Rapid identification of *Legionella* species by mass spectrometry

Claire Moliner,¹ Christophe Ginevra,^{2,3,4,5} Sophie Jarraud,^{2,3,4,5}
Christophe Flaudrops,⁶ Marielle Bedotto,⁶ Carine Couderc,⁶
Jerome Etienne^{2,3,4,5} and Pierre-Edouard Fournier^{1,6}

Correspondence

Pierre-Edouard Fournier
pierre-edouard.fournier@
univmed.fr

¹URMITE CNRS-IRD UMR 6236, Faculté de Médecine, 27 boulevard Jean Moulin, 13385 Marseille Cedex 05, France

²Université de Lyon, Lyon, France

³INSERM, U851, 21 avenue Tony Garnier, 69007 Lyon, France

⁴Université Lyon 1, IFR128, Lyon, France

⁵Hospices Civils de Lyon, Faculté de Médecine Lyon Est Claude Bernard, Lyon, France

⁶Fédération de Microbiologie Clinique, Hôpital de la Timone, 264 rue Saint-Pierre, 13385 Marseille Cedex 05, France

Legionella species are facultative, intracellular bacteria that infect macrophages and protozoa, with the latter acting as transmission vectors to humans. These fastidious bacteria mostly cause pulmonary tract infections and are routinely identified by various molecular methods, mainly PCR targeting the *mip* gene and sequencing, which are expensive and time-consuming. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has emerged as a rapid and inexpensive method for identification of bacterial species. This study evaluated the use of MALDI-TOF-MS for rapid species and serogroup identification of 21 *Legionella* species recognized as human pathogens. To this end, a reference MS database was developed including 59 *Legionella* type strains, and a blind test was performed using 237 strains from various species. Two hundred and twenty-three of the 237 strains (94.1 %) were correctly identified at the species level, although ten (4.2 %) were identified with a score lower than 2.0. Fourteen strains (5.9 %) from eight species were misidentified at the species level, including seven (3.0 %) with a significant score, suggesting an intraspecific variability of protein profiles within some species. MALDI-TOF-MS was reproducible but could not identify *Legionella* strains at the serogroup level. When compared with *mip* gene sequencing, MALDI-TOF-MS exhibited a sensitivity of 99.2 and 89.9 % for the identification of *Legionella* strains at the genus and species level, respectively. This study demonstrated that MALDI-TOF-MS is a reliable tool for the rapid identification of *Legionella* strains at the species level.

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INTRODUCTION

Legionella species are fastidious, Gram-negative bacteria that belong to the *Gammaproteobacteria*. They are facultative, intracellular parasites infecting macrophages and protozoa, and are naturally associated with free-living amoebae, which may act as transmission vectors to humans. *Legionella* species mostly cause pulmonary tract infections. *Legionella pneumophila* is the most common pathogenic species for humans, and thus the most studied. Other proven pathogenic *Legionella* species include *L. anisa*, *L. birminghamensis*, *L. bozeman*, *L. cincinnatiensis*,

L. dumoffii, *L. feeleyi*, *L. gormanii*, *L. hackeliae*, *L. jordanis*, *L. lansingensis*, *L. longbeachae*, *L. lytica*, *L. maceachernii*, *L. micdadei*, *L. oakridgensis*, *L. parisiensis*, *L. sainthelensi*, *L. tucsonensis* and *L. wadsworthii* (Garrity *et al.*, 2005).

Molecular methods have become major tools for the identification of fastidious bacteria. For *Legionella* species, several molecular tools have been described, including PCR targeting the *mip* gene (Ratcliff *et al.*, 1998). This gene, currently the mostly commonly used PCR target, encodes an immunophilin of the FK506-binding protein class (Hacker & Fischer, 1993). The *mip* gene exhibits 3–31 % nucleotide sequence variation among *Legionella* species and appears to be genetically stable (Ratcliff *et al.*, 1998). Other discriminatory molecular methods include PCR

Abbreviation: MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Table 1. *Legionella* type strains used in this study

Strains shaded in grey are those for which spectra are available in the Bruker database.

Species	Subspecies	Strain name	Serogroup	Culture collection no.*
<i>L. pneumophila</i>	<i>pneumophila</i>	Philadelphia-1	1	ATCC 33152
<i>L. pneumophila</i>	<i>pneumophila</i>	Lens	1	CIP 108286
<i>L. pneumophila</i>	<i>pneumophila</i>	Paris	1	CIP 107629
<i>L. pneumophila</i>	<i>pneumophila</i>	Togus-1	2	ATCC 33154
<i>L. pneumophila</i>	<i>pneumophila</i>	Bloomington-2	3	ATCC 33155
<i>L. pneumophila</i>	<i>fraseri</i>	Los Angeles-1	4	ATCC 33156
<i>L. pneumophila</i>	<i>pneumophila</i>	Portland 1	4	LTC-14
<i>L. pneumophila</i>	<i>pneumophila</i>	San Francisco	4	LTC-15
<i>L. pneumophila</i>	<i>pascuelli</i>	MICU B	5	ATCC 33735
<i>L. pneumophila</i>	<i>pascuelli</i>	U8W	5	ATCC 33737
<i>L. pneumophila</i>	<i>pneumophila</i>	Cambridge-2	5	NCTC 11417
<i>L. pneumophila</i>		Reims	5	ATCC 700710
<i>L. pneumophila</i>	<i>fraseri</i>	Dallas 1E	5	ATCC 33216
<i>L. pneumophila</i>	<i>pneumophila</i>	Chicago 2	6	ATCC 33215
<i>L. pneumophila</i>	<i>pneumophila</i>	Chicago 8	7	ATCC 33823
<i>L. pneumophila</i>	<i>pneumophila</i>	Concord 3	8	ATCC 35096
<i>L. pneumophila</i>	<i>pneumophila</i>	IN-23-G1-C2	9	ATCC 35289
<i>L. pneumophila</i>	<i>pneumophila</i>	Leiden 1	10	ATCC 43283
<i>L. pneumophila</i>	<i>pneumophila</i>	797-PA-H	11	ATCC 43130
<i>L. pneumophila</i>	<i>pneumophila</i>	570-CO-H	12	ATCC 43290
<i>L. pneumophila</i>	<i>pneumophila</i>	82A3105	13	ATCC 43736
<i>L. pneumophila</i>	<i>pneumophila</i>	169-MN-H	14	ATCC 43703
<i>L. pneumophila</i>	<i>fraseri</i>	Lansing 3	15	ATCC 35251
<i>L. pneumophila</i>	<i>fraseri</i>			DSM 7514
<i>L. pneumophila</i>	<i>pascuelli</i>			DSM 7515
<i>L. pneumophila</i>	<i>pneumophila</i>			DSM 7513
<i>L. anisa</i>				ATCC 35291
<i>L. anisa</i>				ATCC 35292
<i>L. anisa</i>				DSM 17627
<i>L. beliardensis</i>				DSM 19152
<i>L. birminghamensis</i>				ATCC 43702
<i>L. birminghamensis</i>				ATCC 700709
<i>L. birminghamensis</i>				DSM 19232
<i>L. bozemanai</i>			1	ATCC 33217
<i>L. bozemanai</i>			2	ATCC 35545
<i>L. brunensis</i>				DSM 19236
<i>L. drancourtii</i>				A18532-39
<i>L. dumoffii</i>				ATCC 35850
<i>L. dumoffii</i>				ATCC 33279
<i>L. cherrii</i>				DSM 19213
<i>L. cincinnatiensis</i>				DSM 19233
<i>L. erythra</i>			1	ATCC 35303
<i>L. erythra</i>			2	ATCC 11987
<i>L. erythra</i>				DSM 17644
<i>L. feeleyi</i>				LC 878
<i>L. feeleyi</i>				ATCC 700514
<i>L. feeleyi</i>			1	ATCC 35072
<i>L. feeleyi</i>			2	ATCC 35849
<i>L. feeleyi</i>				DSM 17645
<i>L. gormanii</i>				ATCC 33297
<i>L. hackeliae</i>			1	ATCC 35250
<i>L. hackeliae</i>			2	ATCC 35999
<i>L. hackeliae</i>				DSM 19214
<i>L. impletisoli</i>				DSM 18493

Table 1. cont.

Species	Subspecies	Strain name	Serogroup	Culture collection no.*
<i>L. israelensis</i>				DSM 19235
<i>L. jamestowniensis</i>				DSM 19215
<i>L. jordanis</i>				ATCC 33623
<i>L. jordanis</i>				ATCC 700762
<i>L. jordanis</i>				DSM 19212
<i>L. lansingensis</i>				ATCC 49751
<i>L. lansingensis</i>				DSM 19556
<i>L. londiniensis</i>			1	ATCC 49505
<i>L. londiniensis</i>			2	ATCC 700510
<i>L. longbeachae</i>			1	ATCC 33462
<i>L. longbeachae</i>			2	ATCC 33484
<i>L. longbeachae</i>				DSM 10572
<i>L. maceachernii</i>				ATCC 35300
<i>L. micdadei</i>				ATCC 33218
<i>L. moravica</i>				DSM 19234
<i>L. oakridgensis</i>				ATCC 33761
<i>L. oakridgensis</i>				ATCC 700515
<i>L. parisiensis</i>				ATCC 35299
<i>L. parisiensis</i>				ATCC 700174
<i>L. parisiensis</i>				DSM 19216
<i>L. rubrilucens</i>				DSM 11884
<i>L. sainthelensi</i>			1	ATCC 35248
<i>L. sainthelensi</i>			2	ATCC 700517
<i>L. sainthelensi</i>			2	ATCC 49322
<i>L. sainthelensi</i>				DSM 19231
<i>L. tucsonensis</i>				ATCC 49180
<i>L. tucsonensis</i>				DSM 19246
<i>L. wadsworthii</i>				ATCC 33877
<i>L. yabuuchiae</i>				DSM 18492

*ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; LC, British Legionella Reference Unit: Respiratory and Systematic Infection Laboratory; LTC, Lyon Type Collection, Centre National de Reference des Legionelles.

amplification and sequencing of the 16S–23S rRNA intergenic spacers or tDNA spacers (De Gheldre *et al.*, 2001; Riffard *et al.*, 1998). Ribotyping and PCR fingerprinting have also demonstrated a high discriminatory power for *Legionella* species (Salloum *et al.*, 2002; Van Belkum *et al.*, 1993), but may be more adapted to strain genotyping than species identification. However, although reliable, molecular methods are time-consuming and expensive.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has recently emerged as a rapid and inexpensive bacterial species identification method. It has been shown that most bacterial species exhibit specific protein profiles (Hsieh *et al.*, 2008). MS has been used for efficient discrimination of *Arthrobacter* species (Vargha *et al.*, 2006), *Coxiella burnetii* (Skultety *et al.*, 2007), viridans streptococci (Friedrichs *et al.*, 2007), staphylococci (Carbonnelle *et al.*, 2007), *Neisseria* species (Gudlavalleti *et al.*, 2008; Ilina *et al.*, 2009), *Campylobacter* species (Kolinska *et al.*, 2008),

Burkholderia cepacia (Miñán *et al.*, 2009; Vanlaere *et al.*, 2008), *Clostridium* species (Grosse-Herrenthey *et al.*, 2008), *Erwinia* species (Sauer *et al.*, 2008), *Escherichia coli* (Parisi *et al.*, 2008; Ruelle *et al.*, 2004), *Listeria* species (Barbuddhe *et al.*, 2008), non-fermenting bacteria (Degand *et al.*, 2008; Mellmann *et al.*, 2008), oral anaerobic bacteria (Stingu *et al.*, 2008), *Yersinia enterocolitica* (Parisi *et al.*, 2008), *Salmonella* species (Dieckmann *et al.*, 2008; Ruelle *et al.*, 2004), *Bacteroides* species (Nagy *et al.*, 2009) and *Bartonella* species (Fournier *et al.*, 2009). However, to date, no data are available regarding the identification of *Legionella* species using MALDI-TOF-MS. In the Bruker database (Bruker Daltonics, Bremen, Germany), containing MALDI-TOF-MS profiles of various bacterial species, only a few *Legionella* MS profiles are available, with only one spectrum per species or subspecies for 24 *Legionella* species (Table 1).

In this study, we performed MS analysis on representative strains of all recognized *Legionella* species with recognized pathogenicity for humans. We then developed a database

Table 2. MALDI-TOF-MS identification of 114 blind-tested *L. pneumophila* strains

Species	Sg*	Reference	Source	MS best match*	Score
<i>L. pneumophila</i>	1	LG 0801 5008	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710†	2.484
		LG 0802 3006	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.645
		LG 0803 5002	Clinical	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.461
		LG 0804 4003	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.556
		LG 0808 3013	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.409
		LG 0808 3017	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.350
		LG 0808 5012	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.497
		LG 0813 2012	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.574
		LG 0820 5015	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.560
		LG 0828 3013	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.558
<i>L. pneumophila</i>	2	LG 0735 3006	Clinical	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.494
		HL 0513 3039	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.373
		LG 0801 3006	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.571
		LG 0802 1027	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.587
		LG 0808 2014	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.469
		LG 0811 3024	Environmental	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.373
		LG 0816 4051	Environmental	<i>L. pneumophila pneumophila</i> Leiden 1 sg10 ATCC 43283	2.297
		LG 0827 3049	Environmental	<i>L. pneumophila pascuelli</i> MICU B sg5 ATCC 33735	2.325
		LG 0846 1016	Environmental	<i>L. pneumophila pneumophila</i> Leiden 1 sg10 ATCC 43283	2.406
		HL 0709 3024	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.521
<i>L. pneumophila</i>	3	LG 0805 3021	Environmental	<i>L. pneumophila pneumophila</i> Togus-1 sg2 ATCC 33154	2.206
		LG 0805 4019	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.532
		LG 0819 5001	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.587
		HL 0617 4018	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.447
		HL 0628 3035	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.448
		HL 0638 5023	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.404
		HL 0647 5013	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.498
		HL 0520 3047	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.457
		HL 0302 5038	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.650
		HL 0347 1034	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.515
<i>L. pneumophila</i>	4	LG 0830 1011	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.499
		LG 0805 5010	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.247
		LG 0807 3012	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.526
		LG 0808 3014	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.53
		LG 0809 5015	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.608
		LG 0810 4013	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.576
		LG 0814 2030	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.627
		LG 0830 3012	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.637
		LG 0832 3013	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.566
		LG 0805 5008	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.368
<i>L. pneumophila</i>	5	LG 0813 4029	Environmental	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.399
		LG 0819 3014	Environmental	<i>L. pneumophila fraseri</i> Lansing 3 sg15 ATCC 35251	2.098
		LG 0818 3012	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.539
		LG 0830 2017	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.61
		LG 0719 1001	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.535
		LG 0719 5020	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.503
		HL 0649 3004	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.577
		HL 0545 3090	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.525
		HL 0439 2007	Clinical	<i>L. pneumophila pneumophila</i> San Francisco sg4	2.184
		HL 0418 3001	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.573
<i>L. pneumophila</i>	6	HL 0428 3018	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.626
		HL 0434 2016	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.532
		HL 0519 5025	Clinical	<i>L. pneumophila pneumophila</i> Chicago 2 sg6 ATCC 33215	2.413
		HL 0531 4019	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.521
		HL 0622 5016	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.566
		HL 0640 3012	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.361

Table 2. cont.

Species	Sg*	Reference	Source	MS best match*	Score
<i>L. pneumophila</i>	7	LG 0838 5007	Clinical	<i>L. pneumophila pneumophila</i> 570-CO-H sg12 ATCC 43290	2.485
		LG 0847 2013	Clinical	<i>L. pneumophila pneumophila</i> 570-CO-H sg12 ATCC 43290	2.5
		HL 0314 4021	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.456
		HL 0406 1059	Environmental	<i>L. pneumophila pneumophila</i> DSM 7513_DSM	2.124
		LG 0741 3025	Clinical	<i>L. pneumophila fraseri</i> Dallas 1E sg5 ATCC 33216	2.281
		LG 0751 2019	Clinical	<i>L. pneumophila fraseri</i> Lansing 3 sg15 ATCC 35251	2.493
		HL 0637 3027	Clinical	<i>L. pneumophila fraseri</i> Lansing 3 sg15 ATCC 35251	2.303
		LG 0824 4006	Environmental	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.196
<i>L. pneumophila</i>	8	HL 0605 4006	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.433
		HL 0615 4004	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.463
		LG 0816 3011	Environmental	<i>L. pneumophila pneumophila</i> Bloomington-2 sg3 ATCC 33155	2.089
		LG 0819 3015	Environmental	<i>L. pneumophila pneumophila</i> Leiden 1 sg10 ATCC 43283	2.254
		LG 0821 1033	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.596
		HL 0646 1011	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.553
		LG 0825 2016	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.568
		LG 0729 5031	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.429
<i>L. pneumophila</i>	9	HL 0530 4032	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.388
		HL 0547 4039	Clinical	<i>L. pneumophila pneumophila</i> Philadelphia-1 sg1 ATCC 33152	2.294
		HL 0323 1005	Clinical	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.6
		HL 0330 2004	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.568
		LG 0816 4067	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.596
		LG 0839 3018	Environmental	<i>L. pneumophila fraseri</i> Dallas 1E sg5 ATCC 33216	2.527
		HL 0707 5016	Environmental	<i>L. pneumophila pneumophila</i> Chicago 8 sg7 ATCC 33823	2.376
		LG 0751 4015	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.572
<i>L. pneumophila</i>	10	HL 0619 3023	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.431
		HL 0624 4004	Environmental	<i>L. pneumophila pneumophila</i> Cambridge-2 sg5	2.372
		LG 0814 3013	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.572
		LG 0819 2017	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.567
		LG 0823 4028	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.56
		LG 0824 1004	Environmental	<i>L. pneumophila pneumophila</i> Bloomington-2 sg3 ATCC 33155	2.589
		LG 0828 3018	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.618
		LG 0829 3010	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.533
<i>L. pneumophila</i>	10	LG 0838 5009	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.597
		LG 0849 3011	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.574
		HL 0606 2008	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.519
		HL 0610 2005	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.577
<i>L. pneumophila</i>	11	LG 0841 3010	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.514
<i>L. pneumophila</i>	12	LG 0807 2016	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.62
<i>L. pneumophila</i>	14	LG 0807 5008	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.636
		LG 0814 2033	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.666
		LG 0830 1015	Environmental	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.528
		LG 0840 1014	Environmental	<i>L. pneumophila pneumophila</i> Concord 3 sg8 ATCC 35096	2.566
		HL 0707 1021	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.632
		LG 0721 2025	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.558
		LG 0723 1018	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.537
		LG 0740 4025	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.594
<i>L. pneumophila</i>	15	HL 0608 5019	Environmental	<i>L. pneumophila pneumophila</i> Portland 1 sg4	2.571
		LG 0819 2016	Clinical	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.578
		LG 0840 1003	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.565
		LG 0739 2049	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.592
		LG 0745 3016	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.537
		HL 0611 5011	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.601
		HL 0620 2008	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.589
		LG 0827 3014	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.532
<i>L. pneumophila</i>	15	LG 0839 3017	Environmental	<i>L. pneumophila pneumophila</i> San Francisco sg4	2.473
		HL 0621 3029	Environmental	<i>L. pneumophila pneumophila</i> 169-MN-H sg14 ATCC 43703	2.65

Table 2. cont.

Species	Sg*	Reference	Source	MS best match*	Score
		HL 0505 3004	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.31
		HL 0505 3005	Environmental	<i>L. pneumophila</i> Reims sg5 ATCC 700710	2.649

*Sg, Serogroup.

†Strain used to evaluate reproducibility.

with the generated spectra, which we tested using a large collection of both clinical and environmental *Legionella* strains.

METHODS

Legionella strains. Representative strains of 21 recognized and pathogenic *Legionella* species were included in our study (including 12 species already referenced in the Bruker database). For some species, several serogroups were evaluated: two for *L. bozeman*, *L. erythra*, *L. feeleii*, *L. hackeliae*, *L. londiniensis*, *L. longbeachae* and *L. sainthelensi*, and 15 for *L. pneumophila*. For each species and serogroup, one or several type strains were used. The 59 *Legionella* type strains studied are detailed in Table 1. To evaluate the sensitivity and positive predictive value of MS for the identification of *Legionella* strains, 237 additional *Legionella* strains from 19 species were blind tested by MS. These included 114 *L. pneumophila* (Table 2), seven *L. anisa*, five *L. birminghamensis*, 11 *L. bozeman*, one *L. cincinnatiensis*, 10 *L. dumoffii*, 14 *L. erythra*, seven *L. feeleii*, seven *L. gormanii*, six *L. jordanis*, 12 *L. londiniensis*, six *L. longbeachae*, four *L. maceachernii*, 10 *L. micdadei*, five *L. oakridgensis*, eight *L. parisiensis*, five *L. sainthelensi*, four *L. tucsonensis* and one *L. wadsworthii* (Table 3). All strains were cultured on buffered charcoal yeast extract α -ketoglutarate agar (Oxoid) and incubated for 3–5 days at 35 °C in 2.5% CO₂. Species identification of all studied strains was confirmed using *mip* gene amplification and sequencing as described previously (Ratcliff *et al.*, 1998). The resulting consensus sequences were compared with those in GenBank.

MALDI-TOF-MS. For each strain tested, four isolated colonies were deposited on an MTP 384 target polished steel plate (Bruker Daltonics) and allowed to dry at room temperature. Two microlitres of a matrix solution composed of α -cyano-4-hydroxycinnamic acid (Sigma) saturated with 2.5% trifluoroacetic acid and 50% acetonitrile was added and allowed to co-crystallize with each sample. Specimens were processed in an Autoflex II MALDI-TOF-MS spectrometer (Bruker Daltonics) using Flex Control software (Bruker Daltonics). Positive ions were extracted with an accelerating voltage of 20 kV in linear mode. Each spectrum indicates the sum of the ions obtained from 675 laser shots performed in nine different regions of the same spot. The spectra were analysed in an *m/z* range of 2000–20 000, and the analysis was performed using Flex Analysis and BioTyper software (Bruker Daltonics). The data obtained from the four replicates were added to minimize random effects. The presence and absence of peaks were considered to be specific profiles for a particular isolate. Only those peaks with a relative intensity above 0.1 that were constantly present in all four sets of data obtained for a given strain were retained. The standard deviation for each conserved peak did not exceed 6 *m/z* value. A maximum of 100 peaks with a signal-to-noise ratio of 3 was selected in the range of 3000–15 000 Da. From all spectra generated for a single strain, a reference spectrum was determined. A strain was considered to be classified correctly within a species when their reference spectra shared ≥ 75 peaks, with

a mass deviation of 200 p.p.m. For the blind test, the 237 tested strains were compared with the complete Bruker database, and with the reference spectra obtained for all 21 *Legionella* type species tested. Species identification was obtained when a strain demonstrated a best match with a spectrum in the database with a log score ≥ 2.0 .

The complete set of spectra obtained in the present study is available in the Unité des Rickettsies Mass Spectrometry (URMS) database online (www.ifr48.com).

Evaluation of the sensitivity and reproducibility of MALDI-TOF-MS. Strains correctly identified at the species level, i.e. exhibiting an MS identification identical to their *mip* identification with a log score ≥ 2.0 , were considered true positives, whereas all other strains were considered false negatives. Differences were evaluated using the Mantell–Haenszel test, and were considered significant when $P < 0.05$. To evaluate the reproducibility of MALDI-TOF-MS, we retested one strain per species using the same protocol as described above (Tables 2 and 3).

RESULTS

Study of type strains

Using MALDI-TOF-MS, we obtained protein fingerprints for all 59 reference *Legionella* strains. Although all tested strains exhibited distinct protein profiles at the species level (Fig. 1), no difference was identified among serogroups within species (Figs 1 and 2). When comparing the spectra obtained from the 59 representative strains with those of 2843 bacteria within the Bruker database, including one spectrum from each of 21 *Legionella* species, we observed that 39 strains were correctly identified at the species level with a score of ≥ 2.0 , and seven strains were correctly identified but with a best score of < 2.0 against *Legionella* species. The remaining 13 strains, from nine species, being absent from the Bruker database, obtained a score of < 1.5 against all bacterial species. All spectra obtained from the 59 type strains were added to our MALDI-TOF-MS database.

Blind test

When compared with the complete database, spectra obtained from the 114 *L. pneumophila* strains enabled unambiguous identification at the species level with log scores of ≥ 2.0 against *L. pneumophila* only. However, scores of ≥ 2.0 were obtained for all strains against all *L. pneumophila* serogroups, thus demonstrating that MALDI-TOF-MS is not discriminatory at the serogroup level

Table 3. MALDI-TOF-MS identification of 123 blind-tested strains from various pathogenic *Legionella* species

Strains correctly identified at the species level but with scores <2.0 are shaded in pale grey; strains misidentified at the species level with scores <2.0 are shaded in dark grey; strains misidentified at the species level with scores ≥2.0 are shaded in black.

Species	Sg	Reference	Source	MS best match	Score
<i>L. anisa</i>		HL 0234 1007	Clinical	<i>L. anisa</i> ATCC 35291	2.683
		HL 0248 1032	Clinical	<i>L. anisa</i> ATCC 35291	2.607
		HL 0402 4026	Environmental	<i>L. anisa</i> ATCC 35292	2.302
		HL 0404 2021	Environmental	<i>L. anisa</i> ATCC 35291	1.879
		HL 0404 3014	Environmental	<i>L. anisa</i> ATCC 35292*	2.129
		HL 0404 5031	Environmental	<i>L. anisa</i> ATCC 35291	2.409
		HL 0411 4028	Environmental	<i>L. anisa</i> ATCC 35291	2.406
<i>L. birminghamensis</i>		LG 0714 2030	Environmental	<i>L. birminghamensis</i> ATCC 43702	2.287
		LG 0714 5025	Environmental	<i>L. birminghamensis</i> ATCC 700709	2.295
		HL 0605 3039	Environmental	<i>L. birminghamensis</i> ATCC 700709	2.149
		HL 0628 4037	Environmental	<i>L. birminghamensis</i> ATCC 43702*	2.055
		HL 0550 4026	Environmental	<i>L. birminghamensis</i> ATCC 43702	1.914
<i>L. bozemanæ</i>	1	HL 0538 2034	Clinical	<i>L. parisiensis</i> ATCC 700174	2.425
	1	LG 0810 3002	Environmental	<i>L. bozemanæ</i> sg1 ATCC 33217	2.661
	2	HL 0523 3007	Environmental	<i>L. bozemanæ</i> sg2 ATCC 35745	2.463
	2	HL 0545 4050	Environmental	<i>L. bozemanæ</i> sg2 ATCC 35745	1.894
		96010250	Clinical	<i>L. bozemanæ</i> sg2 ATCC 35745	2.371
		96010251	Clinical	<i>L. bozemanæ</i> sg2 ATCC 35745*	2.307
		HL 0613 3003	Environmental	<i>L. bozemanæ</i> sg2 ATCC 35745	2.578
		HL 0406 2051	Environmental	<i>L. bozemanæ</i> sg2 ATCC 35745	2.484
		HL 0431 3026	Environmental	<i>L. bozemanæ</i> sg2 ATCC 35745	2.33
		HL 0449 5025	Environmental	<i>L. parisiensis</i> ATCC 700174	2.296
		HL 0632 4014	Environmental	<i>L. parisiensis</i> ATCC 700174	1.778
<i>L. cincinnatiensis</i>		HL 0524 4030	Clinical	<i>L. cincinnatiensis</i> DSM 19233	2.188
<i>L. dumoffii</i>		HL 0610 1039	Environmental	<i>L. dumoffii</i> ATCC 33279	2.633
		HL 0610 3032	Environmental	<i>L. dumoffii</i> ATCC 33279	1.366
		HL 0610 3033	Environmental	<i>L. dumoffii</i> ATCC 33279	1.803
		HL 0612 3018	Environmental	<i>L. dumoffii</i> ATCC 33279*	2.118
		HL 0616 2015	Environmental	<i>L. dumoffii</i> ATCC 33279	2.186
		HL 0616 2016	Environmental	<i>L. dumoffii</i> ATCC 33279	2.518
		LG 0727 3011	Environmental	<i>L. dumoffii</i> ATCC 33279	1.713
		LG 0727 3014	Environmental	<i>L. dumoffii</i> ATCC 33279	2.425
		LG 0744 2011	Environmental	<i>L. dumoffii</i> ATCC 33279	1.422
		HL 0610 3042	Environmental	<i>L. dumoffii</i> ATCC 33279	2.341
<i>L. erythra</i>	1	LG 0723 4020	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.558
	1	HL 0635 3006	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.596
	1	HL 0503 4030	Environmental	<i>L. erythra</i> sg1 ATCC 35303*	2.399
	1	HL 0506 4082	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.461
	1	HL 0521 3029	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.531
	2	HL 0709 4019	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.492
	2	LG 0713 3012	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.522
	2	LG 0714 5035	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.56
	2	LG 0727 4014	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.282
	2	LG 0745 2017	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.484
	2	HL 0518 1021	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.558
	2	HL 0525 2044	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.435
	2	HL 0549 5011	Environmental	<i>L. erythra</i> sg1 ATCC 35303	2.537
		HL 0647 5015	Environmental	<i>L. erythra</i> sg2 ATCC 11987	2.545
<i>L. feeleii</i>		HL 0507 4005	Clinical	<i>L. feeleii</i> sg2 ATCC 35849	2.271
	1	HL 0438 3099	Environmental	<i>L. feeleii</i> sg2 ATCC 35849	2.458
	1	LG 0750 3022	Environmental	<i>L. feeleii</i> LC878	2.305
	1	HL 0418 4001	Environmental	<i>L. feeleii</i> LC878*	2.252
	2	LG 0837 2016	Environmental	<i>L. feeleii</i> sg1 ATCC 35072	2.396

Table 3. cont.

Species	Sg	Reference	Source	MS best match	Score
<i>L. gormanii</i>	2	HL 0601 1024	Environmental	<i>L. feeleii</i> sg2 ATCC 35849	2.411
		HL 0648 5009	Environmental	<i>L. feeleii</i> LC878	2.405
		LG 0839 5017	Clinical	<i>L. gormanii</i> ATCC 33297*	2.125
		HL 0625 4010	Environmental	<i>L. gormanii</i> ATCC 33297	2.165
		HL 0404 3029	Environmental	<i>L. gormanii</i> ATCC 33297	1.847
		HL 0506 2025	Environmental	<i>L. gormanii</i> ATCC 33297	1.826
		HL 0408 2043	Environmental	<i>L. drancourtii</i> A18532-39	1.81
		HL 0522 4013	Environmental	<i>L. parisiensis</i> ATCC 700174	1.769
<i>L. jordani</i>	2	HL 0540 3034	Environmental	<i>L. parisiensis</i> ATCC 700174	2.335
		Ly95-90	Clinical	<i>L. jordani</i> ATCC 700762	2.435
		HL 0710 1006	Environmental	<i>L. jordani</i> ATCC 700762	2.35
		HL 0539 2007	Environmental	<i>L. jordani</i> ATCC 700762	2.425
		HL 0407 4026	Environmental	<i>L. jordani</i> ATCC 700762	2.438
		HL 0426 1037	Environmental	<i>L. jordani</i> ATCC 700762*	2.207
		HL 0450 5001	Environmental	<i>L. parisiensis</i> ATCC 700174	1.648
<i>L. londiniensis</i>	2	HL 0546 4029	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.735
		HL 0552 5024	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.583
		HL 0552 5025	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.583
		HL 0604 5014	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.696
		HL 0612 1022	Environmental	<i>L. londiniensis</i> sg2 ATCC 700510	2.575
		HL 0616 3036	Environmental	<i>L. londiniensis</i> sg2 ATCC 700510	2.607
		HL 0622 3040	Environmental	<i>L. londiniensis</i> sg2 ATCC 700510	2.642
		HL 0622 5014	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.634
		HL 0647 3017	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.573
		LG 0721 1028	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.672
		LG 0815 4028	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505*	2.203
		LG 0713 3010	Environmental	<i>L. londiniensis</i> sg1 ATCC 49505	2.365
<i>L. longbeachae</i>	1	HL 0625 5022	Clinical	<i>L. longbeachae</i> sg1 ATCC 33462	2.439
		HL 0422 4011	Clinical	<i>L. longbeachae</i> sg1 ATCC 33462	2.548
		LG 0905 2014	Clinical	<i>L. longbeachae</i> sg1 ATCC 33462*	2.38
		LG 0902 4055	Clinical	<i>L. sainthelensi</i> sg2 ATCC 49322	2.253
		HL 0521 1031	Clinical	<i>L. longbeachae</i> sg1 ATCC 33462	2.62
<i>L. maceachernii</i>	1	HL 0427 5008	Environmental	<i>L. longbeachae</i> sg1 ATCC 33462	2.629
		LG 0736 4032	Environmental	<i>L. maceachernii</i> ATCC 35300	2.205
		HL 0404 3013	Environmental	<i>L. maceachernii</i> ATCC 35300*	2.126
		HL 0540 2029	Environmental	<i>L. maceachernii</i> ATCC 35300	1.691
		HL 0525 3043	Environmental	<i>Pseudomonas hydrocarboxydans</i> B333_UFL	1.322
<i>L. micdadei</i>	1	HL 0646 4012	Clinical	<i>L. micdadei</i> ATCC 33218	1.864
		LG 0801 5022	Environmental	<i>L. micdadei</i> ATCC 33218	2.365
		LG 0818 2002	Environmental	<i>L. micdadei</i> ATCC 33218	2.524
		LG 0843 3030	Environmental	<i>L. micdadei</i> ATCC 33218	2.433
		HL 0605 2007	Environmental	<i>L. micdadei</i> ATCC 33218	2.303
		HL 0612 1016	Environmental	<i>L. micdadei</i> ATCC 33218*	2.215
		HL 0651 3006	Environmental	<i>L. micdadei</i> ATCC 33218	2.244
		HL 0544 1029	Environmental	<i>L. micdadei</i> ATCC 33218	2.521
		HL 0416 4017	Environmental	<i>L. micdadei</i> ATCC 33218	2.612
		HL 0522 5034	Environmental	<i>Myroides odoratus</i> DSM 2811 HAM	1.414
<i>L. oakridgensis</i>	1	LG 0712 2004	Environmental	<i>L. oakridgensis</i> ATCC 700515	2.557
		LG 0716 3020	Environmental	<i>L. oakridgensis</i> ATCC 33761	2.42
		HL 0648 3005	Environmental	<i>L. oakridgensis</i> ATCC 700515*	2.335
		HL 0507 5031	Environmental	<i>L. oakridgensis</i> ATCC 700515	2.498
		HL 0449 4007	Environmental	<i>L. oakridgensis</i> ATCC 33761	2.506
<i>L. parisiensis</i>	1	96010011	Clinical	<i>L. parisiensis</i> ATCC 700174	2.612
		Ly66-88	Clinical	<i>L. parisiensis</i> ATCC 700174	2.667
		LG 0818 2001	Environmental	<i>L. parisiensis</i> ATCC 700174	2.538

Table 3. cont.

Species	Sg	Reference	Source	MS best match	Score
<i>L. sainthelensi</i>	1	LG 0842 4019	Environmental	<i>L. parisiensis</i> ATCC 700174	2.628
		LG 0844 5026	Environmental	<i>L. parisiensis</i> ATCC 700174	2.709
		HL 0632 4015	Environmental	<i>L. parisiensis</i> ATCC 700174*	2.186
		HL 0438 5019	Environmental	<i>L. parisiensis</i> ATCC 700174	2.59
		HL 0438 5084	Environmental	<i>L. parisiensis</i> ATCC 700174	2.726
	1	LG 0827 1057	Environmental	<i>L. sainthelensi</i> sg2 ATCC 700517	2.524
	1	HL 0440 2012	Environmental	<i>L. sainthelensi</i> sg2 ATCC 700517	2.574
	1	HL 0441 3010	Environmental	<i>L. sainthelensi</i> sg2 ATCC 700517	2.537
	1	HL 0442 3001	Environmental	<i>L. sainthelensi</i> sg2 ATCC 700517*	2.449
		HL 0621 3030	Environmental	<i>L. sainthelensi</i> sg2 ATCC 700517	2.453
<i>L. tucsonensis</i>		HL 0239 1038	Clinical	<i>L. bozemanæ</i> sg2 ATCC 35745	2.279
		HL 0449 4038	Environmental	<i>L. tucsonensis</i> ATCC 49180	2.283
		HL 0438 3117	Environmental	<i>L. parisiensis</i> ATCC 700174	2.277
		LG 0849 5014	Environmental	<i>L. tucsonensis</i> ATCC 49180*	2.155
<i>L. wadsworthii</i>		LG 0739 3034	Clinical	<i>L. parisiensis</i> ATCC 700174	2.301

*Strains used to evaluate reproducibility.

(Table 2). Among the other 123 blind-tested strains (Table 3), 99 were identified correctly at the species level with a score of ≥ 2.0 (80.5 %); 17 (13.8 %) exhibited a score of

< 2.0 , comprising 10 against their homologous species, five against an heterologous *Legionella* species and two against a non-*Legionella* bacterial species; and seven strains exhibited

Fig. 1. Virtual gel view of MS reference spectra from the tested *Legionella* species.

Fig. 2. Virtual gel view of MS reference spectra from the tested *Legionella pneumophila* serogroups.

a score of ≥ 2.0 against a heterologous *Legionella* species. As with *L. pneumophila*, spectra obtained from the different serogroups of other species exhibited a score of ≥ 2.0 against all serogroups of their homologous species.

Thus, the overall sensitivity of MALDI-TOF-MS for the identification of *Legionella* strains was 99.2% at the genus level (235/237) and 89.9% at the species level (213/237). In addition, strains with a score of < 2.0 were correctly identified at the species level in 58.8% of cases. However, the sensitivity varied among species. There was 100% sensitivity in eight species: *L. pneumophila* (114/114), *L. cincinnatiensis* (1/1), *L. erythra* (14/14), *L. feeleei* (7/7), *L. londiniensis* (12/12), *L. oakridgensis* (5/5), *L. parisiensis* (8/8) and *L. sainthelensi* (5/5). The sensitivity was lower in the other 11 tested species: 85.7% in *L. anisa* (6/7), 83.3% (5/6) in *L. jordanis* and *L. longbeachae*, 80.0% (4/5) in *L. birminghamensis* and *L. micdadei*, 63.6% in *L. bozemanae* (7/11), 60.0% in *L. dumoffii* (6/10), 50.0% (2/4) in *L. maceachernii* and *L. tucsonensis*, 28.6% in *L. gormanii* (2/7) and 0% in *L. wadsworthii* (0/1). In the reproducibility assay, the 19 strains tested obtained the same species identifications as in the blind test, with scores of > 2.0 for all strains. For seven out of eight of the species with 100% of strains correctly identified, our database included more than two reference spectra, which was the case in only four of the 11 remaining species ($P=0.03$).

DISCUSSION

The ability of various *Legionella* species to cause pneumonia and their potential role in nosocomial infections has stimulated the development of efficient identification tools, enabling rapid and adequate management of patients. However, precise identification techniques commonly used for *Legionella* species require molecular methods that are expensive and time-consuming. Here, we used MALDI-TOF-MS, a rapid and cost-effective identification method, for the identification of *Legionella* at the species level. Obtaining a strain identification took only 10 min from selecting a colony grown on the agar plate, with up to 15 strains being analysed per hour at a total cost of €1.65 per strain (including personnel cost).

We demonstrated that the discriminatory power of this method for identifying *Legionella* strains at the species level was high. We first developed a spectrum database using 59 representative strains from 21 species, and observed that all species could be differentiated (Figs 1 and 2), although some species, such as *L. longbeachae* and *L. sainthelensi*, exhibited closely related protein spectra. Next, we tested the validity of this method by performing a blind test with 237 strains from 19 different species. Among these, we observed that the discriminatory power of MALDI-TOF-MS varied according to the tested species, with strains from eight species being correctly identified in 100% of cases, whereas, for the remaining 11 species, the degree of proper identification varied from 0 to 85.7%. Such differences might be explained by an intraspecific variability of protein profiles among strains. We observed that *Legionella* strains were more likely to be correctly identified at the species level when more than two reference spectra were present in the database ($P=0.03$) (Table 1). In addition, for seven strains, a score of > 2.0 was obtained against a species that differed from but was phylogenetically close to their homologous species (Adeleke *et al.*, 1996; Grattard *et al.*, 2006; Ko *et al.*, 2002). These results suggest that the discriminatory power of MALDI-TOF-MS may be improved by increasing the number of spectra per species in the database. All spectra obtained in this study were added to our database (www.ifr48.com).

According to our results, we estimated the sensitivity of MALDI-TOF-MS for *Legionella* isolate identification to be 99.2 and 89.9% at the genus and species level, respectively (Tables 2 and 3). However, although we demonstrated that MALDI-TOF-MS is a powerful tool for the identification of *Legionella* strains at the species level, notably, for the most common pathogenic species, *L. pneumophila*, it could not discriminate among serogroups. In *Listeria* species, MALDI-TOF has previously been demonstrated to be as discriminatory as PFGE at the serogroup level (Barbuddhe *et al.*, 2008). However, for *Legionella* species, reference spectra from the different serogroups enabled correct species identification, but were too similar to be discriminatory at the serogroup level. Antigenic variations among *Legionella* serogroups have mainly been linked to differ-

ences in the LPS and major outer-membrane protein (Benson & Fields, 1998). Our data suggest that these protein differences are not easily detectable by MALDI-TOF-MS. However, we cannot rule out the possibility that these results were due to our mode of preparation of bacteria.

In conclusion, we determined reference MS spectra for cultivated and recognized pathogenic *Legionella* species. We demonstrated that species identification by MALDI-TOF-MS is reproducible and relevant for the genus *Legionella* and presents several advantages over other identification tools, including speed, discriminatory power and low cost. We added to the existing URMS database all spectra generated from this study, spanning 21 different pathogenic species. For *Legionella* strains showing intraspecific protein profile variability, this database will continuously be supplemented with spectra from additional strains to increase the representativity of species.

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