Identification of *Leishmania* at the species level with matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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Abstract

Matrix-assisted laser desorption ionization time-of-flightMALDI-TOF mass spectrometry (MS) is now widely recognized as a powerful tool with which to identify bacteria and fungi at the species level, and sometimes in a rapid and accurate manner. We report herein an approach to identify, at the species level, *Leishmania* promastigotes from *in vitro* culture. We first constructed a reference database of spectra including the main *Leishmania* species known to cause human leishmaniasis. Then, the performance of the reference database in identifying *Leishmania* promastigotes was tested on a panel of 69 isolates obtained from patients. Our approach correctly identified 66 of the 69 isolates tested at the species level with log (score) values superior to 2. Two *Leishmania* isolates yielded non-interpretable MALDI-TOF MS patterns, owing to low log (score) values. Only one *Leishmania* isolate of *Leishmania peruviana* was misidentified as the closely related species *Leishmania braziliensis*, with a log (score) of 2.399. MALDI-TOF MS is a promising approach, providing rapid and accurate identification of *Leishmania* from *in vitro* culture at the species level.

Keywords: Identification, *Leishmania*, MALDI-TOF MS, mass spectra, parasite
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Introduction

Human leishmaniasis, caused by 21 species of the obligate intracellular protozoan *Leishmania*, is one of the most neglected tropical diseases according to the WHO, despite the 2 million new cases recorded yearly [1]. This vector-borne disease encompasses several clinical syndromes, ranging from self-healing cutaneous infection to life-threatening visceral infections, partly depending on the *Leishmania* species involved [2]. Knowledge of the species involved is critical for predicting the evolution and prognosis, and for determining the appropriate treatment. However, conventional diagnostic techniques, i.e. microscopic examination of Giemsa-stained smears of infected tissues, *in vitro* cultivation of clinical samples on diphasic NNN or axenic media, and *Leishmania* serology assays, do not allow diagnosis at the species level [3]. Identification at the species level requires molecular biology techniques such as PCR or sequencing. These molecular biology techniques remain at an experimental stage, and are confined to research care centres. The reference standard method for identifying *Leishmania* species, as recommended by the WHO, remains isoenzyme analysis, which is labour-intensive, costly, and confined to reference centres [1]. Thus, no rapid and simple method for the species identification of *Leishmania* currently exists.

For approximately 10 years, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been proposed as an alternative to conventional and routine identification methods in clinical microbiological

laboratories. This identification technique was originally adapted for the identification of prokaryotic organisms [4]. New developments have extended its use to a few eukaryotic organisms, such as yeasts and moulds, making this technique a straightforward, fast and reliable identification method for bacteria, yeasts and moulds in a cost-effective way [5–7]. However, MALDI-TOF MS has not yet been adapted for the identification of *Leishmania* or any other protozoan parasites. In this article, we show how we first constructed a reference mass spectra library (MSL) by using cultures of 56 well-characterized *Leishmania* isolates, and then tested it by identifying a panel of 69 isolates with a MALDI-TOF MS-based approach.

Materials and Methods

Leishmania isolates and culture

A panel (panel 1) of 56 isolates was used to construct a mass spectra library, and is summarized in Table 1. The isolates were provided and accurately identified with the isoenzyme method by the Centre National de reference des Leishmanioses (CNR-L) (French National Reference Centre for Leishmaniasis) in Montpellier, France. A different panel (panel 2) of 69 isolates was used to assess identification performance (Table 2). These isolates were provided by the CNR-L, the Institut Pasteur of Alger, Algeria, and the Institut

TABLE 1. Main characteristics of the *Leishmania* isolates furnished by the Centre National de reference des Leishmanioses included in the reference database

Taxon	Isolate	Provenance	Host	CF	Zymodem
Leishmania infantum	MHOM/EG/87/RTC2	Egypt	*Homo sapiens*	VL	MON-98
L. infantum	MHOM/FR/87/RM1	France	*Canis familiaris*	VL	MON-108
L. infantum	MHOM/TN/80/IPT1	Tunisia	*H. sapiens*	VL	MON-1
L. infantum	MHOM/DZ/82/LIPA59	Algeria	*H. sapiens*	CL	MON-24
L. infantum	MHOM/FR/78/LEM75	France	*H. sapiens*	VL	MON-1
Leishmania donovani	MHOM/IN/00/DEVI	India	*H. sapiens*	VL	MON-2
L. donovani	MHOM/IN/96/THAK57	India	*H. sapiens*	VL	MON-2
L. donovani	MHOM/SD/98/LEM3580	Sudan	*H. sapiens*	VL	MON-18
L. donovani	MHOM/ET/67/HU3	Ethiopia	*H. sapiens*	VL	MON-18
L. donovani	MHOM/IN/80/DD8	India	*H. sapiens*	VL	MON-2
Leishmania archibaldi	MHOM/ET/72/GEBRE1	Ethiopia	*H. sapiens*	VL	MON-82
L. archibaldi	MCAN/SD/98/LEM3556	Sudan	*C. familiaris*	VL	MON-82
Leishmania major	MHOM/SU/73/5-ASKH	Turkmenistan	*H. sapiens*	CL	MON-4
L. major	MHOM/IL/81/FRIEDLINa	Israel	*H. sapiens*	CL	MON-103
L. major	MHOM/TN/2001/LEM4286	Tunisia	*H. sapiens*	CL	MON-25
L. major	MHOM/MA/2003/LEM4685	Morocco	*H. sapiens*	CL	MON-25
L. major	MHOM/YE/76/LEM62	Yemen	*H. sapiens*	CL	MON-26
Leishmania aethiopica	MHOM/ET/72/L100	Ethiopia	*H. sapiens*	CL	MON-14
L. aethiopica	MHOM/ET/91/KASSAYE	Ethiopia	*H. sapiens*	DCL	MON-248
Leishmania killicki	MHOM/TN/80/LEM163	Tunisia	*H. sapiens*	CL	MON-8
L. killicki	MHOM/TN/80/LEM180	Tunisia	*H. sapiens*	CL	MON-8
Leishmania tropica	MHOM/MA/95/LEM3015	Morocco	*H. sapiens*	CL	MON-264
L. tropica	MHOM/SU/74/K27	Azerbaijan	*H. sapiens*	CL	MON-60
L. tropica	MHOM/GR/80/GR-L35	Greece	*H. sapiens*	CL	MON-56
L. tropica	0000/00/84/LEM643	NA	NA	VL	MON-104
L. tropica	MRAT/IQ/72/ADHANIS1	Iraq	*Rattus rattus*	VL	MON-5
Leishmania braziliensis	MHOM/BR/75/M2904	Brazil	*H. sapiens*	CL	MON-165
L. braziliensis	MHOM/BR/84/LTB300	Brazil	*H. sapiens*	MCL	MON-166
L. braziliensis	MHOM/BR/82/LTB12JULY82	Brazil	*H. sapiens*	MCL	MON-208
L. braziliensis	MHOM/BR/75/M2903b	Brazil	*H. sapiens*	CL	MON-43
L. braziliensis	MHOM/GF/2003/LAV008	FG	*H. sapiens*	CL	MON-43
Leishmania guyanensis	MHOM/GF/98/LEM3657	FG	*H. sapiens*	CL	MON-45
L. guyanensis	MHOM/GF/2003/LEM4570	FG	*H. sapiens*	CL	MON-45
L. guyanensis	MHOM/GF/2004/LAV016	FG	*H. sapiens*	CL	MON-45
L. guyanensis	MHOM/GF/79/LEM85	FG	*H. sapiens*	CL	MON-45
Leishmania panamensis	MHOM/EC/90/CALDERON	Equador	*H. sapiens*	CL	MON-161
L. panamensis	MDAS/PA/71/LS94	Panama	*H. sapiens*	CL	MON-47
Leishmania peruviana	MHOM/PE/84/CE49	Peru	*H. sapiens*	CL	MON-140
Leishmania lainsoni	IUBI/BR/00/M12025	Brazil	*Lutzomyia ubiquitalis*	None	MON-150
L. lainsoni	MCUN/BR/85/M9342	Brazil	*Cuniculus paca*	None	MON-151
Leishmania naiffi	MDAS/BR/79/M5533	Brazil	*Dasypus novemcinctus*	None	MON-148
Leishmania amazonensis	MHOM/BR/72/M1845	Brazil	*Proechimys* sp.	None	MON-41
L. amazonensis	MHOM/BR/73/M2269	Brazil	*H. sapiens*	CL	MON-132
L. amazonensis garnhami	MHOM/VE/76/JAP78	Venezuela	*H. sapiens*	CL	MON-41
Leishmania mexicana	MNYC/BZ/62/M379	Belize	*Nyctomys sumichrasti*	CL	MON-40
L. mexicana	MHOM/BZ/82/BEL21	Belize	*H. sapiens*	CL	MON-156
L. mexicana pifanoi	MHOM/VE/57/LL1	Venezuela	*H. sapiens*	DCL	MON-40
Leishmania pifanoi	MHOM/VE/57/LL1a	Venezuela	*H. sapiens*	DCL	MON-40
Leishmania deanei	MCOE/BR/78/M5088	Brazil	*Coendou* sp.	None	MON-52
L. deanei	MCOE/BR/74/M2674	Brazil	*Coendou prehensilis*	CL	MON-134
Leishmania arabica	MPSA/SA/83/JISH220	Saudi Arabia	*Psammomys obesus*	VL	MON-99
Leishmania enriettii	MCAV/BR/45/L88	Brazil	*Cavia porcellus*	CL	MON-97
L. enriettii	MCAV/BR/95/CUR2	Brazil	*C. porcellus*	CL	MON-97
Leishmania gerbilli	MRHO/CN/60/GERBILLI	China	*Rhombomys opimus*	CL	MON-22
Leishmania turanica	MRHO/SU/65/VL	Turkmenistan	*R. opimus*	VL	MON-21
L. turanica	MMEL/SU/79/MEL	Georgia	*Meles meles*	None	MON-65

CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; FG, French Guyana; MCL, mucocutaneous leishmaniasis; NA, not available; VL, visceral leishmaniasis.