

Aspects of food control and animal health



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Keywords:

MALDI-TOF MS, database, contact platform, spectrometry, spectra

Abstract

Recent years have seen substantial progress towards the application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in routine microbiological diagnostics. MALDI-TOF MS-based identification relies on the reproducible detection of large biopolymer mass patterns obtained from proteins of whole cells.

Besides species identification in microbiological diagnostics, MALDI-TOF MS can also be used for protein profiling, to screen for metabolites and

contaminants, for epidemiological traceback, for phenotyping resistance and virulence patterns, or for biomarker detection directly from clinical material to mention only a few of the applications.

The fundamental principle of MALDI-TOF MS-based microbial identification analysis relies on a computer-based comparison of mass spectra recorded from unknown pathogens with databases containing mass spectra from samples with a known taxonomic status. Such databases are commercially available and most of the employed software and database solutions allow creating and including custom, user-specific and transferable database entries. The goal of the present work is to introduce a novel user-to-user internet platform – MALDI-User-Platform (MALDI-UP) – which was specifically designed to facilitate the exchange of information about user-specific database entries. Thereby, diagnostic gaps could be closed efficiently and databases for yet under-reported applications, such as the species identification of animal meat, insects or mushrooms via MALDI-TOF MS, could be promoted.

Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful analytical tool in food analysis, veterinary and clinical diagnostics. At the present stage the technique is used mainly for the differentiation, classification and identification of bacteria and other microorganisms in several fields of microbiology [Clark et al., 2013; Randall et al., 2015, Singhal et al., 2015, Zimmermann, 2015]. In contrast to classical microbial identification techniques, like biochemical identifications and molecular approaches, MALDI-TOF MS measurements can be carried out within minutes, coupled with the advantage of minimal material costs per identification [Dihman et al., 2011; Tran et al., 2015]. The method is not restricted to bacterial identification, but can also be utilized to determine eukaryotic cells, fungi, algae, protozoans and insects. MALDI-TOF MS was found to be useful even for species identification based on host proteins [Singhal et al., 2015; Barbano et al., 2015; Cassagne et al., 2014].

The comparison of experimental mass spectra obtained from unidentified samples (e.g. microorganisms) with reference spectra in a given database results in a ranking list containing the best matching database entries. For this approach, commercial databases use several thousands of spectra from broad microbial fields. The success of identification depends on the quality and the species coverage of the entries included in the used database. Manufacturers place large efforts on extending their databases. However, due to economic reasons, the time between validated manufacturer updates can be long. The systems (consisting of hardware, software and the MS database) employed by most users do not only offer the gradual update of an extensive database supplied by the manufacturer, but also allow for the addition of custom data. Such data can be transferred within the device platform, making it possible to close diagnostic gaps more quickly. In medical and veterinary microbiology, this is particularly relevant for various special microorganisms and highly pathogenic species. Although there is a great demand, until now, there are only a few MALDI-TOF MS database entries available for non-clinical applications, such as the species identification of animal meat or of fish.

The reliable exchange of custom database entries requires that the user who creates the entry (creator) provides certain basic information (metadata) to the user who receives the data (recipient). The recipient is primarily interested in what is being offered and of what quality the entry is: Briefly, this aspect covers the accuracy of species assignment by reference methods, the quality of sample preparation, spectra acquisition, selection, and processing.

Only if this basic information (metadata) is reliable, the recipients will accept such an entry and add it to existing MS databases. Therefore, the recipients must be able to identify the creator of the entry. This can be achieved either through personal contacts, or by means of an open information exchange platform. The online MALDI-UP catalog which is available at <http://maldi-up.ua-bw.de> and which is hosted by the Chemisches und Veterinäruntersuchungsamt Stuttgart (CVUAS) was specifically designed for this purpose. The catalog lists user-created database entries that are uniformly presented and contains comprehensive metadata information such as detailed taxonomic

information, isolate numbers and sample preparation parameters. Furthermore, the designations of isolates (e.g. molecular identification, biochemical data) as well as technical details of the entries (instrument, cultivation, preparation, etc.) are given. The summary of database entries and the contact information of the creators of the entries are also presented. The MALDI-UP catalog is not intended to provide free downloads of the spectra. This is important because it assures that the protection of copyrights will remain in the hands of the copyrights owners.

Usage of the catalog and description of the procedure to add spectral entries

The MALDI-UP catalog is open to everyone interested in providing information about personal database entries with the option to exchange or distribute spectra under own merchandising criteria. The catalog can be used, subject to the conditions stated on the homepage. It is also a source of information for everyone who wishes to contact spectra creators.

The catalog (in Microsoft Excel) can be downloaded from the MALDI-UP platform and new data entries can be added in the proposed format. Following the submission of personal new data to maldi-up@ua-bw.de, they will be carefully checked and missing information will be requested. Finally, the latest entries will be included in the next update of the catalog. Creators agree to make their contact information publicly available (institutional and email address) and principally agree to respond to data requests. The procedure ensures that the creators themselves control the exchange of information with their colleagues.

The catalog will be continuously updated on a regular basis, at least monthly, and demands a procedure of disclosed parameters for validation. This concept was developed with assistance from employees at CVUA Stuttgart and the Hessian State Laboratory (for comments and suggestions please contact maldi-up@ua-bw.de).

The MALDI-UP catalog – a detailed description

The following section specifies the information given in the spreadsheets of the MALDI-UP catalog, including relevant column headings.

Spreadsheet Entries – Information fields (Fig. 1)

	A	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
	MALDI-UP					133 entries			18.03.2016 http://maldi-tof-ms-user-platform.ua-bw.de					CVUA Stuttgart (CVUAS)					
1	List of available database-entries by users					http://maldi-up.ua-bw.de					Contact: MALDI-UP@ua-bw.de								
2	Liste verfügbarer Datenbank-Einträge von Anwendern																		
3																			
4																			
5	Basic data for the entry					Taxonomic information about the used material					data for the isolate/material								
6						Taxonomie					Daten zum Isolat / dem Material								
7	No.	Date	system	user responsible for the row (see contact)	domain	phylum	class	order	family	genus	species	subspecies	identifier	isolation source / date / [reference]	isolate tracking	further details on the isolate / material	spore former	verification of assignment	
8	Nr.	Datum des Listeneintrags	System	Verantwortlich für die Zeile (s. contact)	Domäne	Phylum	Klasse	Ordnung	Familie	Genus	Spezies / Art	Unterart	Material- / Isolatbezeichnung	isoliert aus / wann / [Literatur]	Isolat-Historie	weitere Angaben zum Isolat / Material	bildet Sporen	Verifikation der Benennung	
9	FILTER																		
10	0001	22.07.2015	Braker Biotyper LT-microflex	CVUA Stuttgart (CVUAS)	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	cytotoxius		CVUAS 2833	potato puree / 2009 / [0001]	isolate CVUAS	Draft genome sequence [0001]	yes	isolate of species description [0001], sequenced [0001]	
11	0002	22.07.2015	Braker Biotyper LT-microflex	CVUA Stuttgart (CVUAS)	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	cytotoxius		NVH 331-98	[0001]	A. Sorokin (INRA, F) > CVUAS > DSMZ	NVH 331-98; DSM 22305	yes	typestrain [0001]	
12	0003	22.07.2015	Braker Biotyper LT-microflex	CVUA Stuttgart (CVUAS)	Bacteria	Bacteroidetes	Bacteroidia	Proteobacteria	Proteobacteriales	Flavobacterium	psychrophilum		CVUAS 10309	rainbow trout (Oncorhynchus mykiss)	isolate CVUAS		no	sequenced	
13	0004	22.07.2015	Braker Biotyper LT-microflex	CVUA Stuttgart (CVUAS)	Eukaryota	Chordata	Mammalia	Carnivora	Carnivora	scrofa	domestica		M0001	flesh	CVUAS	pork neck		label; zoonosis analysis	

Fig. 1: Screenshot of the first Excel spreadsheet “Entries”, depicting the list of database entries.

In the upper part of the spreadsheet one can find contact information to the editor of the version (defined by the number of entries and the date). There is also a link to the MALDI-UP homepage to get back to the main website. The next rows include the column titles of the table in English and German. The row “FILTER” serves as an Excel filter for each column to facilitate the individual search and adaption of the table after download.

The entries are sorted by a rough taxonomic classification. Following the download of the MALDI-UP catalog, the sorting can be restricted and controlled using the filters.

The columns of the table were grouped in six sections:

Basic data for the entry					
Taxonomic information about the used material					
Data for the isolate or material					
Technical data for the entry					
Results of an independent sample preparation in routine mode, using the new entry for identification					
Information about the number of users who have tested the entry and the type of feedback (positive/ negative)					

Explanation for every data column:

Basic data for the entry	
No.	Serial number of the database entry
Date	Listing date
System	Mass spectrometry system used
User responsible for the line (see contact)	Institution responsible for the information in this row. The contact information of the creator is given on the spreadsheet "contact".

Taxonomic information about the used material	
Domain	Rough classification in taxonomic criteria up to subspecies-level to facilitate searching and filtering
Phylum	
Class	
Order	
Family	
Genus	
Species	
Subspecies	

Data for the isolate/material	
Identifier	Identifier of the isolate, preferably a number of a public strain collection (e.g. DSM, ATCC ...) or an isolate collection number (e.g. of the creators CVUAS 1234)
Isolation source / date / [reference]	Source of the material, literature reference (if available)
Isolate tracking	Known history of the isolate. Isolates should be conserved to allow subsequent verification.
Further details on the isolate	
Spore former	The ability of the isolate to form spores, because of the possible influence on MALDI-TOF MS spectra and preparation requirements.
Verification of assignment	Principle of the verification of the isolate / material (e.g. sequenced, type strain, veterinary post mortem pathology examination)
Results of sequencing	In this field a short version of sequencing results can be provided, which supports the species assignation. Depending on the primer-system used, sequencing results are not always the sequence of the whole target gene (e.g. 16S rDNA, <i>rpoB</i> , <i>gyrB</i>). The resulting match with the first reliable hit in a genetic sequence database should therefore be given with the number of bases. If available in format: 16S rDNA: 1387bp (96.70% of ref-sequence) > 1[difference bp]/1387 = 99.93% similarity with <i>Flavobacterium psychrophilum</i> JIP02/86, 100% completeness (11.06.15; EzBioCloud)
Other results for assignment of the isolate	E.g. biochemical data, or specific PCR results, especially important for species decision (if available)
Isolate / material conserved as	Own isolate collection or reference to public strain collection.

Technical data for the entry	
Agar / medium	Agar (e.g. SBA = sheep blood agar) used for cultivation of the microorganism
Kind of medium	Solid / liquid
Cultivation temperature	In degree Celsius
Cultivation time	In hours (h) or days (d)
Cultivation, other conditions	e.g. atmospheric conditions (aerobic, anaerobic)
Sample preparation [reference]	Sample preparation protocol used, with reference if available
Matrix	e.g. HCCA
Creation of the entry according to the manufacturer's specifications by trained personnel	Yes or no
Full name of entry	Full name of the new entry
Date of entry (year/day/month)	Creation date of the new entry

Results with the entry	
	Result of an independent sample preparation in routine use
Sample preparation > date of independent spectrum	e.g. direct transfer (DT); date in format day/month/year
Commercial database version	Commercial database version used (e.g. DB 5898)
Result with commercial database version	Result of the independent spectrum identified with the commercial database e.g. Biotyper-format: <i>Staphylococcus aureus</i> (1.782) B: 1.782 is the score value, A/B/C the consistency category of the Genus / Species according to [Anonymous, 2012]
Result with the new entry	Result of the independent spectrum identified with the database including the new entry
Remarks for interpretation	Remarks or information about the interpretation of this entry (e.g. "closely related to <i>Staphylococcus aureus</i> ")

Information about the number of users who have tested the entry and the type of feedback (positive/ negative)	
	Feedback from other users of the entry
Number of known external users of the entry	Number of recipients, with whom the entry has been shared
Number of users who gave feedback about the entry. Green: positive experiences; red: negative experiences	Number of reported experiences for the entry: positive experiences confirm the result found with the new entry; negative experiences fail to confirm the new entry

Spreadsheet Contact (Fig. 2)

The spreadsheet "contact" contains addresses of creators and their institutions, in alphabetical order, who have deposited information on own database entries.






	A	B	C	D	E	F	G	H	I	J	K	L
1	MALDI-UP											
2	contact address											
3	Kontakte / Ansprechpartner											
4	institution			address			e-mail institution	contact person	e-mail contact person	MALDI-system	institution category	
5	Institution			Adresse			e-mail Institution	Ansprechpartner MALDI	e-mail Ansprechpartner	MALDI-System	Art der Institution	
6	FILTER											
7	CVFR	CVUA Freiburg		Chemisches und Veterinäruntersuchungsamt Freiburg, Dienstgebäude Tierhygiene	Am Moosweiher 2	79108 Freiburg	D	poststelle@cvuaf.bwl.de	Dr. Christine Wind	christine.wind@cvuaf.bwl.de	Bruker Biotyper LT-microflex	Food and veterinary inspection service
8	CVKA	CVUA Karlsruhe		Chemisches und Veterinäruntersuchungsamt Karlsruhe	Weissenburger Str. 3	76187 Karlsruhe	D	poststelle@cvuka.bwl.de	Dr. med. vet. Franziska Eisenbeiss	franziska.eisenbeiss@cvuka.bwl.de	Bruker Biotyper LT-microflex	Food and veterinary inspection service
9	CVUAS	CVUA Stuttgart		Chemisches und Veterinäruntersuchungsamt Stuttgart	Schaafflandstr. 3/2	70367 Fellbach	D	poststelle@cvuas.bwl.de	Dr. Jörg Rau	joerg.rau@cvuas.bwl.de	Bruker Biotyper LT-microflex	Food and veterinary inspection service
10	LGL	LGL Oberschleißheim		Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit, LHS-Zentrale Analytik	Veterinärstrasse 2	85764 Oberschleißheim	D	poststelle@lgl.bayern.de	Dr. Ingrid Huber, Dr. Melanie Pavlovic	ingrid.huber@lgl.bayern.de; melanie.pavlovic@lgl.bayern.de	Bruker Biotyper LT-microflex	Food and veterinary and health inspection service
11	LHL	LHL Giessen		Landesbetrieb Hessisches Landeslabor	Schubertstraße 60 - Haus 13	35392 Giessen	D	poststelle@lhl.hessen.de	Dr. Tobias Eisenberg	Tobias.Eisenberg@lhl.hessen.de	Bruker Biotyper LT-microflex	Food and veterinary inspection service
12	RIP	RIPAC-LABOR		RIPAC Labor GmbH	Am Mühlenberg 11	14476 Potsdam - Golm	D		Dr. Marcel Erhard	merhard@ripac-labor.de	VITEK MS Plus (Axima + SARAMIS)	Private veterinary laboratory
13	RKI	RKI Berlin		Robert Koch-Institut, Center for Biological Threats and Special Pathogenes (ZBS); ZBS 6 - Proteomics and Spectroscopy	Nordufer 20	13353 Berlin	D		Dr. Peter Lasch	LaschP@rki.de	Bruker Autoflex	Governmental institution

Fig. 2: Screenshot of the second spreadsheet, depicting the list of creators with contact information.

The Row “FILTER” serves as Excel-filter for every column, to facilitate the individual search and adaption of the table after download.

Institution	Name in abbreviated form
	Name in short form
	Logo of the institution
Address	Official name of institution
	Street
	Area code and city
	Country code
E-mail institution	E-mail address of the institute, without one specific recipient
Contact person	Physical person, who works with the MALDI-TOF MS / provides entries
E-mail contact person	Email address of the contact person
MALDI-System	The type of MALDI-TOF MS equipment available
Institution category	Private / Governmental / Inspection service...

Spreadsheet References (Fig. 3)

This page summarizes the references used for the identification of certain species. It also refers to the extraction methods used. Sequential numbers are given, followed by a column with the complete citation of the article.

If a new reference is essential for the creator, it should be submitted together with the database entry. The reference number will be edited by the webmaster of the MALDI-UP list.

A	B	C
1	References	
2		
3	[BRU01] DT	Brucker Protocol direct transfer, Anonymous, MALDI Biotyper 3.1 User manual Revision 1, 2012 Appendix D2.1
4	[BRU02] eDT	Brucker Protocol extended direct transfer, Anonymous, MALDI Biotyper 3.1 User manual Revision 1, 2012 Appendix D2.1
5	[BRU03] extr	Brucker Protocol extraction, Anonymous, MALDI Biotyper 3.1 User manual Revision 1, 2012 Appendix D2.2
6	[BRU04] Fextr	Brucker Protocol Filamentous-Fungi-Cultivation ProcedureV1.0, Anonymous
7	[0007] OSextr	Organic solvent extraction for meat
8		
9	[0001] Guinebreiere et al., 2013	Guinebreiere MH, Auger S, Galleron N, Contzen M, De Sarrau B, De Buyser ML, Lamberet G, Fagerlund A, Granum PE, Lereclus D, De Vos P, Nguyen-The C, Sorokin A
10		<i>Bacillus cytotoxicus</i> sp. nov. is a new thermotolerant species of the <i>Bacillus cereus</i> group occasionally associated with food poisoning
11		Int. J. Syst. Evol. Microbiol. 2013 ; 63: 31-40
12	[0002] Bertsch et al., 2013	Bertsch D, Rau J, Eugster MR, Hug MC, Lawson PA, Lacroix C, Meile L
13		<i>Listeria fleischmannii</i> sp. nov., isolated from cheese
14		Int. J. Syst. Evolut. Microbiol. 2013 ; 63: 526-532
15	[0003] DSMZ Catalogue	Anonymous
16		Catalogue of microorganisms. Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.
17		DSMZ, Braunschweig, Germany
18	[0004] Eisenberg et al., 2014	Eisenberg T, Nessler A, Nicklas W, Spamer V, Seeger H, Zschöck M
19		<i>Streptobacillus</i> sp. isolated from a cat with pneumonia
20		Journal of Medical Microbiology Case Reports, 2014 ; 1-7. DOI 10.1099/jmmcr.0.000562
21	[0005] Eisenberg et al., 2015a	Eisenberg T, Glaeser S, Nicklas W, Mauder N, Contzen M, Aledelbi K, Kämpfer P
22		<i>Streptobacillus felis</i> sp. nov., isolated from a cat with pneumonia, and emended descriptions of the genus <i>Streptobacillus</i> and of <i>Streptobacillus moniliformis</i> .
23		Int. J. Syst. Evolut. Microbiol. 2015 ; 65: 2172-2178. DOI: 10.1099/ijs.0.000238
24	[0006] Glaeser et al., 2013	Glaeser SP, Galatis H, Martin K, Kämpfer P.
25		<i>Niabella hirudinis</i> and <i>Niabella drilacis</i> sp. nov., isolated from the medicinal leech <i>Hirudo verbana</i> .
26		Int. J. Syst. Evolut. Microbiol. 2013 ; 63: 3487-3493. DOI 10.1099/ijs.0.050823-0
27	[0007] Stoll & Rau, 2015	Stoll P, Rau J
28		Tierartendifferenzierung von Fleisch mittels MALDI-TOF MS
29		Poster auf Deutscher Lebensmittelchemikertag Karlsruhe 14.-16.09.2015. Lebensmittelchemie 69: 142.
30	[0008] Eisenberg et al., 2015b	Eisenberg T, Nicklas W, Mauder N, Rau J, Contzen M, T Semmler, Hofmann N, Aledelbi K, Ewers C
31		Phenotypic and genotypic characteristics of members of the genus <i>Streptobacillus</i>
32		Plos One August 7, 2015 10(8): e0134312. journal.pone.0134312

Fig. 3: Screenshot of the third spreadsheet, depicting the list of references.

Spreadsheets History (Fig. 4)

This page provides an overview of the history of uploads and the total sum of entries. Additionally, it states the next scheduled update of the list.

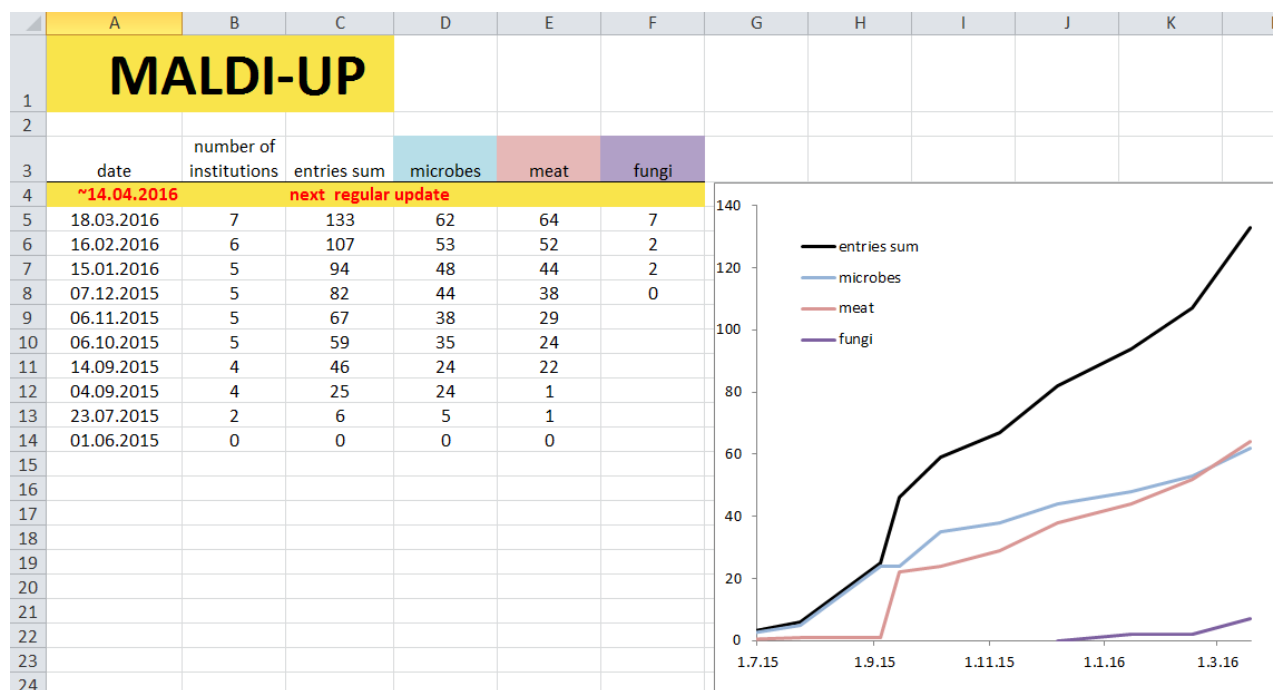


Fig. 4: Screenshot of the fourth spreadsheet, depicting the history of the increase and the material groups of the MALDI-UP entries.

Row 3 contains several columns:

Date	Chronological list of update-dates
Number of institutes	Number of institutes that have provided information about database entries (see page contact)
Entries sum	Total number of entries
Microbes	Number of entries for microorganisms
Meat	Number of entries for meat
Fungi	Number of entries for fungi
	Still open, for other types of entries (e.g. plants, algae etc.)

This sheet also contains a graphic overview of the progression of entries [Fig. 4].

Discussion

Universal use of databases depends on size and quality of data entries. The purpose of the MALDI-User-Platform (MALDI-UP) is to provide MALDI-TOF MS users with an easily accessible possibility to exchange database entries. At the time of writing (March 2016), altogether 133 entries by users of seven institutions have been added to the catalog (Version 133/18.03.2016) since its first release (September 2015). These database entries originate from different mass spectrometers (e.g. Bruker LT microflex and autoflex, and bioMérieux VITEK MS Plus). Furthermore, since the database has been launched in November 2015 the number of downloads has increased continuously (Fig. 4).

This open access catalog does not claim to substitute the manufacturers' continued efforts to provide database extensions. It allows, however, to gain a quick overview on available database entries from users working in the same area of research or diagnostics with special interests, such as animal meat, insects or mushrooms. Furthermore, the contact to colleagues can be easily established, independently of whether the creator is from a research institution, a hospital or an official or private diagnostic laboratory. The interest during the first months is reflected by the number of deposited entries and the growing number of downloads in the last months (Fig. 4, Fig. 5).

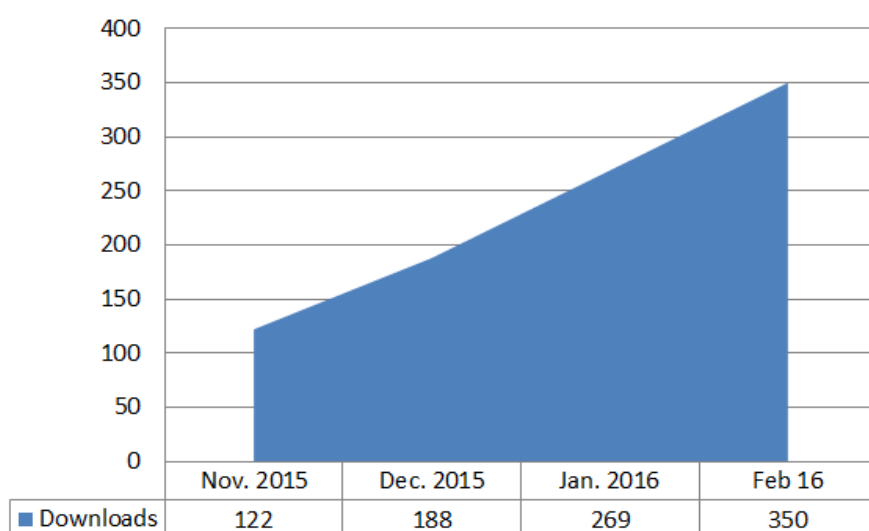


Fig. 5: Downloads of the MALDI-UP file since the launch.

The interest in the catalog and its benefit for the user will increase for both, creators and recipients, as the number of approved data entries increases. Additionally, fruitful collaborations can arise beyond the exchange of database entries alone.

The potential of finding interested creators, capable of offering high-quality entries for exchange, is considered high, for the following reasons:

- the number of marketed MALDI-TOF mass spectrometers increases world-wide (2015 > 2.000),
- a higher level of training of MALDI-users, offered by the manufacturers or other institutions, ensures an increase in knowledge and
- a wide range of research projects deal with MALDI-TOF MS use and validation.

Increasing numbers of catalog entries and database downloads are promising and an incentive to consolidate the MALDI-UP project as a source for current data as a pillar of up-to-date diagnostics. We have already proven that MALDI-TOF MS can be a suitable tool to be integrated in the description of novel species. Therefore, it is increasingly important to quickly provide MS data of newly detected species. This has been common practice for several years for data on taxonomically important genetic sequences. As an example, we refer to the MALDI-UP catalog entries for the recently described bacterial species *Bacillus cytotoxicus* CVUAS 2833 (entry 0001; Guinebretière et al., 2013) and *Listeria fleischmanni* (entry 0006; Bertsch et al., 2013) as well as for the entries of *Streptobacillus (S.) felis* (entry 0013, Eisenberg et al., 2015 a), *S. notomys* (entry 0119; Eisenberg et al., 2016 a) and *S. ratti* (entry 0120; Eisenberg et al., 2016 b).

Additionally, the database focused on the identification of species of food animals has recently been augmented by spectral data for pork meat (entry 0004; Stoll et al, 2015).

In conclusion, the launch of the MALDI-UP catalog aims to support networking and expansion of the community of MALDI users based on mutual sharing of information.

Acknowledgements

The authors like to thank M. Contzen, E. Hiller (CVUAS), and Andy Schneider (RKI Berlin) for helpful discussions and S. Böttcher (CVUAS) for preparing the MALDI-UP web-page.

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Impressum / Editorial Board

Aspects of food control and animal health

Free eJournal

ISSN: 2196-3460

Volume 2016 Issue 01

Published April 2016

Publisher:

Chemisches und Veterinäruntersuchungsamt Stuttgart
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GERMANY

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Internet: www.cvuas.de

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Chemisches und Veterinäruntersuchungsamt Stuttgart

V.i.S.d.P: Maria Roth
Editor in Chief and Layout: Stefan Böttcher
Corporate Design: Maja Lindemann



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