at 37°C, except for *M. marinum*, *M. brumae*, *M. septicum*, *M. alvei*, *M. goodii*, and *M. ulcerans* (30°C), and the MGIT tubes were incubated at 37°C. To work with viable mycobacteria, biosafety level 3 containment is required. However, the spectrometer used for identification of routine bacteria is located outside this safety room. Therefore, we first developed a method to kill the mycobacteria before placing them on the target plate, allowing us to work with the inactivated mycobacteria outside the level 3 room. Several colonies grown on solid medium were harvested in 40 μl of 70% ethanol in order to obtain a rich suspension. When an MGIT tube was detected as positive, the mycobacteria were pelleted (4,500 × *g*; 10 min), the supernatant was discarded, and the pellet was resuspended in 30 μl of 70% ethanol. To check the efficiency of the inactivation, we used different strains of *M. tuberculosis*, which were suspended in either ethanol or sterile water. These suspensions were stored at room temperature for 10 min. The ethanol was then discarded after a centrifugation step, and the pellet was inoculated on LJ medium and incubated at 37°C for 4 months. The cultures were positive in 3 weeks for all tubes inoculated with water suspensions, whereas they were negative after 4 months of incubation for all the tubes inoculated with ethanol suspensions.

Instrumentation and data analysis. For each tested strain, the 70% ethanol suspension was deposited in five replicates on a target plate (Bruker Daltonics, Bremen, Germany) and allowed to dry at room temperature. On each well, 1 μl of matrix solution SA (sinapinic acid, 20 mg/ml; acetonitrile, 30%; trifluoroacetic acid, 10%) was added and allowed to crystallize with the sample at room temperature. One microliter of 10 mM ammonium phosphate was then added to each well. Samples were processed in the MALDI-TOF MS spectrometer (Microflex; Bruker Daltonics, Bremen, Germany) with the flex control software (Bruker Daltonics). Positive ions were extruded at an accelerating voltage of 20 kV in linear mode. Each spectrum was the sum of the shots performed in 6 different regions of the same well. The spectra were analyzed in an *m/z* range of 3,640 to 19,055. The analysis was performed with the flex analysis software and calibrated with protein calibration standard I (Bruker Daltonics). Numerical data obtained from the spectrometer (the peak value and relative intensity for each peak) were analyzed using the Andromas software and compared with the Andromas database, which was engineered using a previously described strategy (3, 5). Briefly, for each species used to engineer the database (Table 1), five isolates of each of the selected strains listed in Table 1 grown on Löwenstein-Jensen medium were analyzed by MALDI-TOF MS. We retained the peaks with a relative intensity above 0.07, which were present in all the replicates.

In order to validate this database, MALDI-TOF MS was performed using bacteria grown on Löwenstein-Jensen medium (Table 3) at different growth steps. Fast-growing bacteria were analyzed every day between 1 and 14 days after the positivity of the culture. Slow growers were analyzed the first day and 2 months after the positivity of the culture. For mycobacteria grown in MGIT, MALDI-TOF MS was performed after the positive detection. The profiles of the tested isolates were compared with those of species included in the database by using the Andromas software, taking into account a possible error of ±10 *m/z* units. For all tested strains, we determined the percentage of common peaks

TABLE 1. Strains used to establish the MALDI-TOF MS mycobacterial database

Species (n = 53)	Complex	Source[a]
Mycobacterium abscessus	*M. abscessus* complex	ATCC 19977
Mycobacterium alvei		CIP 103464
Mycobacterium africanum	*M. tuberculosis* complex	NEM Hospital
Mycobacterium arupense		NRC
Mycobacterium asiaticum		ATCC 25276
Mycobacterium aurum		ATCC 25793
Mycobacterium avium	*M. avium* complex (MAC)	CIP 109829
Mycobacterium bohemicum		CIP 105811
Mycobacterium bolletii	*M. abscessus* complex	CIP 108541
Mycobacterium bovis	*M. tuberculosis* complex	NRC
Mycobacterium bovis bacillus Calmette-Guérin (BCG)	*M. tuberculosis* complex	NEM Hospital
Mycobacterium brumae		CIP 103465
Mycobacterium celatum		CIP 106109
Mycobacterium chelonae		NEM Hospital
Mycobacterium chitae		ATCC 25805
Mycobacterium confluentis		CIP 105510
Mycobacterium diernhoferi		ATCC 19344
Mycobacterium flavescens		ATCC 14474
Mycobacterium fortuitum	*M. fortuitum* complex	NEM Hospital
Mycobacterium gastri		NEM Hospital
Mycobacterium genavense		AP Hospital
Mycobacterium goodii		CIP 106349
Mycobacterium gordonae		NEM Hospital
Mycobacterium hassiacum		CIP 105218
Mycobacterium immunogenum	*M. abscessus* complex	CIP 106684
Mycobacterium intermedium		CIP 104542
Mycobacterium intracellulare	*M. avium* complex (MAC)	CIP 104243
Mycobacterium kansasii		ATCC 25053
Mycobacterium lentiflavum		CIP 105465
Mycobacterium mageritense		CIP 104973
Mycobacterium marinum		CIP 104528
Mycobacterium massiliense	*M. abscessus* complex	CIP 108297
Mycobacterium microti	*M. tuberculosis* complex	NRC
Mycobacterium mucogenicum		NEM Hospital
Mycobacterium neoaurum		ATCC 25795
Mycobacterium palustre		CIP 107748
Mycobacterium parafortuitum		ATCC 19686
Mycobacterium peregrinum	*M. fortuitum* complex	NEM Hospital
Mycobacterium phlei		CIP 105389
Mycobacterium porcinum		CIP 105392
Mycobacterium rhodesiae		CIP 106806
Mycobacterium scrofulaceum		CIP 105416
Mycobacterium septicum		CIP 106642
Mycobacterium simiae		ATCC 25273
Mycobacterium smegmatis		MC2 155
Mycobacterium szulgai		CIP 104532
Mycobacterium terrae		CIP 104321
Mycobacterium thermoresistibile		ATCC 19529
Mycobacterium triplex		CIP 106108
Mycobacterium tuberculosis	*M. tuberculosis* complex	NEM Hospital
Mycobacterium ulcerans		GEIHP
Mycobacterium vaccae		ATCC 23014
Mycobacterium xenopi		NEM Hospital

[a] AP, Microbiology Department of Ambroise Paré Hospital, Boulogne-Billancourt, France; ATCC, American Type Culture Collection (Manassas, VA); CIP, Collection Institut Pasteur (Paris, France); GEIHP, Groupe d'Etude des Interactions Hôte-Pathogène, Angers University, Angers, France; NRC, National Reference Center for Surveillance of Mycobacterial Diseases and Drug Resistance, Pitié-Salpétrière Hospital, Paris, France; NEM, Microbiology Department of Necker-Enfants Malades Hospital, Paris, France.

TABLE 2. Identification of mycobacteria grown on solid medium by MALDI-TOF MS

Species	No. of strains tested	No. of correct MALDI-TOF MS identifications		Absence of identification[e]
		Species	Complex	
M. tuberculosis	18[b]		17	1
M. bovis BCG	14[b]		14	
M. africanum	3[b,c]		3	
M. avium	25[b,c]	24		1
M. intracellulare	19[a,b,c]	17	2	
M. xenopi	26[a,b,c]	26		
M. kansasii	14[c]	14		
M. abscessus	47[b,c,d]		46	1
M. massiliense	6[b,d]		6	
M. bolletii	3[d]		3	
M. chelonae	26[c]	25		1
M. fortuitum	26[a,b,c]	25		1
M. peregrinum	16[a,b,c]	13	2	1
M. mucogenicum	20[b,c]	19		1
M. gordonae	27[b,c]	26		1
M. diernhoferi	1[b]	1		
M. gastri	2[b]	2		
M. thermoresistibile	1[f]	1		
M. confluentis	1[a]	1		
M. neoaurum	1[f]	1		
M. chitae	1[f]	1		
M. smegmatis	1[f]	1		
M. vaccae	1[f]	1		
M. aurum	1[f]	1		
M. porcinum	1[b]	1		
M. scrofulaceum	1[f]	1		
M. brumae	1[a]	1		
M. septicum	1[a]	1		
M. marinum	2[f]	2		
M. parafortuitum	1[f]	1		
M. ulcerans	4[g]	4		

[a] Microbiology Department of Ambroise Paré Hospital, Boulogne-Billancourt, France.
[b] Microbiology Department of Necker-Enfants Malades Hospital, Paris, France.
[c] National Reference Center for Surveillance of Mycobacterial Diseases and Drug Resistance, Pitié-Salpétrière Hospital, Paris, France.
[d] Microbiology department of Raymond Poincaré Hospital, Garches, France.
[e] No. of strains for which no spectra, and thus no identification, were obtained.
[f] Reference strains.
[g] Groupe d'Etude des Interactions Hôte-Pathogène, Angers University, Angers, France.

obtained with each of the reference strains. The identification of the tested strain corresponded to the species of the reference strain with the best match in the database. The first best match had to be ≥60% in order to be retained. We considered a difference of ≥10% between the first and second match the minimum required to give a correct identification.

RESULTS

Engineering of the mycobacterial database. The 53 selected strains grown on Löwenstein-Jensen medium were analyzed by MALDI-TOF MS as described in Materials and Methods. The standard deviation for each conserved peak did not exceed 10 *m*/*z* units. For each selected strain, we found a species-specific spectral profile, allowing identification at the species level for 44 species and at the mycobacterial clade level for the 9 strains belonging to the *M. abscessus* complex and the *M. tuberculosis* complex.

In order to assess whether the length of the growth period could affect the spectral profile, MALDI-TOF MS was performed with cultures of different ages. Figure 1 represents the spectra of 4 different species: 2 fast growers (*M. abscessus* and *M. fortuitum*) at 1 and 14 days after the positivity of the culture and 2 slow growers (*M. tuberculosis* and *M. avium*) at 1 day and 2 months after the positivity of the culture. Despite the fact that the age of the culture influences the MALDI-TOF MS spectra, this delay did not affect the species-specific profiles.

TABLE 3. Identification of mycobacteria grown on liquid medium by MALDI-TOF MS

Species	No. of strains tested	No. of correct MALDI-TOF MS identifications		Absence of identification[a]
		Species	Complex	
M. tuberculosis	7		7	
M. bovis BCG	2		2	
M. africanum	1		1	
M. avium	5	4		1
M. intracellulare	7	2		5
M. xenopi	5	2		3
M. kansasii	7	3		4
M. abscessus	6		5	1
M. massiliense	2		2	
M. bolletii	1		1	
M. chelonae	8	6		2
M. fortuitum	8	6		2
M. mucogenicum	8	8		
M. peregrinum	7	6		1
M. gordonae	8	8		

[a] No. of strains for which no identification was obtained.

Validation of the mycobacterial database. (i) Number of replicates. The number of replicates of a given strain necessary to obtain good spectral acquisition and thus correct identification was determined. The probability of obtaining correct acquisition increases with the number of replicates of the same suspension. The results are shown in Table 4. Five replicates allowed correct identification in 99% and 79% of the strains tested for fast- and slow-growing mycobacteria, respectively.

(ii) Identification of tested strains. Among the 311 tested strains grown on solid medium, MALDI-TOF MS allowed 97% good identifications. In 3% of the tested strains, no identification could be obtained due to the poor quality of the spectra. No misidentification was noted. Some species are only distinguishable at the complex level because of high similarities between their spectra. For example, *M. abscessus*, *M. massiliense*, and *M. bolletii* have a high degree of genetic similarity that results in a unique mass spectral profile for the three subspecies. The same observation has been established for *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*, and *M. africanum*. Overall, correct identifications were obtained for 210 strains at the species level and 93 strains at the complex level (Table 2). When bacteria were grown on liquid media, good identifications were observed for 63/82 strains (77%), whereas 19/82 strains could not be identified (Table 3).

DISCUSSION

MALDI-TOF MS has been shown to greatly improve routine bacterial identification (3, 5, 8). In the present work, we

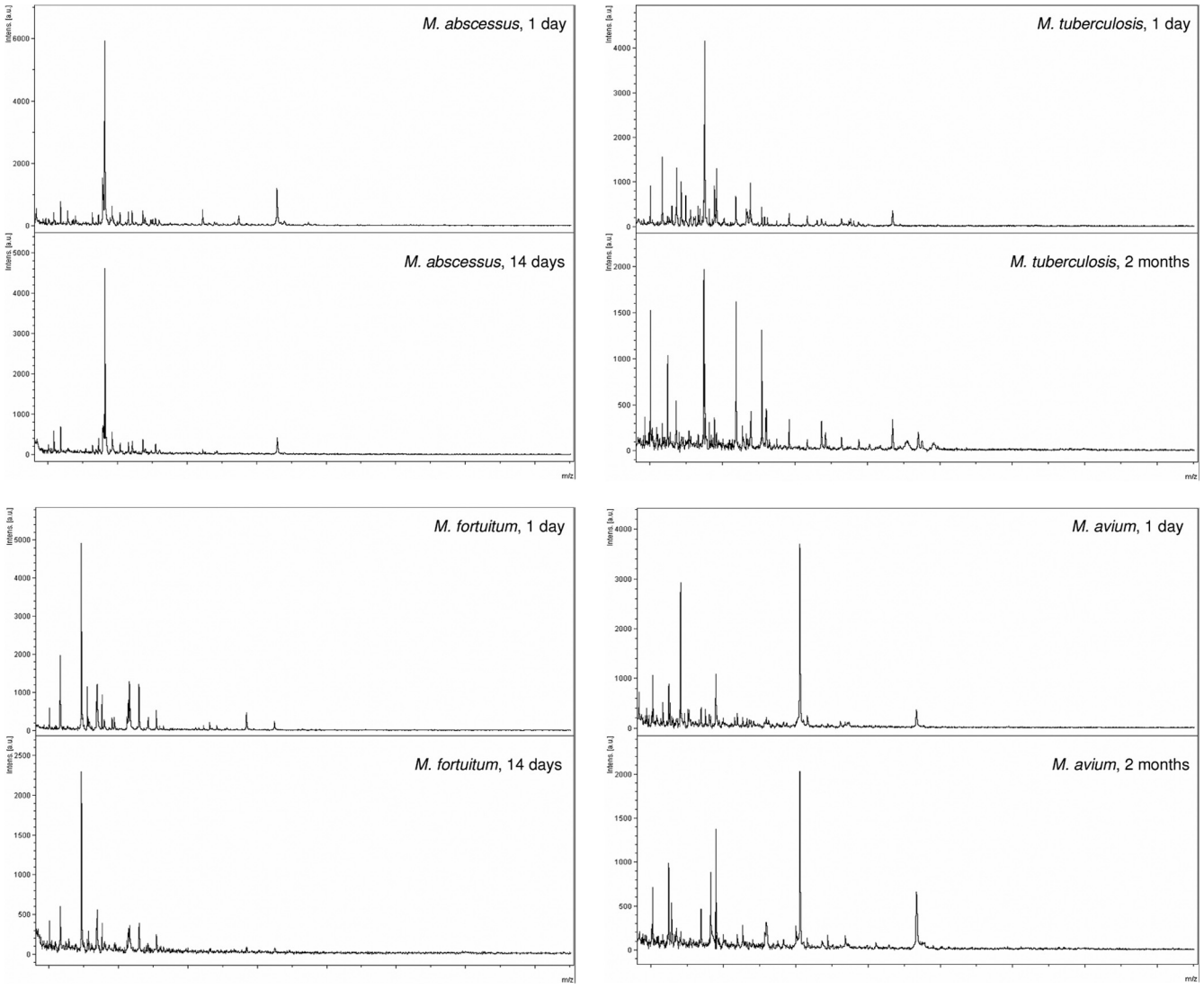

FIG. 1. Spectra obtained for different mycobacterial species according to the incubation time after the positivity of the culture.

demonstrated that our previously described strategy for bacterial identification by MALDI-TOF MS can be extended to mycobacterial species. Our strategy based on using intact cells avoids time-consuming extraction steps. Correct identifications were obtained for 97% (solid media) and 77% (liquid media) of all tested strains. For 3% of mycobacteria tested in Löwenstein-Jensen medium, no identification was obtained due to poor spectral acquisition, probably because of insufficient culture material and/or the frequency of the mucoid character of the strains to be identified. The results in liquid medium are not as good as those obtained in solid medium. This is mostly due to spectral acquisition failures, due either to the low number of bacteria or to potential interference of the supplements included in this complex medium (PANTA and OADC). It should be pointed out that only the MGIT medium was tested in this study. It would be interesting to test the performance of this method on other commercially available liquid media. An extended incubation in the MGIT medium after a first negative assay may increase the sensitivity of this technique, as is the case for AccuProbe and an immunochromatographic kit, which can give positive results after prolonged incubation (13).

It should be pointed out that none of the strains were misidentified in this study, regardless of the medium used, contrary to the results obtained using rapid identification techniques, such as hybridization or DNA strips (33).

TABLE 4. Probabilities of obtaining good acquisition depending on the number of replicates

Type (no.) of species	Probability (%) of obtaining good acquisition with:			
	2 replicates	3 replicates	4 replicates	5 replicates
All species tested (337)	67	74	80	87
Fast-growing mycobacteria (159)	79	86	92	99
Slow-growing mycobacteria (178)	57	65	72	79

We have also shown that a high number of replicates increases the probability of good identification, especially for slow-growing mycobacteria: in some cases, five replicates were required to obtain one good spectral acquisition. This is a striking difference from the identification of routinely isolated bacteria by MALDI-TOF MS, which usually requires only one replicate (3, 5).

The identification of the *M. tuberculosis* complex strains relies mainly on the use of DNA hybridization (AccuProbe). Recently, an immunochromatographic test has been introduced (11). Both of these techniques are efficient, with excellent sensibility and specificity (13, 16). DNA strip technologies, such as Inno-LiPa Mycobacteria or GenoType Mycobacterium, are also available. These techniques do not allow identification to the species level among this complex. MALDI-TOF MS technology can also identify the strains belonging to the *M. tuberculosis* complex, but not to the species level. The identification of the bacterial species belonging to this complex can be made using a specific DNA strip kit (GenoType MTBC) (22).

Among the slow-growing mycobacteria, *M. avium* complex (MAC), *M. xenopi*, and *M. kansasii* are frequently implicated in clinical pathology and then need to be correctly identified. The precise identification of the *M. avium* complex is difficult. Among the three probes used in the AccuProbe technique (MAC, *M. avium*, and *M. intracellulare*), a lack of specificity of the MAC and *M. intracellulare* probes has been reported. In addition, misidentifications and no identification have been reported with the DNA strip technologies (9, 19, 25, 33). The MALDI-TOF MS strategy can differentiate between the two species of the MAC in one step, unlike the AccuProbe technique, which needs two probes to precisely identify these bacterial species. The identification of *M. xenopi* from solid medium gave 100% good results with MALDI-TOF MS, equivalent to those obtained using DNA strip technologies. *M. kansasii* is classically identified by hybridization methods (33). *M. kansasii* grown on LJ medium is reliably identified using MALDI-TOF MS. On the other hand, using DNA strips, misidentifications have been reported with *M. gastri* (19, 33).

Currently, identification of the fast-growing mycobacteria is performed using DNA strip techniques or amplification, followed by sequencing. Among the fast-growing mycobacteria, it is important to rapidly distinguish between *M. abscessus*, *M. chelonae*, *M. fortuitum*, and the frequent contaminant *M. gordonae.* The Inno-LiPa Mycobacteria v2 kit is not able to differentiate *M. chelonae* from *M. abscessus* (33), and GenoType Mycobacterium CM is not able to differentiate *M. chelonae* from *M. immunogenum* and *M. abscessus* from *M. immunogenum* (22). However, based on MALDI-TOF MS spectral profiles and according to cultural characteristics, *M. abscessus*, *M. immunogenum*, and *M. chelonae* can be distinguished. *M. abscessus* can grow in 5% NaCl medium, unlike *M. immunogenum* and *M. chelonae* (15). MALDI-TOF MS can differentiate *M. immogenum* and *M. chelonae*. However, *M. abscessus* is not distinguished from *M. bolletii* or *M. massiliense*. *M. abscessus*, *M. massiliense*, and *M. bolletii* present identical spectra, which is consistent with results recently reported by Leao et al. (15). These authors proposed a revision of the taxonomic status of the *M. abscessus* complex, i.e., that *M. abscessus*, *M. massiliense*, and *M. bolletii* should belong to a single species (*M. abscessus*).

M. fortuitum can be falsely identified with DNA strips, leading to many erroneous identifications (24, 32, 33). On the other hand, MALDI-TOF MS correctly identifies the species *M. fortuitum*, and only 2/16 strains of *M. peregrinum* were identified only to the *M. fortuitum* complex level. *M. gordonae* can be perfectly identified by AccuProbe (16). With the DNA strip test Inno-LiPa Mycobacteria v2, there was some misidentification (33) or no identification (23). With MALDI-TOF MS, 34/35 good identifications were obtained regardless of the media used.

Bacteria that are rarely isolated in clinical laboratories can also be identified by our database: as soon as mycobacterial strains are not recognized because their spectra do not match with any reference strain present in the database, their identification is performed by a molecular biology approach. These new spectra are then easily added to our database.

Under some circumstances, fast-growing mycobacteria grow on the usual media (e.g., blood agar). In these cases, MALDI-TOF MS can be performed immediately after acid-fast staining with the same equipment used for routine bacteria or fungi, thus avoiding misidentifications (27, 35).

Other studies have demonstrated the potential of MALDI-TOF MS for mycobacterial identification (12, 18, 21). These works highlighted the reproducibility and specificity of these methods. Pignone et al. described a strategy based on root mean square (RMS) values in order to compare different profiles (21). Hettick et al. developed a biostatistical analysis in order to identify mycobacterial species. This strategy was unable to distinguish closely related species as belonging to the MAC (12). Lefmann et al. used MALDI-TOF MS as a method for 16S rRNA gene-based mycobacterial identification, which first requires PCR and transcription-RNase cleavage steps (18). This method is still time-consuming.

In this study, we did not work on spectral differences at the strain level. Subsequent studies on the spectral profiles are required in order to determine specific markers that allow us to distinguish drug-sensitive and -resistant mycobacteria. Currently, some strains belonging to the same species complex are not distinguishable by MALDI-TOF MS.

The strategy described in this work using whole bacterial cells is simple and rapid. It can easily be implemented in routine clinical laboratories. The technique can be performed within a few minutes by any operator, whereas sequencing requires an average of 3 days and qualified technicians, and 6 h are necessary for DNA strip technology assays. The cost saving of MALDI-TOF MS is considerable (1 euro/sample) compared to other molecular methods (40 times more expensive). Finally, it should be pointed out that systems based on MALDI-TOF MS generate less waste than methods based on molecular biology that use many disposable materials.

ACKNOWLEDGMENTS

We thank Gilles Quesnes for his technical assistance and Laurent Marsollier for kindly providing us with the strains *M. ulcerans*.

This work was supported by grants from the PHRC (Programme Hospitalier de Recherche Clinique; no. BOS07001 and AOM08181) and was cofinanced by the DGA (Direction Générale de l'Armement).

Brunhilde Dauphin is employed by Andromas. Xavier Nassif is a shareholder of Andromas.

REFERENCES

1. **Ashford, D. A., E. Whitney, P. Raghunathan, and O. Cosivi.** 2001. Epidemiology of selected mycobacteria that infect humans and other animals. Rev. Sci. Tech. **20:**325–337.
2. **Butler, W. R., and L. S. Guthertz.** 2001. Mycolic acid analysis by high-performance liquid chromatography for identification of Mycobacterium species. Clin. Microbiol. Rev. **14:**704–726.
3. **Carbonnelle, E., J. L. Beretti, S. Cottyn, G. Quesne, P. Berche, X. Nassif, and A. Ferroni.** 2007. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **45:**2156–2161.
4. **Claydon, M. A., S. N. Davey, V. Edwards-Jones, and D. B. Gordon.** 1996. The rapid identification of intact microorganisms using mass spectrometry. Nat. Biotechnol. **14:**1584–1586.
5. **Degand, N., E. Carbonnelle, B. Dauphin, J. L. Beretti, M. Le Bourgeois, I. Sermet-Gaudelus, C. Segonds, P. Berche, X. Nassif, and A. Ferroni.** 2008. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. J. Clin. Microbiol. **46:**3361–3367.
6. **Devulder, G., M. Perouse de Montclos, and J. P. Flandrois.** 2005. A multigene approach to phylogenetic analysis using the genus Mycobacterium as a model. Int. J. Syst. Evol. Microbiol. **55:**293–302.
7. **Djelouadji, Z., D. Raoult, M. Daffe, and M. Drancourt.** 2008. A single-step sequencing method for the identification of Mycobacterium tuberculosis complex species. PLoS Negl. Trop. Dis. **2:**e253.
8. **Dupont, C., V. Sivadon-Tardy, E. Bille, B. Dauphin, J. L. Beretti, A. S. Alvarez, N. Degand, A. Ferroni, M. Rottman, J. L. Herrmann, X. Nassif, E. Ronco, and E. Carbonnelle.** 2010. Identification of clinical coagulase-negative staphylococci, isolated in microbiology laboratories, by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and two automated systems. Clin. Microbiol. Infect. **16:**998–1004.
9. **Gitti, Z., I. Neonakis, G. Fanti, F. Kontos, S. Maraki, and Y. Tselentis.** 2006. Use of the GenoType Mycobacterium CM and AS assays to analyze 76 nontuberculous mycobacterial isolates from Greece. J. Clin. Microbiol. **44:**2244–2246.
10. **Hance, A. J., B. Grandchamp, V. Levy-Frebault, D. Lecossier, J. Rauzier, D. Bocart, and B. Gicquel.** 1989. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. **3:**843–849.
11. **Hettick, J. M., M. L. Kashon, J. P. Simpson, P. D. Siegel, G. H. Mazurek, and D. N. Weissman.** 2004. Proteomic profiling of intact mycobacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Chem. **76:**5769–5776.
12. **Hettick, J. M., M. L. Kashon, J. E. Slaven, Y. Ma, J. P. Simpson, P. D. Siegel, G. N. Mazurek, and D. N. Weissman.** 2006. Discrimination of intact mycobacteria at the strain level: a combined MALDI-TOF MS and biostatistical analysis. Proteomics **6:**6416–6425.
13. **Ismail, N. A., K. Baba, D. Pombo, and A. A. Hoosen.** 2009. Use of an immunochromatographic kit for the rapid detection of Mycobacterium tuberculosis from broth cultures. Int. J. Tuberc. Lung Dis. **13:**1045–1047.
14. **Kim, B. J., S. H. Lee, M. A. Lyu, S. J. Kim, G. H. Bai, G. T. Chae, E. C. Kim, C. Y. Cha, and Y. H. Kook.** 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (rpoB). J. Clin. Microbiol. **37:**1714–1720.
15. **Leao, S. C., E. Tortoli, C. Viana-Niero, S. Y. Ueki, K. V. Lima, M. L. Lopes, J. Yubero, M. C. Menendez, and M. J. Garcia.** 2009. Characterization of mycobacteria from a major Brazilian outbreak suggests that revision of the taxonomic status of members of the Mycobacterium chelonae-M. abscessus group is needed. J. Clin. Microbiol. **47:**2691–2698.
16. **Lebrun, L., F. Espinasse, J. D. Poveda, and V. Vincent-Levy-Frebault.** 1992. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30:**2476–2478.
17. **Lee, H., H. J. Park, S. N. Cho, G. H. Bai, and S. J. Kim.** 2000. Species identification of mycobacteria by PCR-restriction fragment length polymorphism of the rpoB gene. J. Clin. Microbiol. **38:**2966–2971.
18. **Lefmann, M., C. Honisch, S. Bocker, N. Storm, F. von Wintzingerode, C. Schlotelburg, A. Moter, D. van den Boom, and U. B. Gobel.** 2004. Novel mass spectrometry-based tool for genotypic identification of mycobacteria. J. Clin. Microbiol. **42:**339–346.
19. **Mäkinen, J., M. Marjamaki, H. Marttila, and H. Soini.** 2006. Evaluation of a novel strip test, GenoType Mycobacterium CM/AS, for species identification of mycobacterial cultures. Clin. Microbiol. Infect. **12:**481–483.
20. **Maugein, J.** 2007. Identification moléculaire des mycobactéries non tuberculeuses. Rev. Francophone Lab. **37:**57–62.
21. **Pignone, M., K. M. Greth, J. Cooper, D. Emerson, and J. Tang.** 2006. Identification of mycobacteria by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. J. Clin. Microbiol. **44:**1963–1970.
22. **Richter, E., M. Weizenegger, A. M. Fahr, and S. Rusch-Gerdes.** 2004. Usefulness of the GenoType MTBC assay for differentiating species of the Mycobacterium tuberculosis complex in cultures obtained from clinical specimens. J. Clin. Microbiol. **42:**4303–4306.
23. **Ringuet, H., C. Akoua-Koffi, S. Honore, A. Varnerot, V. Vincent, P. Berche, J. L. Gaillard, and C. Pierre-Audigier.** 1999. hsp65 sequencing for identification of rapidly growing mycobacteria. J. Clin. Microbiol. **37:**852–857.
24. **Russo, C., E. Tortoli, and D. Menichella.** 2006. Evaluation of the new GenoType Mycobacterium assay for identification of mycobacterial species. J. Clin. Microbiol. **44:**334–339.
25. **Sarkola, A., J. Makinen, M. Marjamaki, H. J. Marttila, M. K. Viljanen, and H. Soini.** 2004. Prospective evaluation of the GenoType assay for routine identification of mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **23:**642–645.
26. **Seng, P., M. Drancourt, F. Gouriet, B. La Scola, P. E. Fournier, J. M. Rolain, and D. Raoult.** 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis. **49:**543–551.
27. **Short, W. R., C. Emery, M. Bhandary, and J. A. O'Donnell.** 2005. Misidentification of Mycobacterium peregrinum, the causal organism of a case of bacteremia and automatic implantable cardioverter defibrillator-associated infection, due to its unusual acid-fast staining characteristics. J. Clin. Microbiol. **43:**2015–2017.
28. **Song, S. H., K. U. Park, J. H. Lee, E. C. Kim, J. Q. Kim, and J. Song.** 2009. Electrospray ionization-tandem mass spectrometry analysis of the mycolic acid profiles for the identification of common clinical isolates of mycobacterial species. J. Microbiol. Methods **77:**165–177.
29. **Springer, B., L. Stockman, K. Teschner, G. D. Roberts, and E. C. Bottger.** 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J. Clin. Microbiol. **34:**296–303.
30. **Suffys, P. N., A. da Silva Rocha, M. de Oliveira, C. E. Campos, A. M. Barreto, F. Portaels, L. Rigouts, G. Wouters, G. Jannes, G. van Reybroeck, W. Mijs, and B. Vanderborght.** 2001. Rapid identification of Mycobacteria to the species level using INNO-LiPA Mycobacteria, a reverse hybridization assay. J. Clin. Microbiol. **39:**4477–4482.
31. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Bottger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
32. **Tortoli, E., A. Mariottini, and G. Mazzarelli.** 2003. Evaluation of INNO-LiPA MYCOBACTERIA v2: improved reverse hybridization multiple DNA probe assay for mycobacterial identification. J. Clin. Microbiol. **41:**4418–4420.
33. **Tortoli, E., M. Pecorari, G. Fabio, M. Messino, and A. Fabio.** 2010. Commercial DNA probes for mycobacteria incorrectly identify a number of less frequently encountered species. J. Clin. Microbiol. **48:**307–310.
34. **van Veen, S. Q., E. C. Claas, and E. J. Kuijper.** 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol. **48:**900–907.
35. **Williamson, J. C., T. A. Miano, M. R. Morgan, and E. L. Palavecino.** 2010. Fatal Mycobacterium abscessus endocarditis misidentified as Corynebacterium spp. Scand. J. Infect. Dis. **42:**222–224.
36. **Woo, P. C., S. K. Lau, J. L. Teng, H. Tse, and K. Y. Yuen.** 2008. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect. **14:**908–934.
37. **Zolg, J. W., and S. Philippi-Schulz.** 1994. The superoxide dismutase gene, a target for detection and identification of mycobacteria by PCR. J. Clin. Microbiol. **32:**2801–2812.