Identification of *Brucella* by MALDI-TOF mass spectrometry

Dr. De Maio Flavio

27/02/2015

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2001, p. 235–240 Vol. 39, No. 1
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.1.235–240.2001

Limited Genetic Diversity of *Brucella* spp.

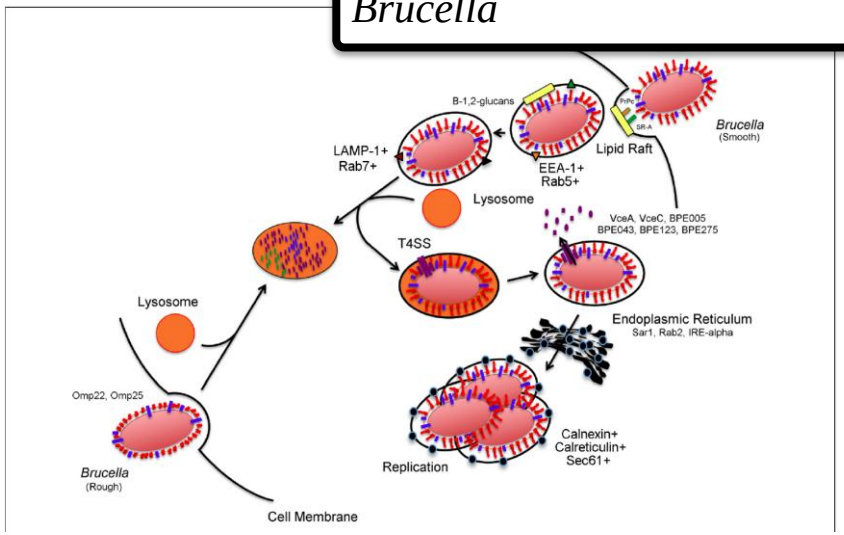
BENJAMÍN GÁNDARA,[1,2] AHIDÉ LÓPEZ MERINO,[1] MARCO ANTONIO ROGEL,[3] AND ESPERANZA MARTÍNEZ-ROMERO[3]*

Escuela Nacional de Ciencias Biológicas, IPN,[1] and Centro de Investigación sobre Fijación de Nitrógeno, UNAM,[3] Cuernavaca, and Servicios de Salud de Zacatecas, S.S.A., Zacatecas,[2] Mexico

Received 30 June 2000/Returned for modification 29 August 2000/Accepted 16 October 2000

Multilocus enzyme electrophoresis (MLEE) highligths a limitated genetic diversity and supports the proposal of a monospecific genus

- *Alphaproteobacteria*
  - *Rhizobiales*
    - *Brucellaceae*
      - *Brucella*
        - *B. abortus biovar 1, 2, 3, 4, 5, 6, 9*
        - *B. melitensis biovar 1, 2, 3*
        - *B. suis biovar 1, 2, 3, 4, 5*
        - *B. canis*
        - *B. ovis*
        - *B. neotomae*
        - *B. inopinata*
        - *B. ceti*
        - *B. microti*
        - *B. pinnipedialis*



Serological agglutination with smooth lipopolysaccharide and associated antigens A and M allows the biovar clissification

Why is important to identify *Brucella* species?

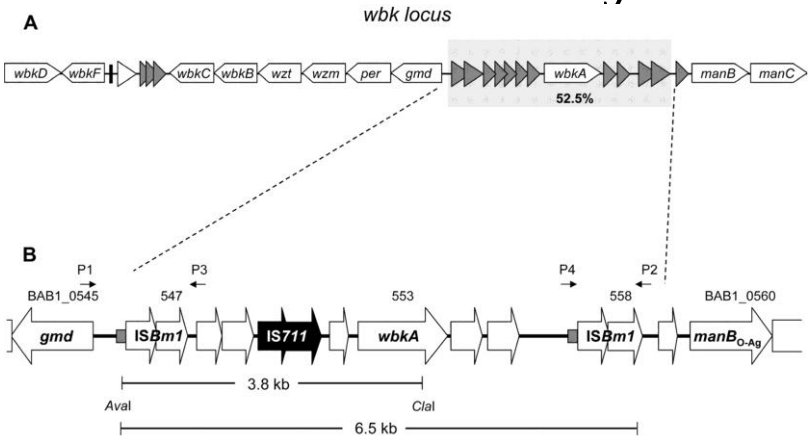
• The identification of the *Brucella* species is important and necessary for the different pathogenic impact for the host (specie-specificity)

• Identify the circulating *Brucella* species and biovar may be important for epidemiological studies, to identify the reservoir and deploy informed control strategies to prevent the spread of the infection (relevant economic impact in the primary sector and for public health)

• Rapid automated system often give misidentification

• PCR shows high sensitivity and specificity but remains cumbersome and with standardization problems

AMOS PCR assay



095-1137/94/$04.00+0

Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR

BETSY J. BRICKER* AND SHIRLEY M. HALLING

National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received 15 April 1994/Returned for modification 12 July 1994/Accepted 2 August 1994

Assay based on the amplification of species-specific-sized products using five primers: one hybridizes to IS711 element and the others to one of the species-specific regions adjacent to the element

Bruce-ladder PCR assay

Species- or strain-specific genetic differences used to design PCR primers

095-1137/08/$08.00+0 doi:10.1128/JCM.00837-08

Evaluation of a Multiplex PCR Assay (Bruce-ladder) for Molecular Typing of All *Brucella* Species, Including the Vaccine Strains[▽]†

I. López-Goñi,[1]* D. García-Yoldi,[1] C. M. Marín,[2] M. J. de Miguel,[2] P. M. Muñoz,[2] J. M. Blasco,[2] I. Jacques,[3,4] M. Grayon,[4] A. Cloeckaert,[4] A. C. Ferreira,[5] R. Cardoso,[5] M. I. Corrêa de Sá,[5] K. Walravens,[6] D. Albert,[7] and B. Garin-Bastuji[7]

[1]obiología y Parasitología, Universidad de Navarra, 31008 Pamplona, Spain[1]; Centro de Investigación y [2]ria de Aragón (CITA), 50080 Zaragoza, Spain[2]; Institut Universitaire de Technologie, Université François Rabelais,

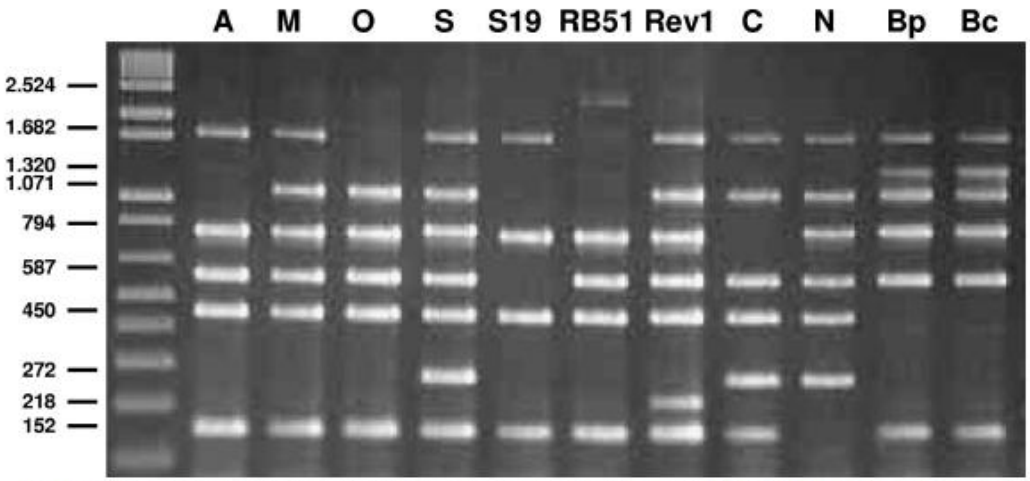


FIG. 1. Differentiation of all *Brucella* species and S19, RB51, and Rev.1 vaccine strains by Bruce-ladder multiplex PCR. Lane 1 (A), *B. abortus*; lane 2 (M), *B melitensis*; lane 3 (O), *B. ovis*; lane 4 (S), *B. suis*; lane 5 (S19), *B. abortus* S19 vaccine strain; lane 6 (RB51), *B. abortus* RB51 vaccine strain; lane 7 (Rev.1), *B. melitensis* Rev.1 vaccine strain; lane 8 (C), *B. canis*; lane 9 (N), *B. neotomae*; lane 10 (Bp), *B. pinnipedialis*; lane 11 (Bc), *B. ceti*.

Mw (bp)	A	M	O	S	S19	RB51	Rev 1	C	N	P	C
2524						■					
1682	■	■		■	■		■	■	■	■	■
1320										■	■
1071		■	■	■			■		■	■	■
794	■	■	■	■	■	■	■		■	■	■
587	■	■	■	■		■	■	■	■	■	■
450	■	■	■	■	■	■	■	■	■		
272				■				■	■		
218							■				
152	■	■	■	■	■	■	■	■		■	■

Multi locus variable number tandem repeats (MLVA)assay

BMC Microbiology

BioMed Central

Research article

Open Access

Evaluation and selection of tandem repeat loci for a *Brucella* MLVA typing assay

Philippe Le Flèche[1,2], Isabelle Jacques[3,4], Maggy Grayon[3], Sascha Al Dahouk[5], Patrick Bouchon[1,2], France Denoeud[2], Karsten Nöckler[6], Heinrich Neubauer[5], Laurence A Guilloteau[3] and Gilles Vergnaud*[1,2]

TABLE 1. Repeat copy numbers at each locus in the MLVA assay for 58 *B. suis* representative isolates

Marker[a]	No. of repeat copies at each locus in the following pattern[b]:						
	S1	S2.1	S2.2	S32.3	S2.4	S3	S4
bruce04	2–7	9	2	2	2–7	7	4
bruce06	2	2	2	2	2	2	2
bruce07	0–8	9	5	0–7	0–10	5	5
bruce08	5	4	7	7	7	3	3
bruce09	0–8	18	3	0–21	5–17	10	9
bruce11	6	8	8	8	8	4	9
bruce12	10	15	15	9	9	11	11
bruce16	0–5	2	2	2	2	4	6
bruce18	0–4	6	4	5–6	5–6	4	5
bruce21	0–9	9	9	9	9	9	9
bruce30	3	4	8	0–8	0–8	5	3
bruce42	4	6	5	5	5	3	3
bruce43	1	1	1	1	1	1	1
bruce45	5	5	5	5	5	5	5
bruce55	3	2	8	0–5	5–6	2	2

[a] MLVA markers are defined as described in references 8 and 12.
[b] Patterns are defined as described in the legend to Fig. 1. See text for details.

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2007, p. 4070–4072 Vol. 45, No. 12
0095-1137/07/$08.00+0 doi:10.1128/JCM.01096-07

Comparison of Multiple-Locus Variable-Number Tandem-Repeat Analysis with Other PCR-Based Methods for Typing *Brucella suis* Isolates[▽]

David García-Yoldi,[1] Philippe Le Fleche,[2,3] María J. De Miguel,[4] Pilar M. Muñoz,[4] José M. Blasco,[4] Zeljko Cvetnic,[5] Clara M. Marín,[4] Gilles Vergnaud,[2,3] and Ignacio López-Goñi[1]*

Departamento de Microbiología y Parasitología, Universidad de Navarra, 31008 Pamplona, Spain[1]; Université Paris-Sud, Institut de Génétique et Microbiologie, Orsay F-91405, and CNRS, Orsay F-91405, France[2]; Centre d'Etudes du Bouchet, 5 rue Lavoisier, 91710 Vert le Petit, France[3]; Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Gobierno de Aragón, 58080 Zaragoza, Spain[4]; and Croatian Veterinary Institute, Department of Immunology, Savska Cesta 143, 10000, Zagreb, Croatia[5]

Received 31 May 2007/Returned for modification 31 July 2007/Accepted 30 September 2007

Perfect correlation of classical typing, omp31 PCR-RFLP, AMOS-ery-PCR and MLVA assay

<u>MLVA was the only assay to highlight epidemiological relationship between strains</u>

OPEN ACCESS Freely available online

PLOS ONE

Biotyping and Genotyping (MLVA16) of *Brucella abortus* Isolated from Cattle in Brazil, 1977 to 2008

Sílvia Minharro[1,2], Juliana P. Silva Mol[1], Elaine M. S. Dorneles[1], Rebeca B. Pauletti[1], Heinrich Neubauer[3], Falk Melzer[3], Fernando P. Poester[1], Maurício G. Dasso[4], Elaine S. Pinheiro[5], Paulo M. Soares Filho[6], Renato L. Santos[7], Marcos B. Heinemann[1], Andrey P. Lage[1]*

1 Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **2** Escola de Medicina Veterinária e Zootecnia, Universidade Federal do Tocantins, Araguaína, Tocantins, Brazil, **3** Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit, Institut für bakterielle Infektionen und Zoonosen, Jena, Germany, **4** Fundação Estadual de Pesquisa Agropecuária, Instituto de Pesquisas Veterinárias Desidério Finamor, Eldorado do Sul, Rio Grande do Sul, Brazil, **5** Instituto Biológico, Centro de Pesquisa e Desenvolvimento de Sanidade Animal, São Paulo, São Paulo, Brazil, **6** Laboratório Nacional Agropecuário, Ministério da Agricultura, Pecuária e Abastecimento, Pedro Leopoldo, Minas Gerais, Brazil, **7** Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Host specificity

Pathogenicity

Minor phenotypic differences

Identification

"**AMOS PCR**"

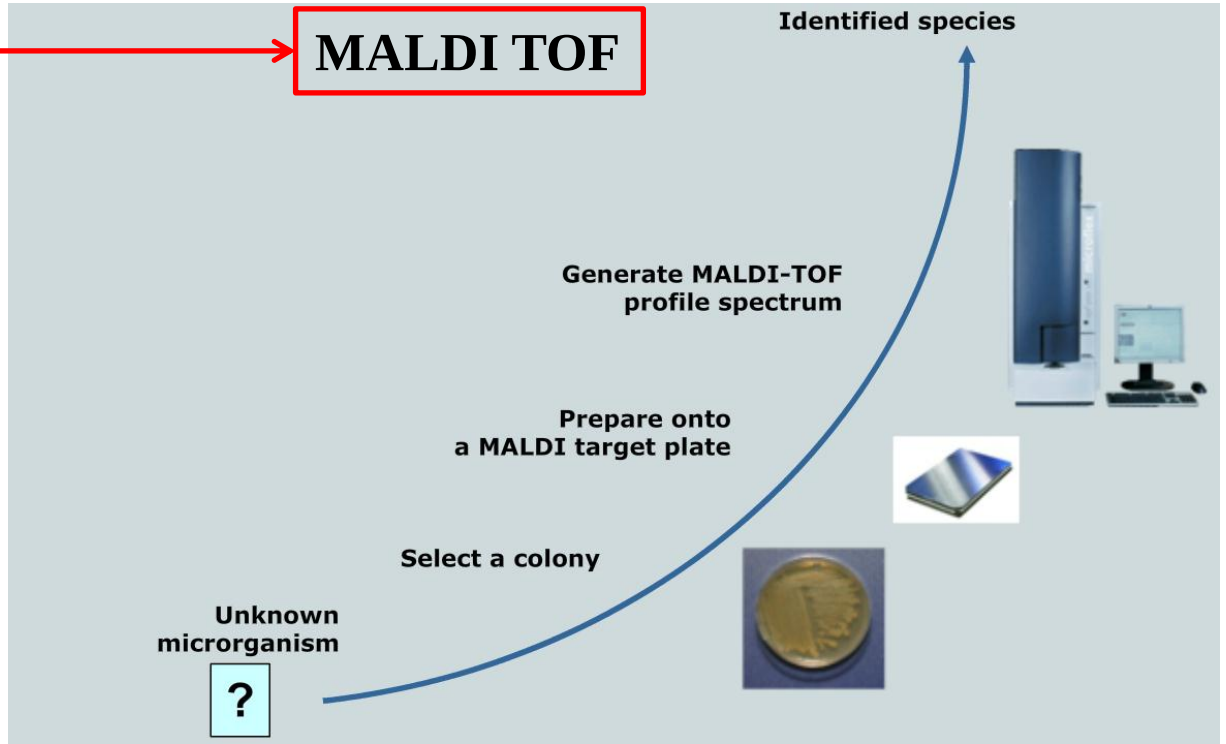
"***Bruce-ladder***"

(Multiplex PCR)

DNA based approaches

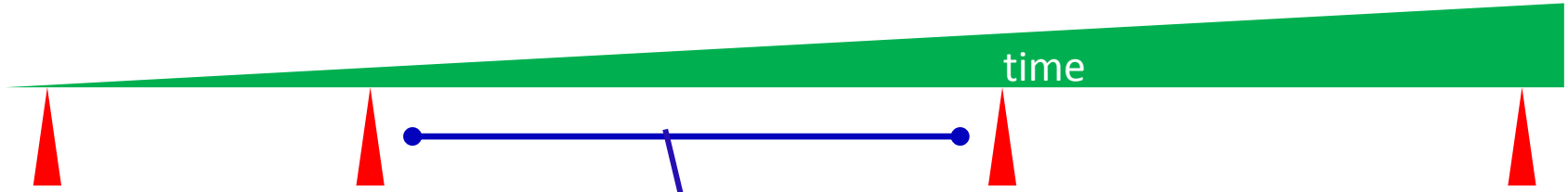
MLVA
(Multi locus variable number tandem repeat analysis

MALDI TOF



5 minutes

Mass spectrometry and bacteria identification



1975: Anhalt and Fenselau combined pyrolysis and MS to characterize some pathogenic bacteria

1980: the develop of soft ionization allows to analyze large molecules

1996: MALDI-TOF bacteria protein spectra can be rapidly and simply obatained from whole cells without a previous treatment

Today: MALDI-TOF used for routine in clinical microbiology laboratories

- Proteome is very dynamic in living cells: the pattern of proteins could change in response to growth conditions
- Doubts on the differences and similarities in mass spectral patterns to establish taxonomy
- Lack of comprehensive databases covering all clinically relevant species
- From the point of view of microbiologists, the identification procedures seemed too simple relative to the complexity of the task
- Identify an organism in a sample in only a fraction of the time required by an expert seemed unreliable
- Most of the early publications involving MALDI-TOF MS-based identification appeared in journals most microbiologists do not access regularly

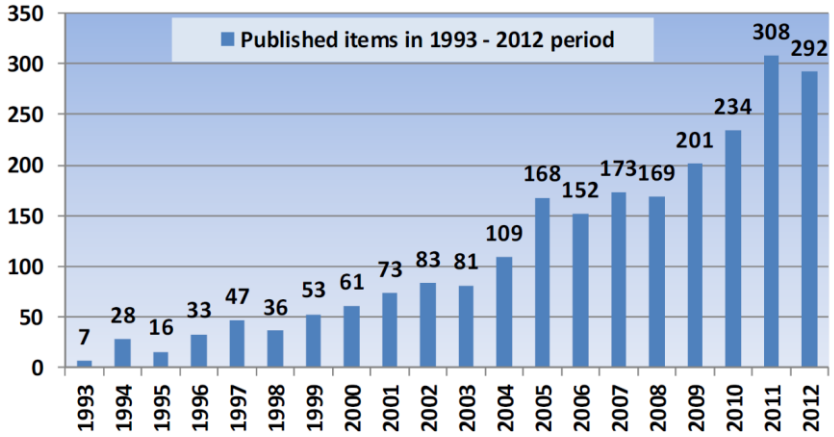


Figure 1: Published item report accessed from Web of Science on Oct 30, 2012 illustrates the number of papers published annually in the field of mass spectral identification of bacteria. In the 1975-1992 time interval, less than 20 papers were published every year.

Analytical chemistry 2012

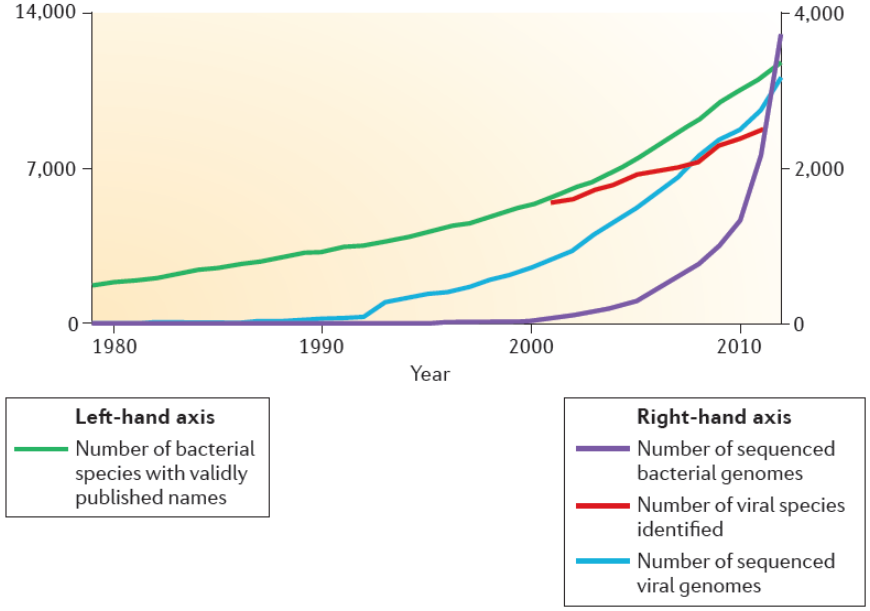
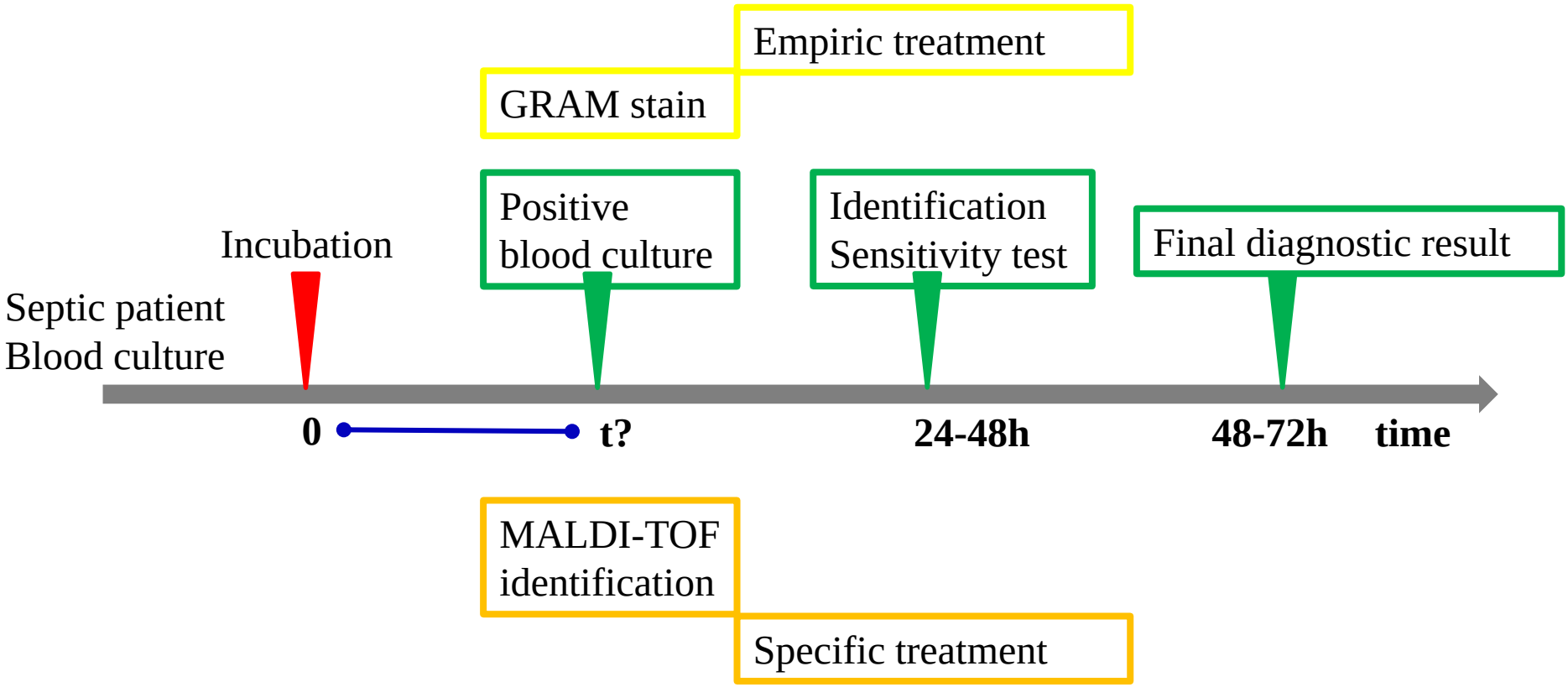


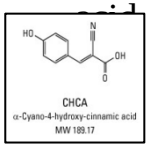
Figure 1 | **The number of identified microbial species from 1979 to 2012.** The development of new technologies has had a substantial impact on the number of microbial species that are identified each year.

Nature reviews microbiology 2013

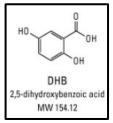
Standard assay vs new MALDI-TOF assay for clinical diagnosis

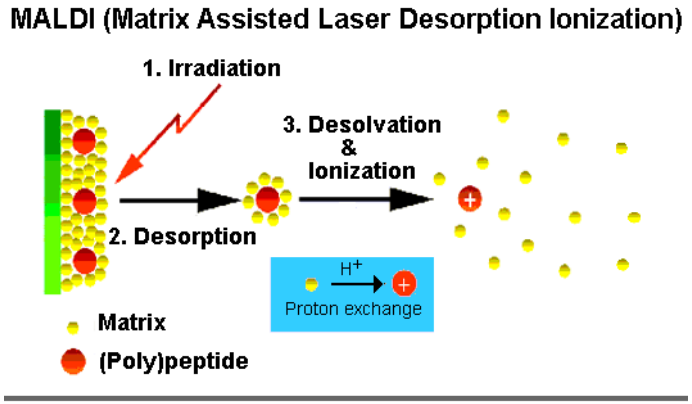


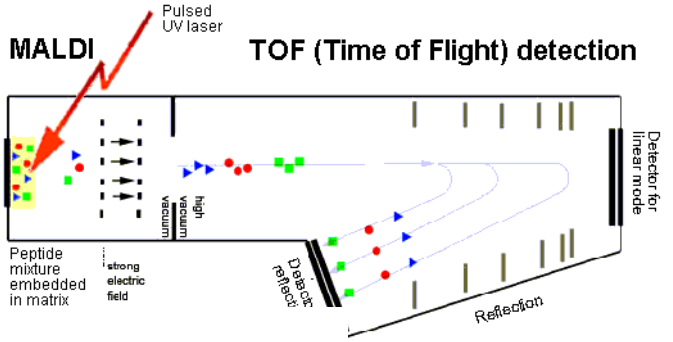
- Alphacyano-4-hydroxycinnamic acid



- 2,5-dihydroxybenzoic acid

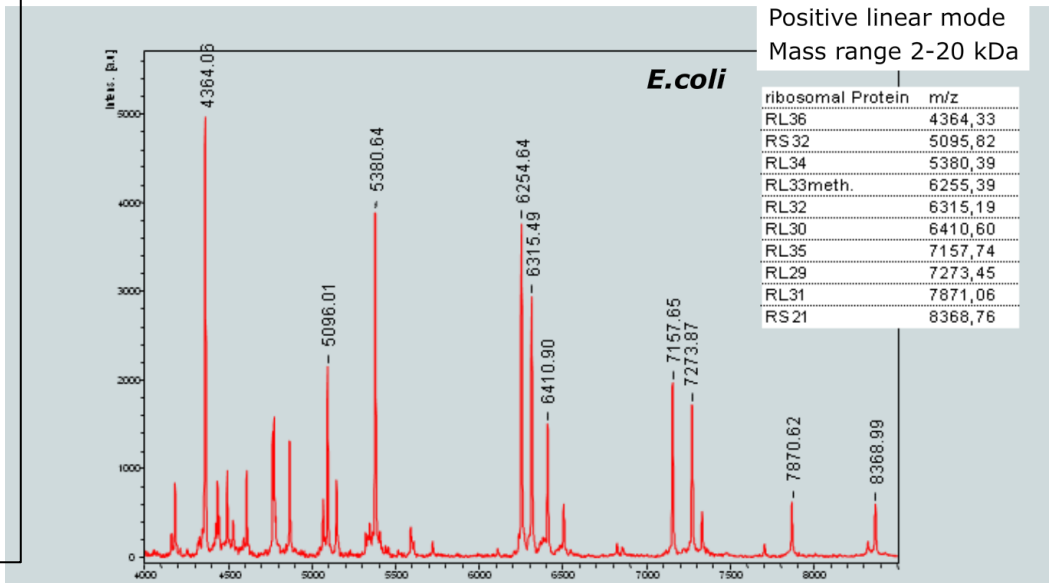






1. Spectral fingerprints vary between microorganisms
2. Among the compounds detected in the spectra, some peaks (molecular masses) are specific to the genus, species, and sometimes subspecies levels.
3. Spectra are reproducible as long as the bacteria are grown under the same conditions

MALDI-TOF MS profile spectrum



Biomarkers

- **Cellular compounds detected:**

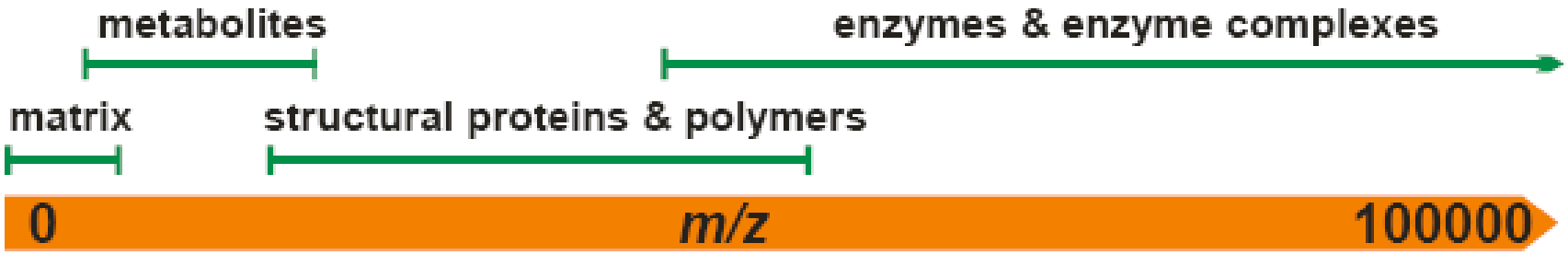
mostly ribosomal proteins or DNA-binding proteins but also complex lipids and polysaccharydes

- **Proteins detected:**

extractable, soluble, moderately **hydrophilic, stable, and abundant**

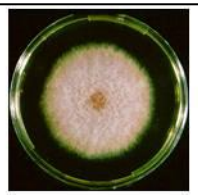
- **Determination of proteins mass signal intensities:**

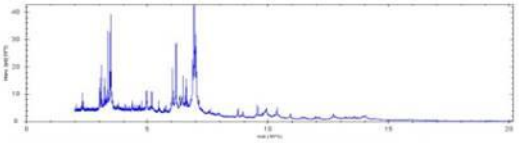
favored by abundance, stability, amino acid composition (esp. Arg and Lys)



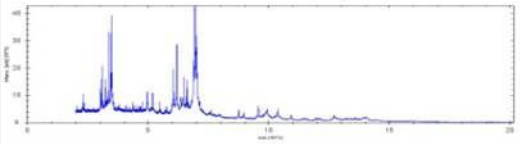
Range of detection MALDI-TOF (2-20kDa)

MALDI-TOF applications



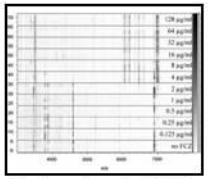


Purification procedure and extraction

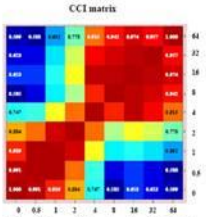


Direct identification

Antifungal resistance detection



Marinach et al. 2010

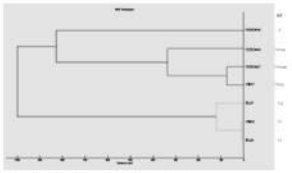


De Carolis et al. 2012

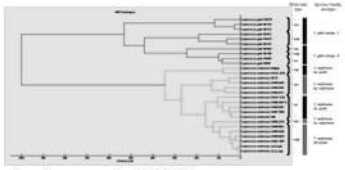
Identification

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(++)	1	*F. solani*	2.136	*F. solani*	2.006
(++)	2	*C. albicans*	2.302	*C. albicans*	2.201

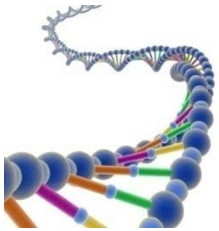
Typing



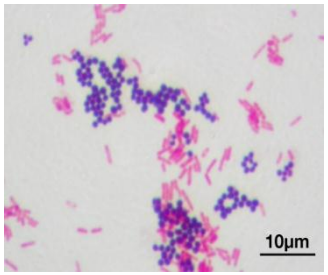
De Carolis et al. 2012

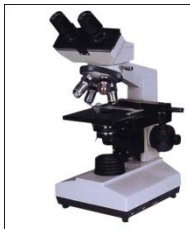


Posteraro et al. 2012



Molecular
(*16s rRna*)





Microscopy

Growth on selective media

It could take 48 h to identification

BACTERIA IDENTIFICATION

Antigen detection

Biochemical
Metabolic assays

MASS SPECTROMETRY

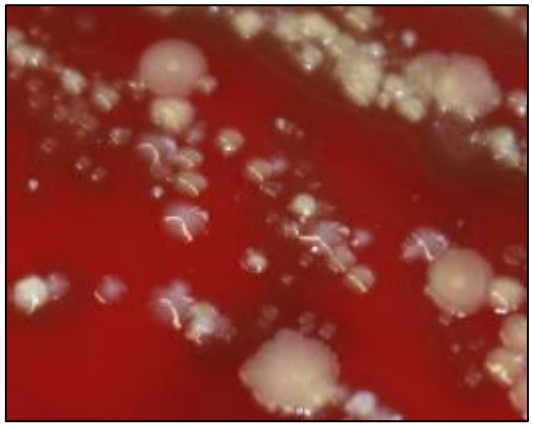
old technology with new applications

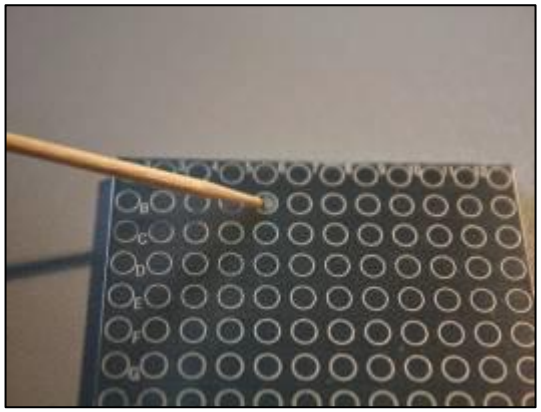
MALDI-TOF sample preparation

Direct method:

- Touch colony with transfer device, such as toothpick
- Transfer a small amount onto spot
- Cover with 1µL of HCCA Matrix, let air dry
- Analyze

Bacteria identification



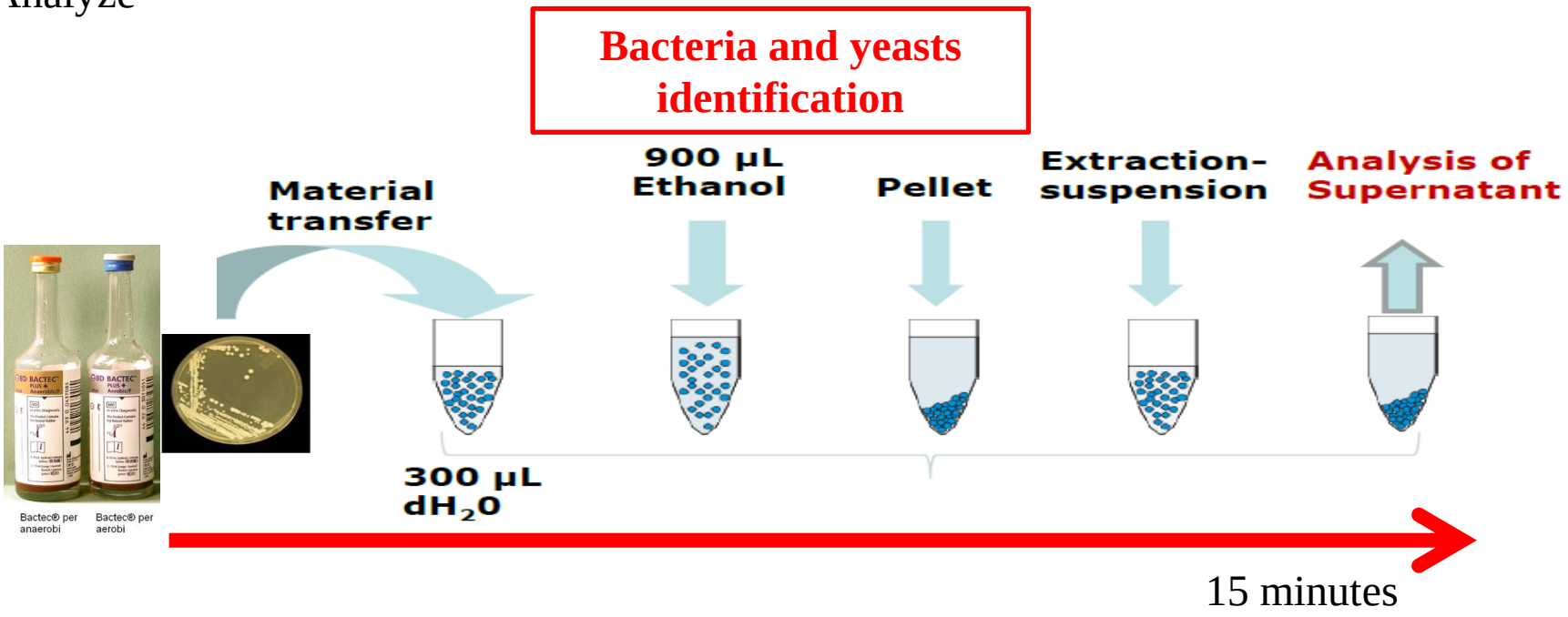


5 minutes

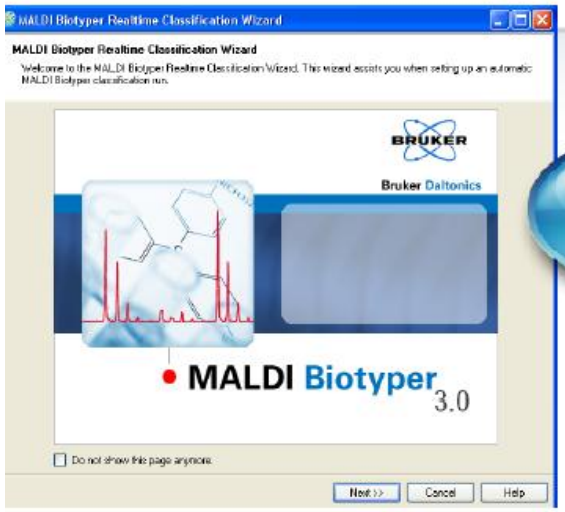
MALDI-TOF sample preparation

Ethanol/Formic Acid Extraction:

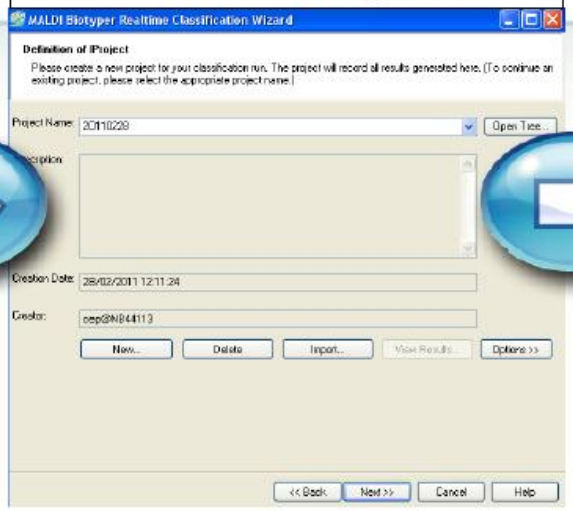
- Pick colony
- Resuspend in 300µl H_2O
- Add 900µl ethanol
- **Inactivation/storage/shipment**
- Add 70% formic acid and ACN
- Centrifuge
- Pipette 1µl onto target
- Cover with 1µL of HCCA Matrix, let air dry
- Analyze



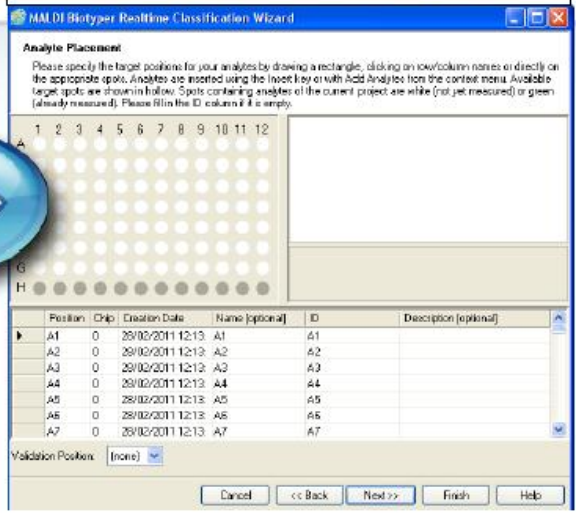
Software MALDI Biotyper



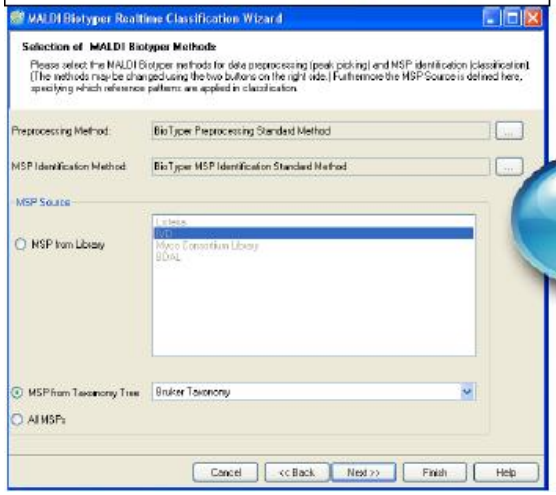
Define Project



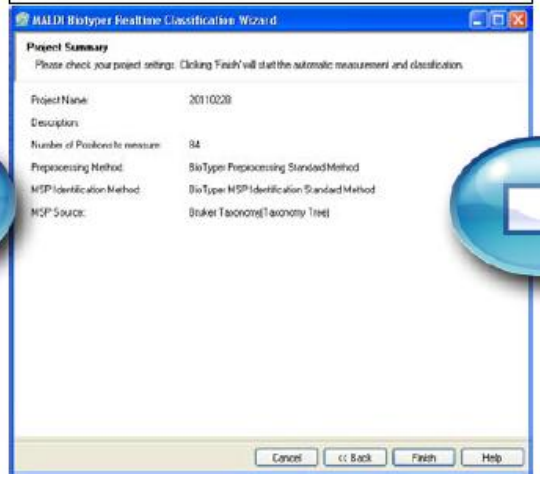
Analyte Placement



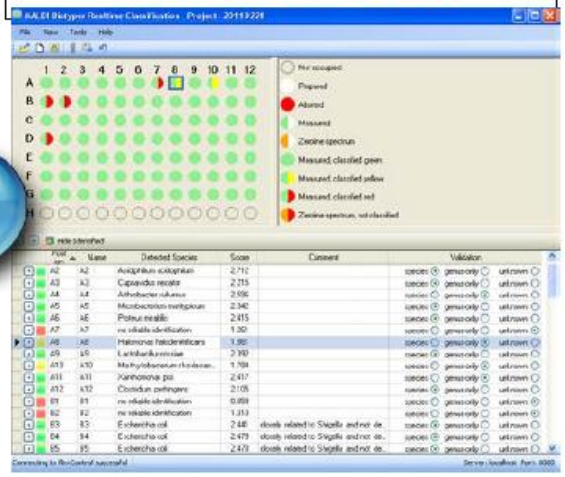
Select Database



Start



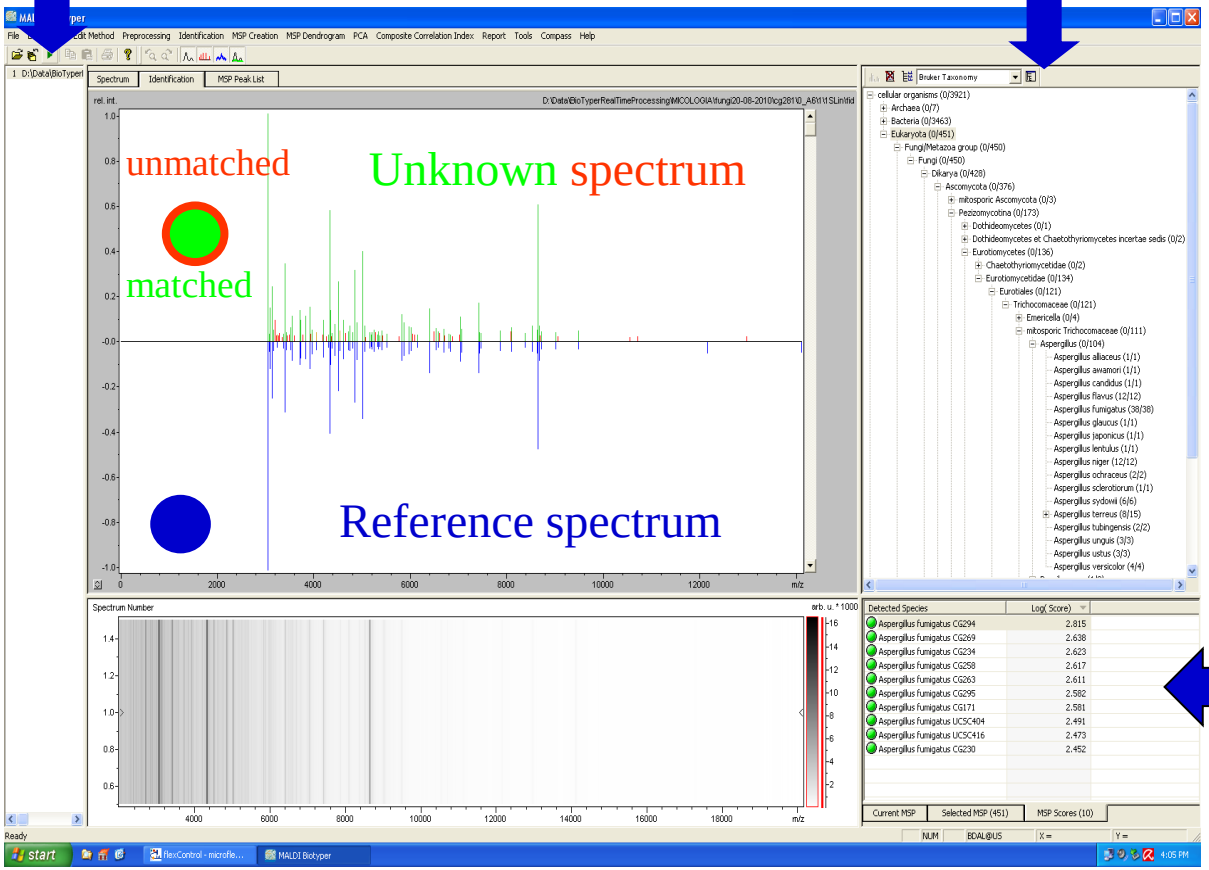
Get the result



Software MALDI Biotyper

Unknown isolate

Match against reference database



Identification result

Calculation of matching score:

Rel. score = % matches of the reference sample

Rel. P-Num = % matches of the unknown spectrum

I-Corr = value of intensity correlation

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	no reliable identification	(-)	red

Analyte Name	Organism (best match)	Score Value	Organism (second best match)	Score Value
Enterococcus faecalis XY_123 BRB (+++) (A)	Enterococcus faecalis	2.348	Enterococcus faecalis	2.198
Enterococcus faecalis XY_123 BRB (+++) (A)	Enterococcus faecalis	2.331	Enterococcus faecalis	2.229
Proteus mirabilis XY_789 BRB (+++) (A)	Proteus mirabilis	2.579	Proteus mirabilis	2.378
Proteus mirabilis XY_789 BRB (+++) (A)	Proteus mirabilis	2.634	Proteus mirabilis	2.394
Pseudomonas aeruginosa MZyme BRB (+++) (A)	Pseudomonas aeruginosa	2.407	Pseudomonas aeruginosa	2.31
Pseudomonas aeruginosa MZyme BRB (+++) (A)	Pseudomonas aeruginosa	2.456	Pseudomonas aeruginosa	2.227
Staphylococcus aureus DSM_19050 BRB (++) (A)	Staphylococcus aureus	2.136	Staphylococcus aureus	2.09
Staphylococcus aureus DSM_19050 BRB (++) (A)	Staphylococcus aureus	2.288	Staphylococcus aureus	2.173

JCM
FAST-TRACK COMMUNICATION

Importance of Using Bruker's Security-Relevant Library for Biotyper Identification of *Burkholderia pseudomallei*, *Brucella* Species, and *Francisella tularensis*

Scott A. Cunningham,[a] Robin Patel[a,b]
Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[a] and Division of Infectious Diseases, Department of Medicine,[b] Mayo Clinic, Rochester, Minnesota, USA

Benefits:
- rapidity
- specificity
- cost
- standardization
- possibility to use *Biotyper* reference or home-made *Brucella* library

OPEN ACCESS Freely available online
PLoS one

Identification of *Brucella* by MALDI-TOF Mass Spectrometry. Fast and Reliable Identification from Agar Plates and Blood Cultures

Laura Ferreira[1], Silvia Vega Castaño[2], Fernando Sánchez-Juanes[1], Sandra González-Cabrero[3], Fabiola Menegotto[3], Antonio Orduña-Domingo[3], José Manuel González-Buitrago[1,4]*, Juan Luis Muñoz-Bellido[2,5]*
Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain, 2 Departamento de Microbiología, Hospital Universitario de Salamanca, Salamanca, Spain, 3 Departamento de
Departamento de Bioqui
Médica, Universidad de Sa

Table 2. Identification by MALDI-TOF mass spectrometry and conventional identification of 131 blind-coded *Brucella*.

Conventional Identification (n° isolates)	MALDI-TOF Identification				
	Correlation at the genus level (%)	Correlation at the species level (%)			
		3/3*	2/3*	1/3*	0/3*
B. abortus (17)	100	82.4	11.8	0	5.9
B. melitensis (112)	100	10.7	8.9	23.2	57.1
B. suis (2)	100	50	0	0	50
Total (131)	100	20.6	9.2	19.8	50.4

Each strain was spotted three times (replicates 1, 2 and 3).
• **No. of replicates.**
doi:10.1371/journal.pone.0014235.t002

.ista et al. BMC Microbiology 2011, 11:267
ıttp://www.biomedcentral.com/1471-2180/11/267

BMC Microbiology

RESEARCH ARTICLE Open Access

Reliable identification at the species level of *Brucella* isolates with MALDI-TOF-MS

Florigio Lista[3], Frans AG Reubsaet[2], Riccardo De Santis[3], Rene R Parchen[1], Ad L de Jong[1], Jasper Kieboom[1], Anton L van der Laaken[1], Ingrid AI Voskamp-Visser[1], Silvia Fillo[3], Hugo-Jan Jansen[4], Jan Van der Plas[4] and Armand Paauw[1*]

- Home-made reference library
- Bruker standard protocol
- 152 strains analyzed
- 98% was correctly identified

JCM

Interlaboratory Comparison of Intact-Cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Results for Identification and Differentiation of *Brucella* spp.

Axel Karger,[a] Falk Melzer,[b] Markus Timke,[c] Barbara Bettin,[a] Herbert Tomaso,[b] Heinrich Neubauer,[b] Sascha Al Dahouk[d,f]

Friedrich-Loeffler-Institut, Institute of Molecular Biology, Greifswald-Insel Ri... Germany[a]; Bruker Daltonik GmbH, Bremen, Germany[c]; Federal Institute for ... Animal Facilities and Biorisk Management, Greifswald-Insel Riems, Germany

- Bruker reference library
- Bruker standard protocol
- 207 strains analyzed
- 92% was correctly identified at species level

TABLE 1 Summary of the classification results obtained with MALDI Biotyper and CPT software

Sample	MALDI Biotyper query result			
	No. correct/total	No. incorrect/total[a]	Misdiagnosis[b]	CPT classification
B. abortus	41/44	3/44	*B. melitensis*	NA[c]
B. canis	21/21			
B. ceti	5/9	3/9	*B. pinnipedialis*	*B. ceti* vs *B. pinnipedialis*
		1/9	*B. canis*	*B. canis* vs *B. ceti*
B. melitensis	52/53	1/53	*B. abortus*	NA
B. microti	12/12			
B. ovis	10/10			
B. pinnipedialis	8/8			
B. suis biovar 1	9/10	1/10	*B. canis*	*B. canis* vs *B. suis* biovar 1
B. suis biovar 2	29/29			
B. suis biovar 3 or 4	2/6	4/6	*B. canis*	*B. canis* vs *B. suis* biovar 3/4
B. suis biovar 5	3/5	1/5	*B. abortus*	*B. suis* biovar 5 vs *B. abortus*/*melitensis*
		1/5	*B. melitensis*	*B. suis* biovar 5 vs *B. abortus*/*melitensis*

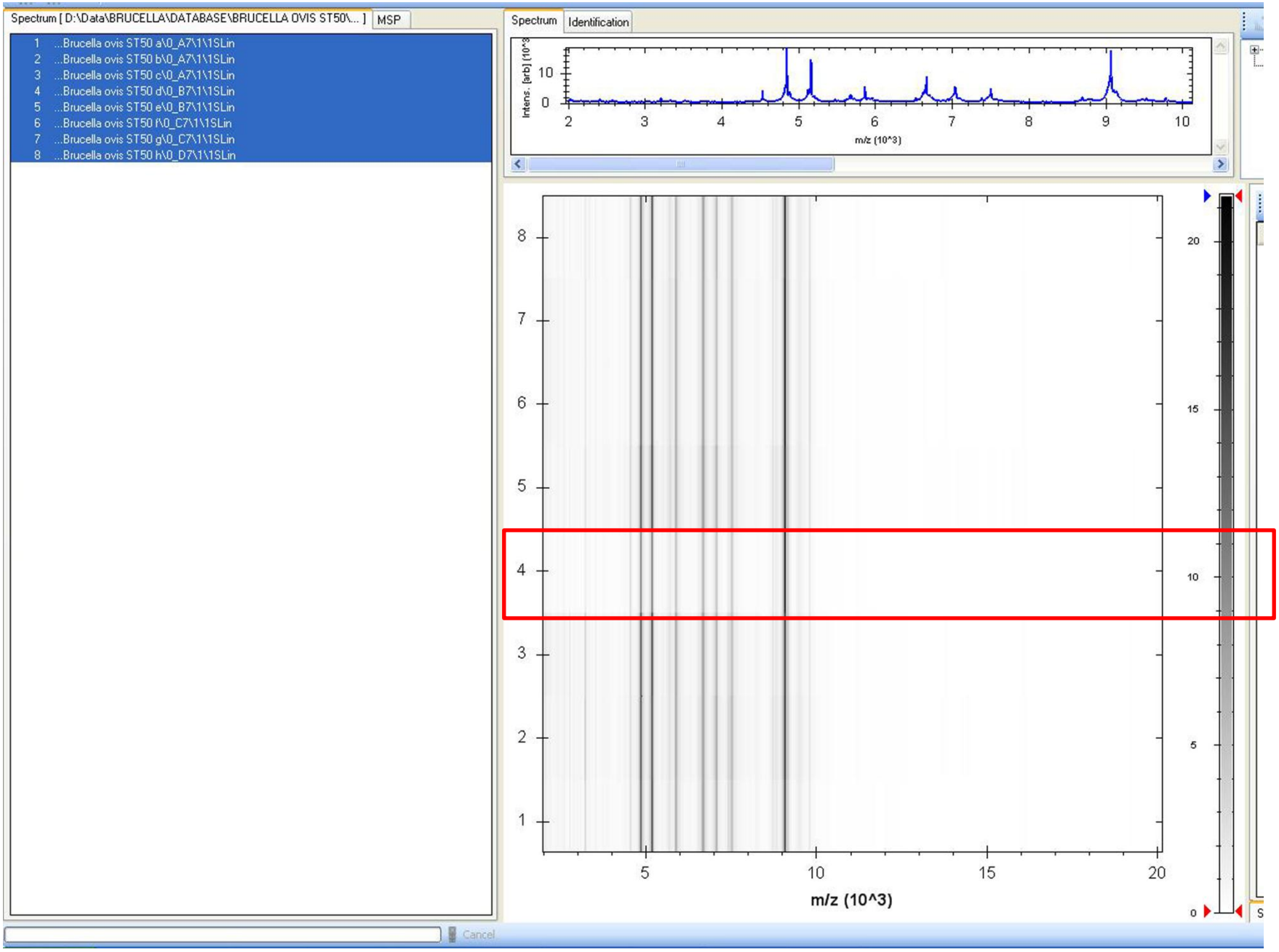
[a] For *Brucella* species and for the biovars of *B. suis* of which not all representatives were correctly identified by MALDI Biotyper software, statistical models were developed that allowed unambiguous identification. For the parameters of the models (CPT classification column), see Table S2 in the supplemental material.
[b] The misdiagnosis column contains the top hits of the MALDI Biotyper query in cases of incorrect species identification.
[c] NA indicates that no models could be deduced with CPT software that performed better than MALDI Biotyper.

Unknown strains

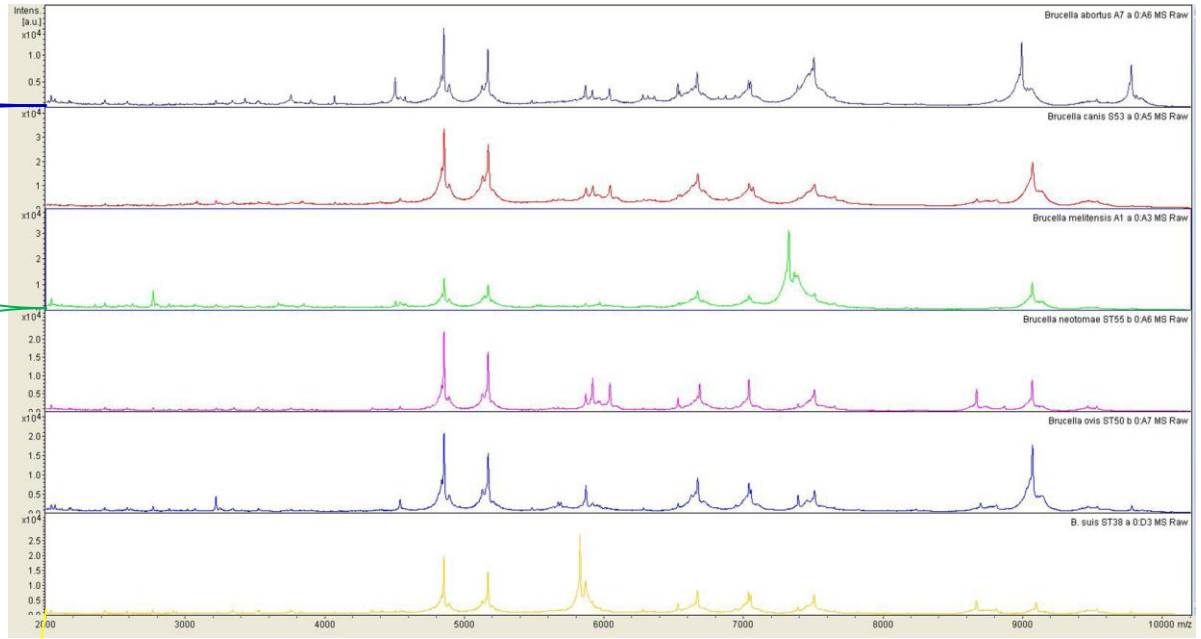
=

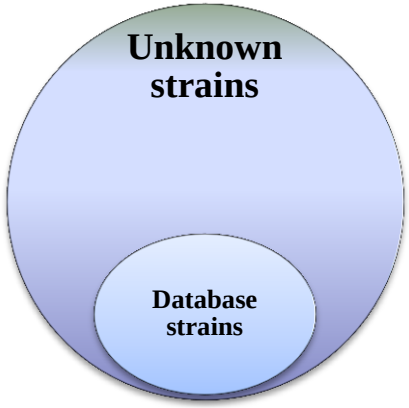
Database strains

Database creation



Database strains	Biovar
B.abortus RB51/ST14	
B.abortus/A7	
B.abortus/ST19	**1**
B.abortus/ST20	
B.abortus/ST21	
B.abortus/A5	**6**
B.abortus/ST29	
B.abortus/ST31	**7**
B.abortus/ST33	**9**
B.abortus/ST35	
B.canis/ST53	
B.melitensis/ST1	
B.melitensis/ST4	**1**
B.melitensis/ST7	
B.melitensis/ST10	**2**
B.melitensis /A1	
B.melitensis /A2	
B.melitensis /A4	**3**
B.melitensis/PU8	
B.melitensis/PU9	
B.melitensis/PU11	
B.neotomae/ST55	
B.ovis/ST50	
B.suis/ST38	**1**
B.suis/ST46	**3**
B.suis/ST49	**5**





At the species level, a total of 93% of bacterial samples were correctly identified…

	MALDI Biotyper results		
Sample	**No. Correct/ total**	**No. Incorrect/ total**	**Misdiagnosis (No)**
B. melitensis	53/53		
B. abortus	36/39	3/39	*B. suis* (3)
B. suis	4/5	1/5	*B. melitensis*
B. ovis	1/1		
B. canis	0/1	1/1	*B. abortus*
B. neotomae	0/1	1/1	*B. abortus*

Incorrect biovar assignments were found in:

- ***B. abortus* 23/39**
- ***B. melitensis* 4/53**

- Automatic identification
- Analogue identification in at least two well
- View the highest score

Phenotyping

- Multiple susceptibility tests
- Phagetyping
- Serotyping
- Biochemical typing

MALDI-typing

Without additional step
Cheap
Easy to perform
Reproducibility

Lack of guidelines

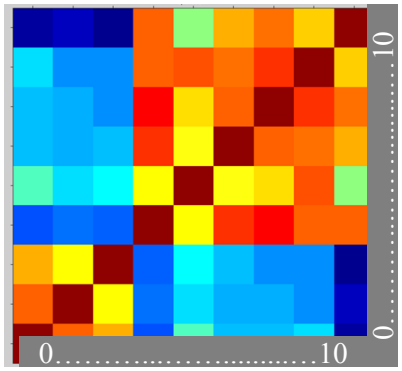
Genotyping

- Pulsed field gel electrophoresis (PFGE)
- Whole genome sequencing

Used to understand the epidemiology of a small community or healthcare – associated infections

Developed for individual bacteria taxa and not transferable to other taxa without considerable adaptation

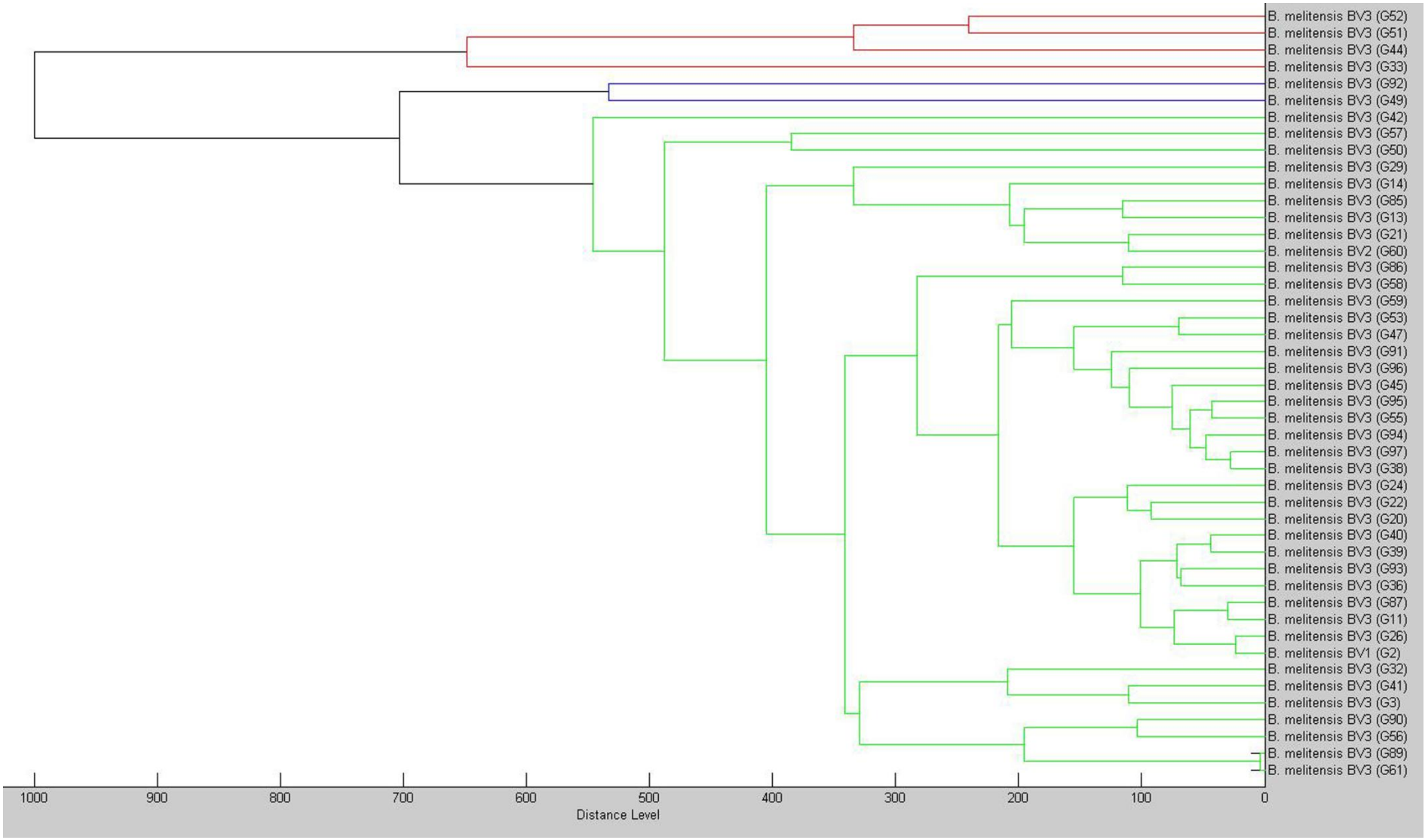
Correlation index



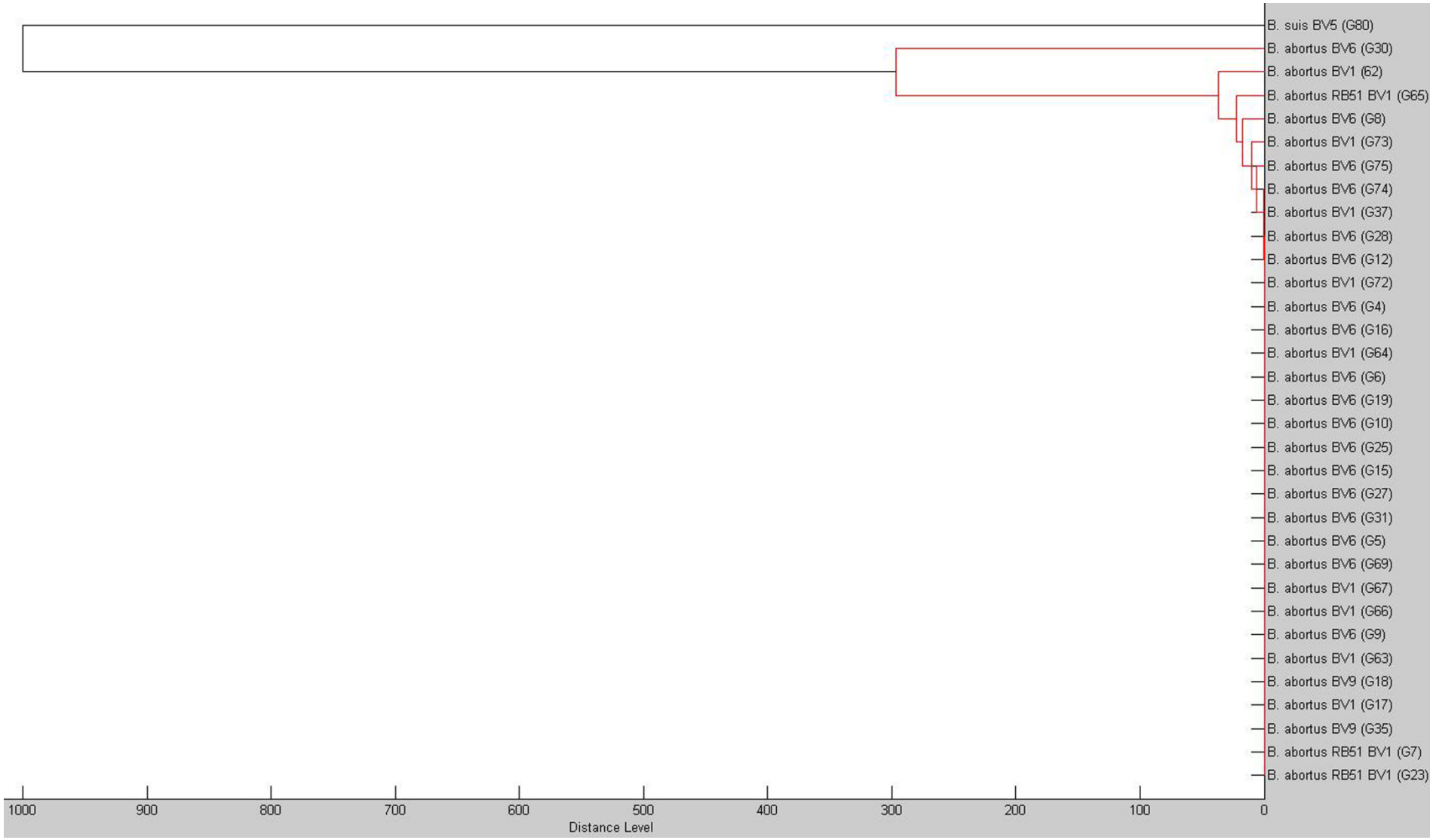
Possibility to look for epidemiological relationships between strains in a larger population range

More complex approach

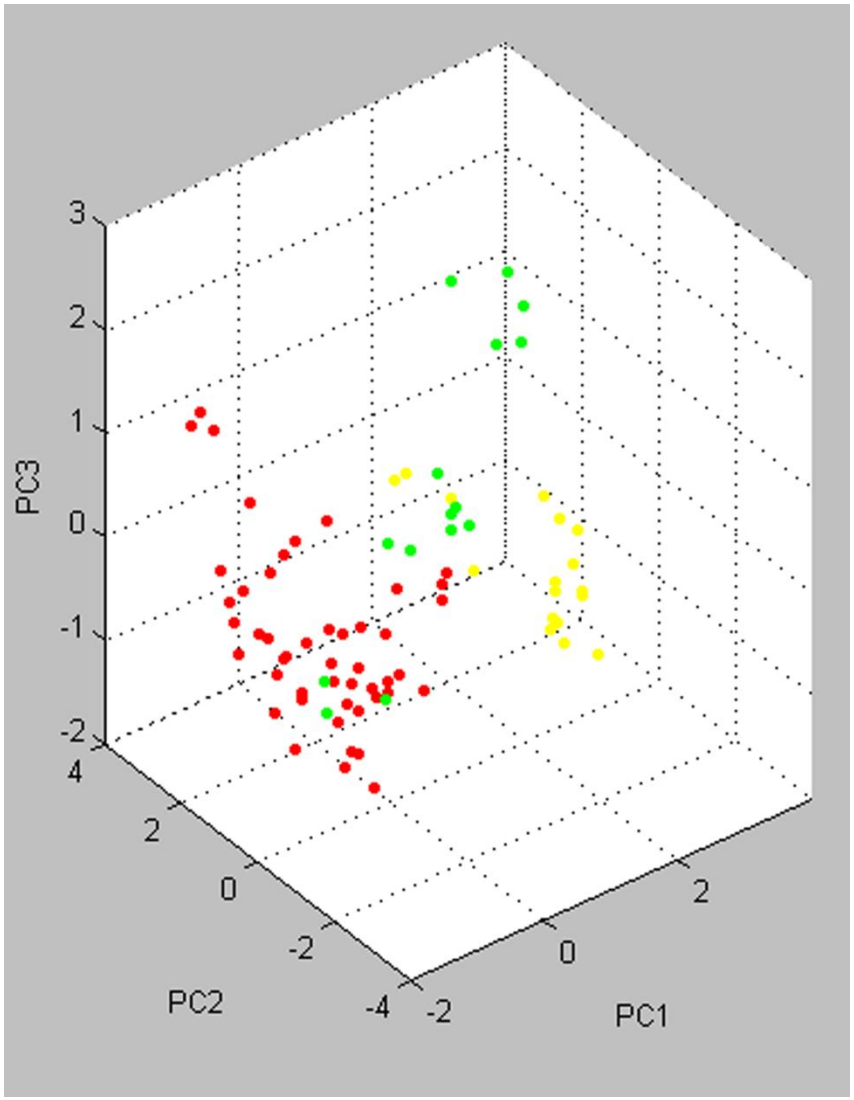
Brucella melitensis dendrogram

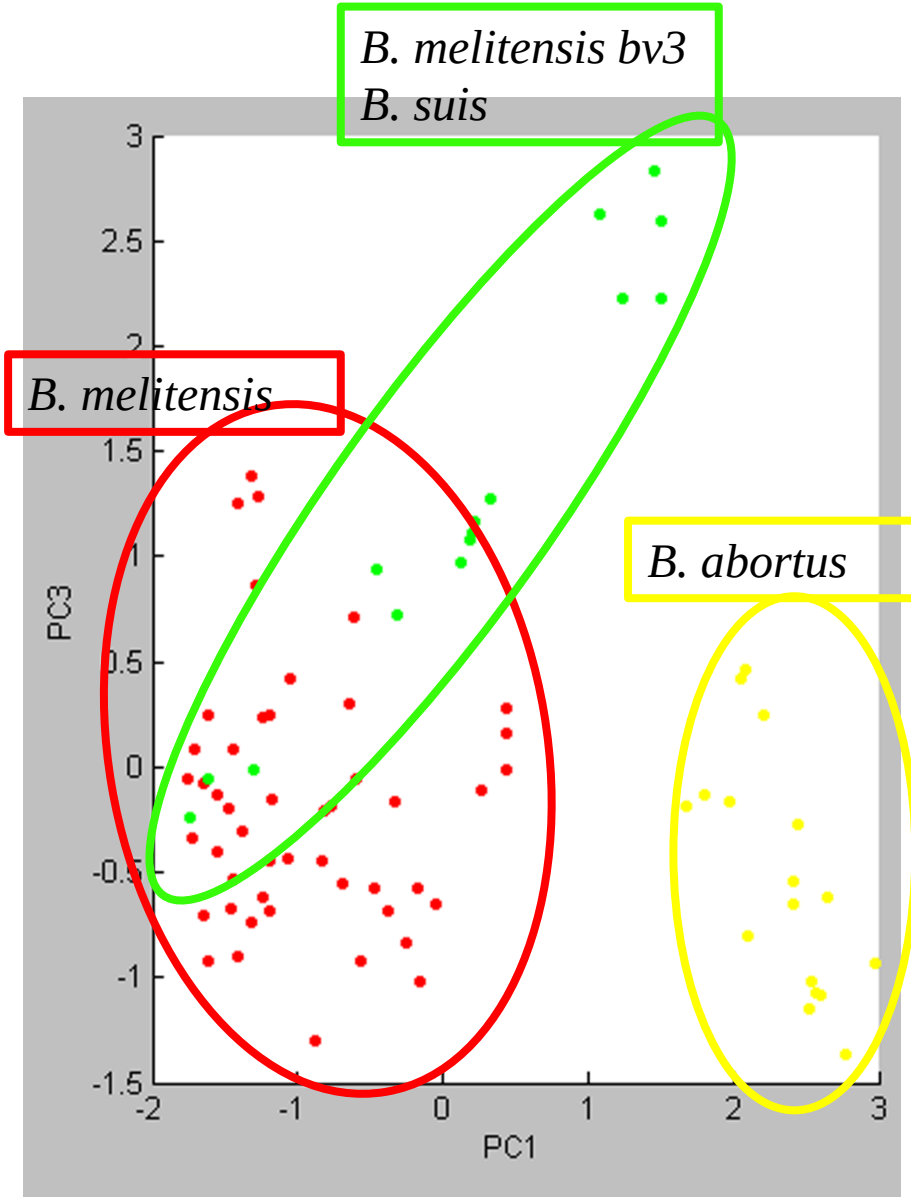


Brucella abortus dendrogram



Brucella MALDI-Typing





Conclusions

✓ Growth conditions show a good standardization of sample treatment

✓ Our protein extraction method is faster and safer than “old” methods, with inactivated sample after cell lysis

✓Possibility to analyze inactivated samples shipped from other centers

✓ New home-made database highlights an excellent resolution as species identification

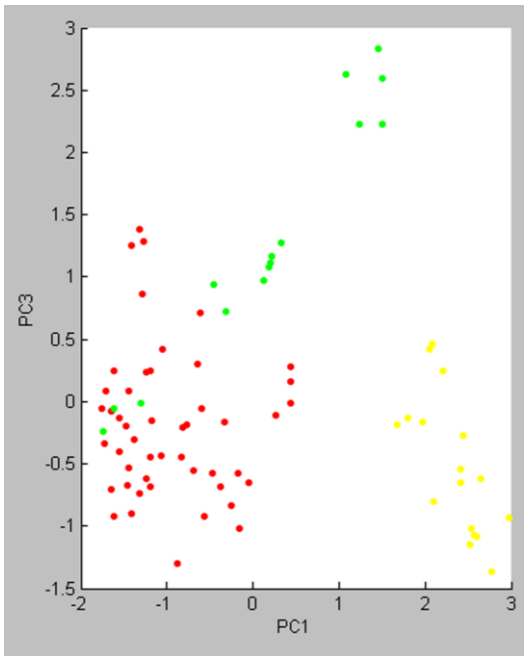
For the next future…

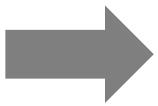
✓ Include negative controls in the database (*Rhizobium*, *Ochrobactrum*)

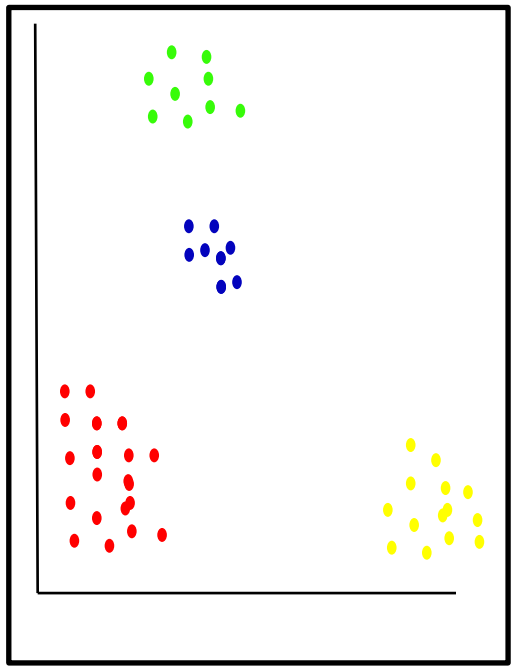
✓ Increase the number of *Brucella* strains in the database

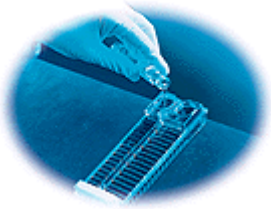
✓ Increase the number of *Brucella* species with different biovar

✓ Assess the clustering of different strains using MALDI-TOF assay with gold standard MLVA analysis and more recent whole genome sequencing to better understand the relationship between circulating strains and infected hosts









Commercial methods

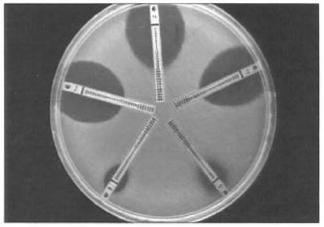


Figure 1-Photograph of a 15 centimeter long Mueller-Hinton plate with five E tests strips (ciprofloxacin, ceftazidime, piperacillin, tircacillin/clavulanic acid and trimethroprim/sulfamethoxazole. The microrganism being tested was Xanthoma maltophilia.

E-Test Kirby-Bauer

BACTERIA
SUSCEPTIBILITY DETERMINATION

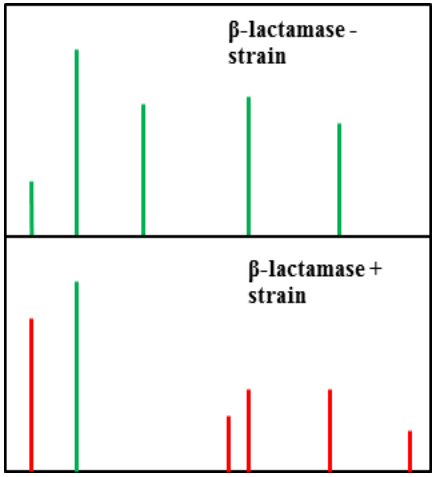
MASS SPECTROMETRY

New generation of susceptibility tests

New generation of susceptibility test…

Enterobacteriaceae

Resuspension in antibiotic solution

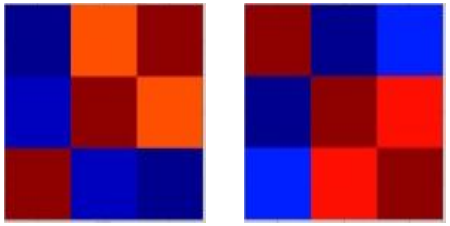


β-lactamic ring hydrolysis evaluation

MALDI-TOF spectra acquisition

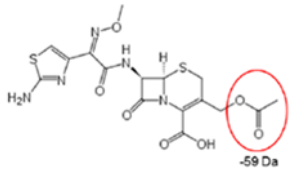
Candida

Resuspension in antifungal solution



Composite correlation index (CCI) evaluation

Cefotaxime hydrolysis



Negative β-lactamase = S

Hydrolyzed form

Not hydrolyzed form

Positive β-lactamase = R

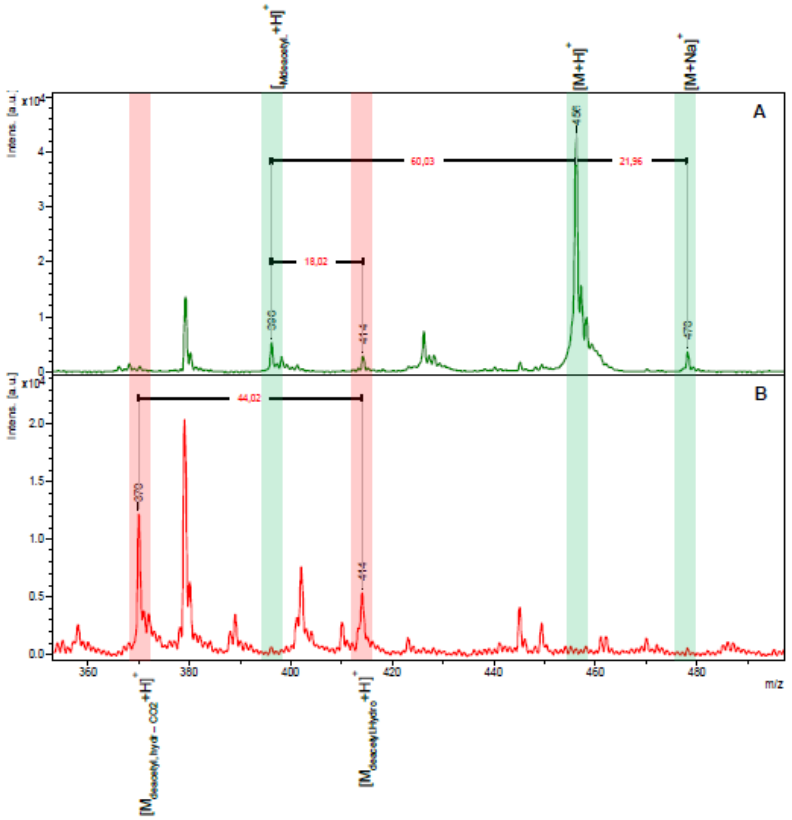


Fig. 5: Hydrolysis of Cefotaxime by a ß-lactamase positive strain (B). DH5a, as negative control (A), shows only slight background hydrolysis. Green-highlighted peaks correspond to the non-hydrolyzed form and red-highlighted peaks correspond to the hydrolyzed form.

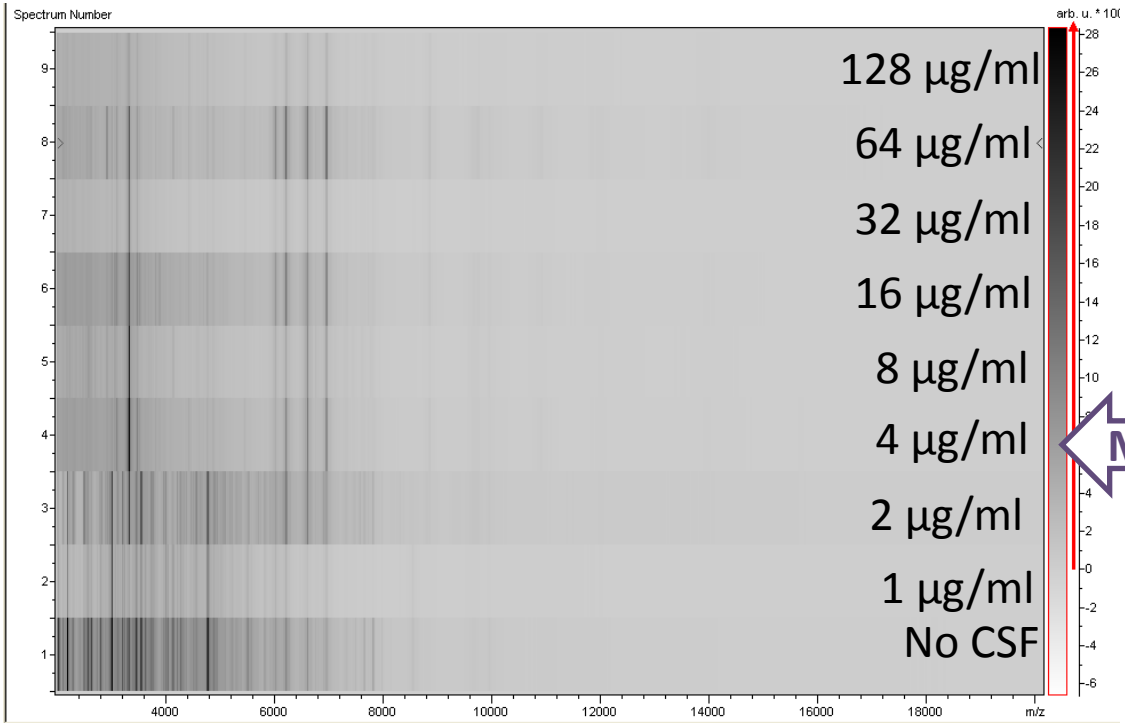
	MW	Sensitivity pattern								Resistance pattern								
	[g/mol]	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$	$[M+2\,Na]^+$	$[M+Na+K]^+$	$[M+3\,Na]^+$	$[M-X^{\cdot}+H]^+$	$[M-X^{\cdot}+Na]^+$	$[M_{hydr.}+H]^+$	$[M_{hydr.}+Na]^+$	$[M_{hydr.}+2\,Na]^+$	$[M_{hydr.}+Na+K]^+$	$[M_{hydr.decarb.}+H]^+$	$[M_{hydr.}-X^{\cdot}+H]^+$	$[M_{hydr.decarb.}-X^{\cdot}+H]^+$	$[M_{hydr.decarb.}+Na]^+$	$[M_{hydr.decarb.}+K]^+$
Ampicillin	349.4	350.4	372.4		394.4					368.4	390.4	412.4		324.4				
Piperacillin	517.5	518.5	540.5		562.5			398.5	420.5	536.5	558.5	580.5		(492.5)				
Cefotaxime	455.5	456.5	478.5					396.5							414.5	370.5		
Ceftazidime	546.6	547.6						468.6							486.6	442.6		
Ertapenem	475.5	476.5	498.5	514.5	520.5	536.5	542.5			494.5	516.5	538.5	554.5	450.5			472.5	488.5
Imipenem	299.4	300.4																
Meropenem	383.4	384.5	406.5		428.5													

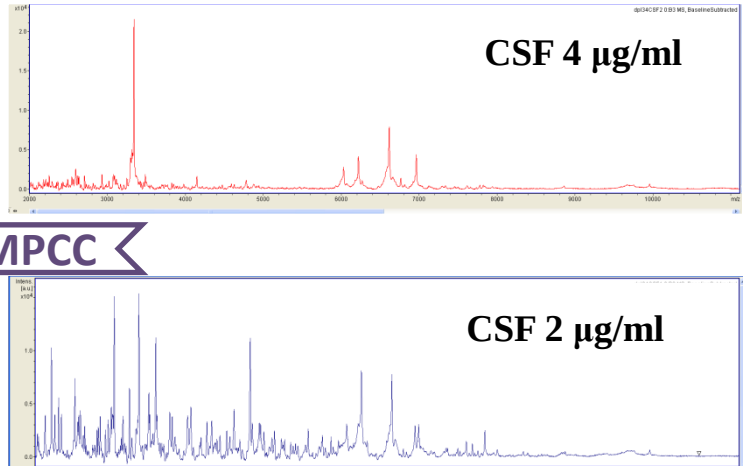
JCM
Journals.ASM.org

Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Caspofungin Susceptibility Testing of *Candida* and *Aspergillus* Species

Elena De Carolis,[a] Antonietta Vella,[a] Ada R. Florio,[a] Patrizia Posteraro,[b] David S. Perlin,[c] Maurizio Sanguinetti,[a] and Brunella Posteraro[d]

Institute of Microbiology[a] and Institute of Hygiene,[d] Università Cattolica del Sacro Cuore, Rome, Italy; Clinical Laboratory, Ospedale San Carlo, Rome, Italy[b]; and Public Health Research Institute, New Jersey Medical School, UMDNJ, Newark, New Jersey, USA[c]





C. albicans cells exposed to CSF scalar dilutions

MALDI-TOF in microbiological diagnostic

- Discrimination of highly related species
- Reduced time of identification compared to conventional methods *(Vlek AL et al. 2012)*
- Reduced time (11.9 vs 9.3 days) and costs of hospitalization ($ 45,709 vs $ 26,126) *(Perez KK et al. 2013)*
- Reduction of mortality: 20.3 % vs 14.5 % *(Huang AM et al . 2013)*

- Exhaustive and shared database
- Typing intra – species
- Revelation bacterial and fungal toxins
- Rapid and automated detection of drug resistance

Characterization of culture supernatant proteins from *Brucella abortus* and its protection effects against murine brucellosis

Jin Ju Lee[a,b,1], Jeong Ju Lim[b,1], Dae Geun Kim[b], Hannah Leah Simborio[b], Dong Hyeok Kim[b], Alisha Wehdnesday Bernardo Reyes[b], WonGi Min[b], Hu Jang Lee[b], Dong Hee Kim[c], Hong Hee Chang[d], Suk Kim[b,d,*]

[a] Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do 430-757, Republic of Korea
[b] College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Republic of Korea
[c] School of Medicine, Gyeongsang National University, Jinju 660-701, Republic of Korea
[d] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Republic of Korea

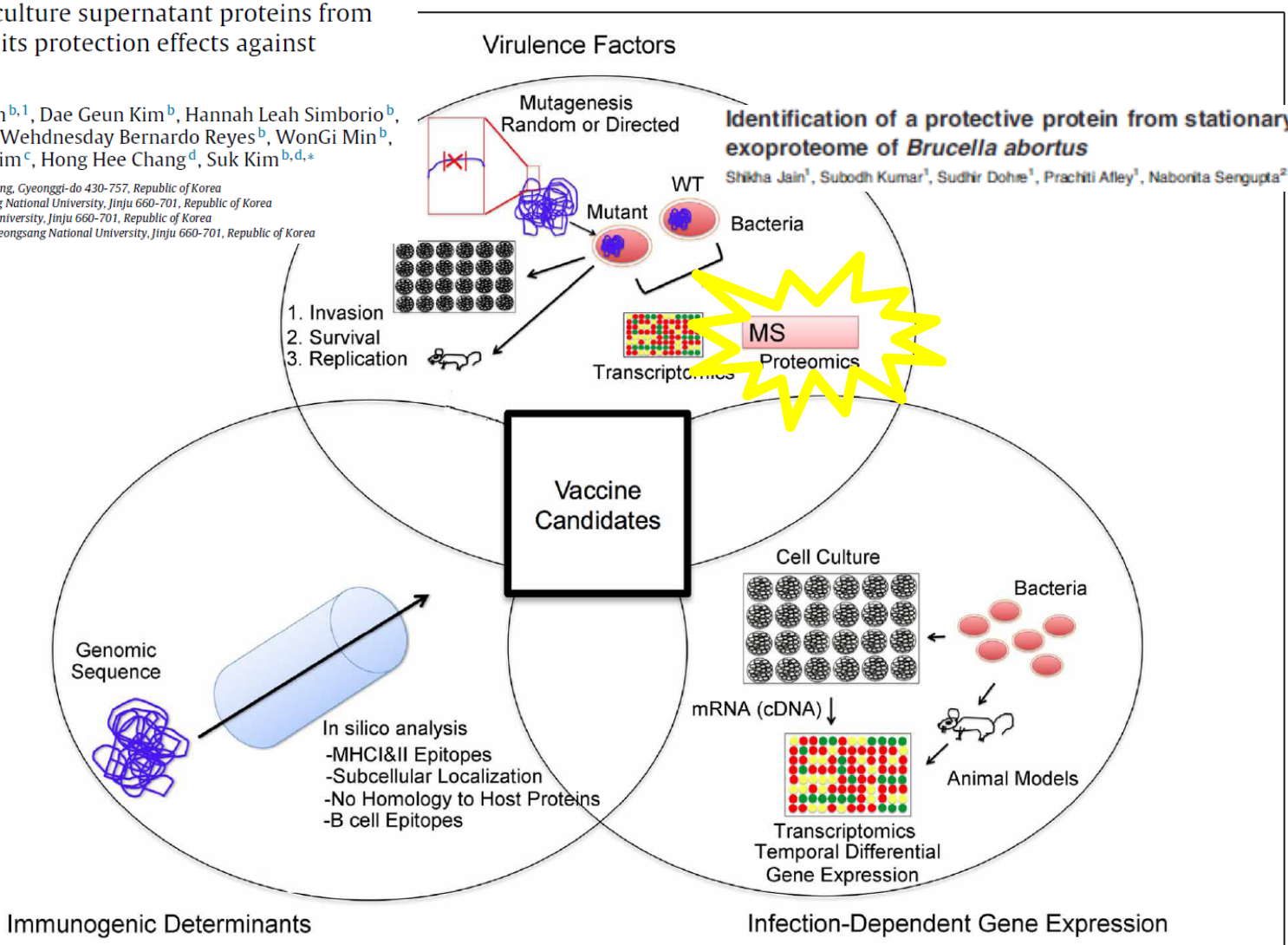


FIGURE 2 | Vaccine candidate selection approach. An important aspect of a vaccine candidate is antigenicity. *In silico* analysis of available genomic sequences can aid in the selection of open reading frames that code for desired properties such as T and B cell epitopes, subcellular localization (i.e., outer membrane proteins), and a lack of homology to host proteins. Secondly, antigens with evidence for a role in pathogenesis are often targeted in the identification of vaccine candidates. Identification of factors important for invasion, survival, and replication can be performed via mutagenesis studies in the mouse or cell culture systems. Additionally, comparative transcriptomic and proteomic studies of wild type and mutant pathogen strains can be carried out to identify potential virulence factors. Lastly, the priming of an immune response to a specific antigen relies on its availability. In order to identify antigenic targets present during infection, infection-dependent gene expression studies may reveal suitable targets.

Gabriel Gomez et al, 2013

Dr. Michela Sali
Dr. Elena De Carolis
Prof. Giovanni Delogu
Prof. Maurizio Sanguinetti

Università Cattolica Sacro Cuore - Roma

Dr. Rosanna Adone
Dr. Paolo Pasquali

Istituto Superiore di Sanità - Roma

Dr. Garofolo Giuliano

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"- Teramo