



MALDI Biotyper

Changing Microbiology

David Burian

MBT Application Specialist

Bruker s.r.o.



Bruker Corporation Today



★ Headquarters

● Worldwide Offices and Distributors



Bruker Corporation Overview

Technology Platforms

Bruker AXS

- X-ray Analysis
 - X-ray Diffraction
 - X-ray Crystallography
 - X-ray Fluorescence
 - EDS Microanalysis
 - Spark OES

Bruker Daltonics

- Mass Spectrometry
 - MALDI-TOF(/TOF)
 - Ion Trap MSⁿ
 - ESI-(Qq)-TOF, FTMS
 - IMS

Bruker Optics

- Vibrational Spectroscopy
 - FT-IR
 - FT-NIR
 - Raman

Bruker BioSpin

- NMR and EPR spectroscopy
 - NMR / TD-NMR
 - EPR
 - MRI
 - Analytical Services

Major Applications

- Materials Identification
- Materials Research
- Structural Proteomics
- Nanotechnology

- Small Molecules Analysis
- Proteomics
- Food quality & safety
- Clinical Microbiology
- Homeland Security/Defense

- PAT & Quality Control
- Food
- Materials Identification
- Materials Research
- Pharma 'Forensics'
- Analytical Chemistry
- Pharmaceuticals
- Life Science
- Food & beverages
- Metabolomics

GLOBAL FOOD TESTING. COM

discover. Collaborate. Succeed.



- A website for current events in food testing
- Community/user driven
- A platform for local testing labs to collaborate and share
- Sponsored by Bruker, operated by the community



REGISTER FOR COMPLETE SOLUTIONS

Food Testing Community Success Stories Forums Featured Lab Event



Event
47th Florida Pesticide Residue Workshop
TradeWinds Island Grand Resort
St Pete Beach, Florida
July 18-21, 2010



Featured Lab
ABC Research Corp
3437 S/W 24th Ave
Gainesville, FL 32607

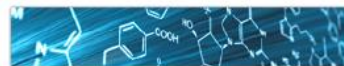


Featured Success Stories
Case studies from food testing needs to solutions



Featured Method
The optimal measurement and validation solutions

Forums



Pesticide Measurement

Current News



Your Link to Food Testing News

Food Testing Community



Resource Hub

SHARE YOUR SUCCESS STORIES

successstories@globalfoodtesting.com

Bruker History



- 1960** —● Bruker was founded by Prof. Laukien.
- 1985** —● Establishment of the mass spectrometry business in Bremen, Germany
- 1991** —● Introduction of the first MALDI-TOF mass spectrometer for routine purposes
- 1998** —● The microbiological R&D department starts operation
- 2004** —● Launch MALDI Biotyper 1.0 as research tool
- 2006** —● Start collaboration with the German Collection of Micro-organisms and Cell Cultures
- 2008** —● First MALDI Biotyper systems at microbiological diagnostic labs and industry sites.
- 2011** —● >500 MALDI Biotypers installed at microbiological diagnostic labs and industry sites
- 2012** —● Bruker completes Patent Portfolio for AST / ESBL testing with MALDI-TOF
- 2013** —● >1000 MALDI Biotyper installed at microbiological diagnostic labs and industry sites

Identifying Micro-organisms for Food Safety



- Consumer products (e.g. food, water) are checked for microbial contaminations => to prevent healthcare problems.
- **Enumeration (colony counting)** => Important to determine contamination level (vs set threshold)
- **Identity Confirmation** of micro-organisms is important to determine:
 - Risk level
 - Contamination source
 - Decontamination treatment
 - Comparison of contaminations
- **Quick identification helps to:**
 - Stop production / Close the source earlier=> Prevent further product contamination.
 - Accurate/earlier decontamination treatment. => Quickly pick-up production again.
 - Earlier recall of products => lower exposure.

Wouldn't it be great, ...



- **if you had a technology that can analyze gram+ and gram- bacteria without prior knowledge?**
- **if this technology could also analyze yeasts, fungi and mycobacteria?**
- **if this method would be a molecular approach and not a phenotypical one?**
- **if this method would give the ID in less than a minute?**
- **if this method had a much better taxonomical resolution than biochemical testing?**
- **if this would be available at a very acceptable price per sample?**



MALDI biotyper

- Microorganism Identification and Classification

MALDI Biotyper workflow:

?

- **Taxonomical Resolution comparable to sequencing**
- **Faster than conventional techniques**
- **Cost-effective**

Identification

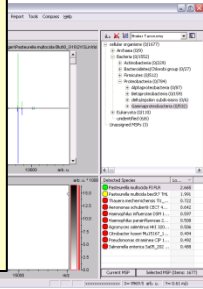


Unknown
Microorganism

Preparation onto
MALDI target plate

MALDI-TOF MS

Data interpretation

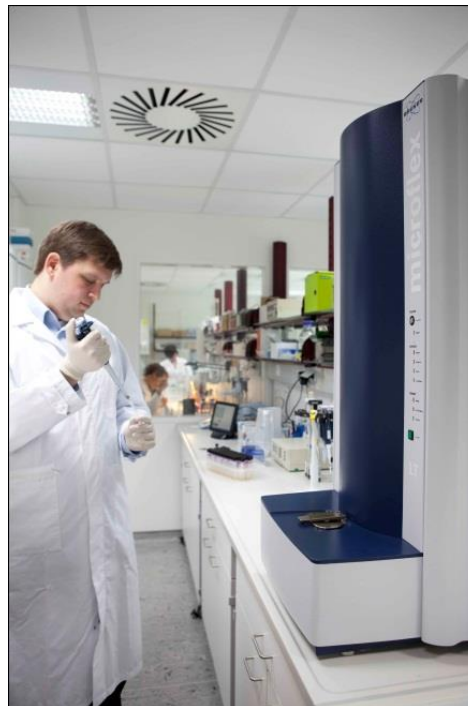
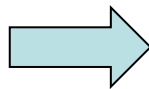
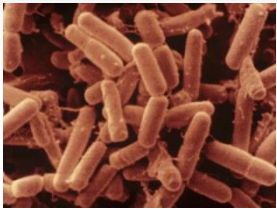


time to result for one sample: ~ 10 min

MALDI Biotyper



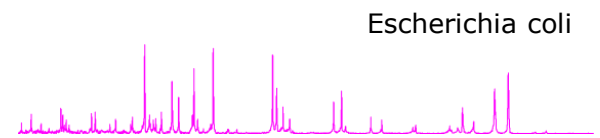
Identify gram+, gram- bacteria, yeasts and fungi by their unique proteomic fingerprint



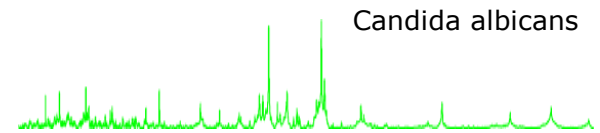
1 min



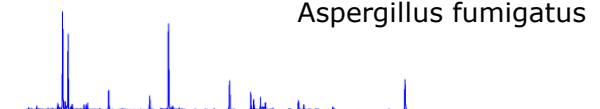
Bacillus subtilis



Escherichia coli

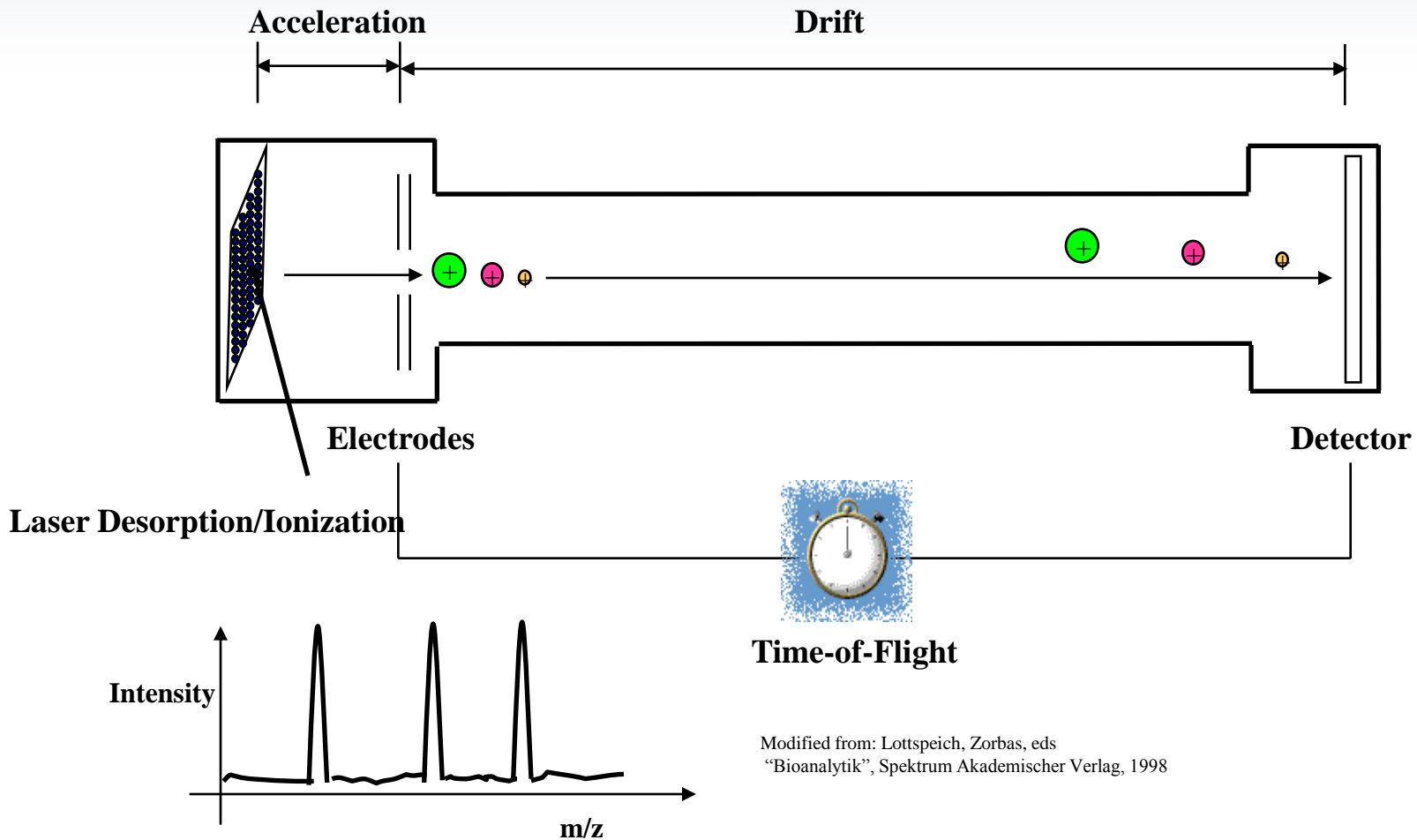


Candida albicans



Aspergillus fumigatus

MALDI-TOF Mass Spectrometry



Modified from: Lottspeich, Zorbas, eds
"Bioanalytik", Spektrum Akademischer Verlag, 1998

Unknown Organism Specifications...

- Classifications of organisms that can be analyzed: Bacteria, yeast, molds, mycobacteria
- Cultivation Media: proved that different media types do not affect results.
- Cultivation Temperatures: demonstrated that different air temperatures, composition, and humidity do not affect results.
Age of Organism: Although fresh cultures are always better, age of the organism doesn't create misidentifications.

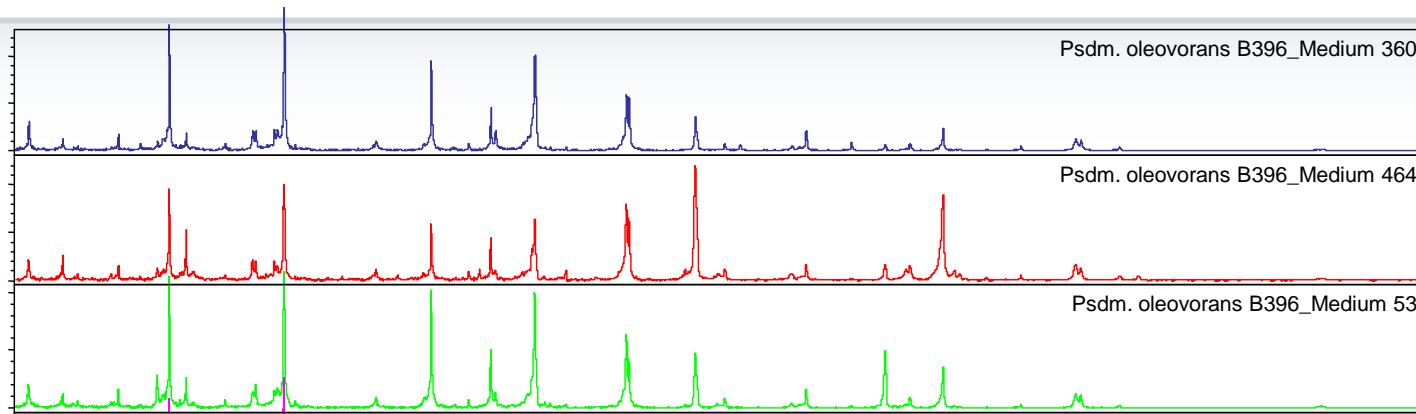


MALDI Biotyper - Basics

Low influence of cultivation conditions



Pseudomonas oleovorans grown on different media



Species ID is not dependent upon the age of culture, growth conditions, or medium selection (e.g.):

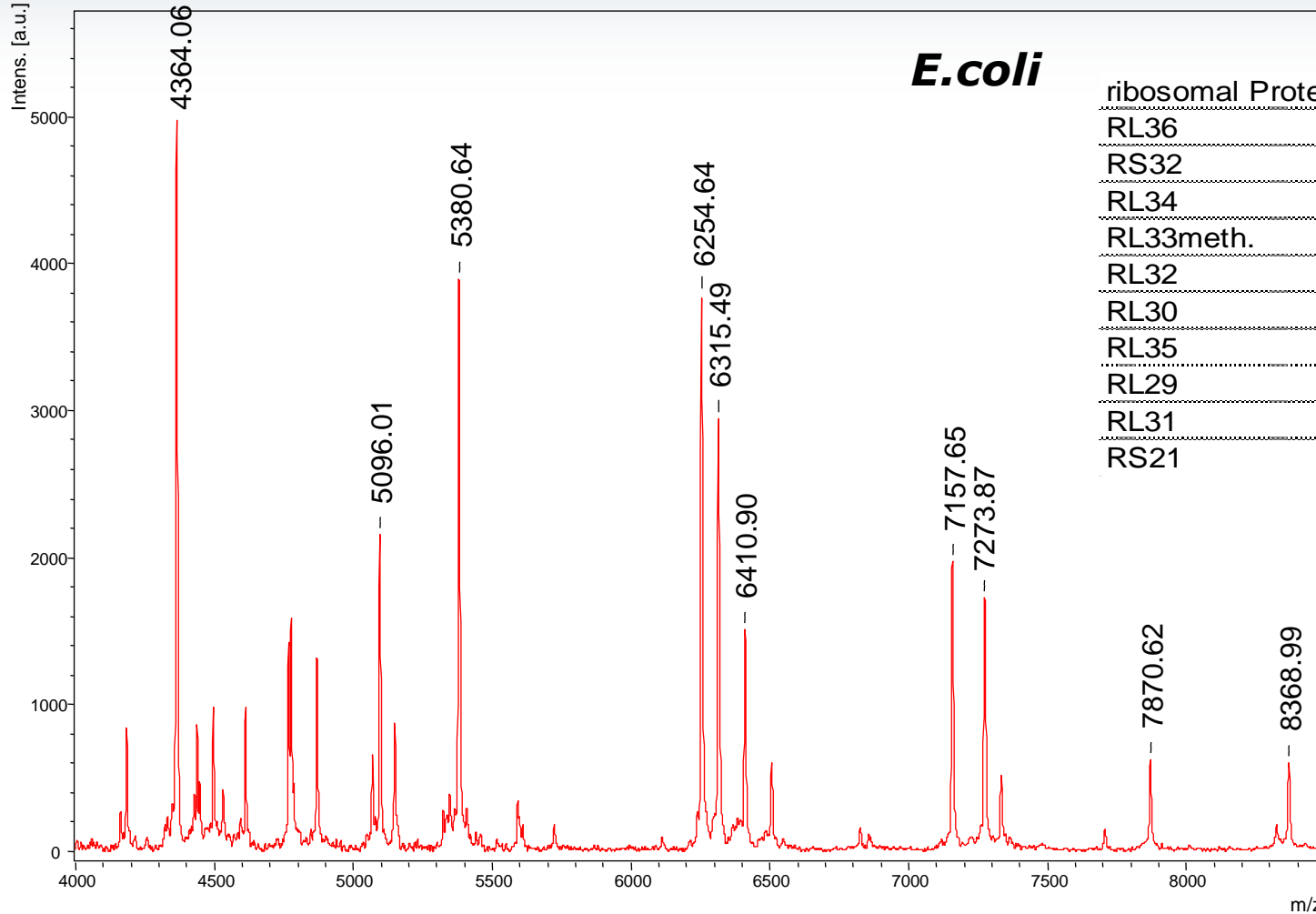
Mellmann et al. *JCM* **2008**

Veloo et al. *Clinical Microbiology and Infection* **2011**

Lartigue et al. *JCM* **2009**

Valentine et al. *Appl. Environ. Microbiol.* **2005**

Each microbial species has a unique proteomic fingerprint.

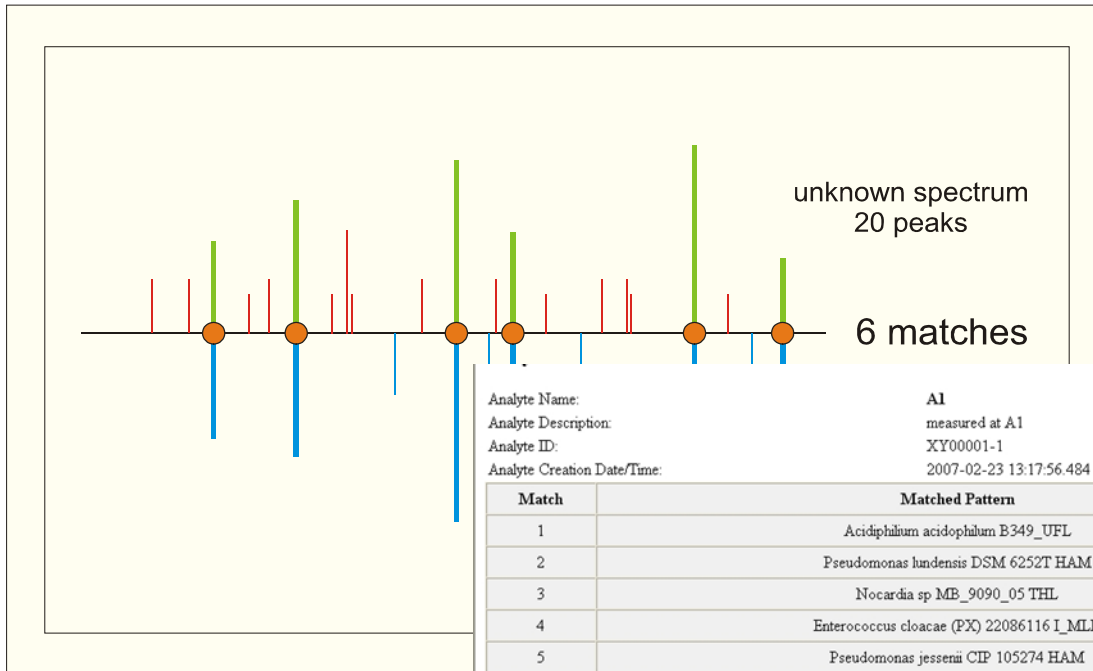


| ribosomal Protein | m/z |
|-------------------|---------|
| RL36 | 4364,33 |
| RS32 | 5095,82 |
| RL34 | 5380,39 |
| RL33meth. | 6255,39 |
| RL32 | 6315,19 |
| RL30 | 6410,60 |
| RL35 | 7157,74 |
| RL29 | 7273,45 |
| RL31 | 7871,06 |
| RS21 | 8368,76 |

MALDI Biotyper – score based pattern matching



Unknown microorganism is matched against a library of more than 2300 microbial species



Analyte Name: A1
Analyte Description: measured at A1
Analyte ID: XY00001-1
Analyte Creation Date/Time: 2007-02-23 13:17:56.484

| Match | Matched Pattern | Score Value | NCBI Identifier |
|-------|---|-------------|-----------------------|
| 1 | <i>Acidiphilium acidophilum</i> B349_UFL | 2.527 | 76588 |
| 2 | <i>Pseudomonas lundensis</i> DSM 6252T HAM | 0.626 | 86185 |
| 3 | <i>Nocardia</i> sp MB_9090_05 THL | 0.408 | 1817 |
| 4 | <i>Enterococcus cloacae</i> (PX) 22086116 I_MLD | 0.407 | 550 |
| 5 | <i>Pseudomonas jessenii</i> CIP 105274 HAM | 0.383 | 77298 |
| 6 | <i>Escherichia coli</i> ATCC 25922 THL | 0.369 | 562 |
| 7 | <i>Klebsiella pneumoniae</i> ssp pneumoniae 9295_1 CHB | 0.358 | 72407 |
| 8 | <i>Pseudomonas abietaniphila</i> CIP 106708 HAM | 0.31 | 89065 |
| 9 | <i>Pseudomonas savastanoi</i> ssp savastanoi LMG 5011 HAM | 0.306 | 29438 |
| 10 | <i>Pseudomonas rhodesiae</i> DSM 14020T HAM | 0.251 | 76760 |

Color-coded identification result.

MALDI Biotyper - Multicenter Studies Evaluation Performance Study



Weighted Laboratory Profile Tests:

| Microorganism group | MBT ID Acc. (BC ID Acc.) |
|---------------------------------------|-----------------------------|
| Non-Fermenting Gram Negative Bacteria | 94 % (86%) |
| Enterobacteriaceae | 99 % (97%) |
| Other Gram Negative Bacteria | 96 % (91%) |
| Gram Positive Bacteria | 97 % (92%) |
| Yeasts | 97 % (97%) |
| Sum / Overall for ID Acc. | 97% (93%) |

- 0,61% Identification only on Genus Level (Biochemical: 0,52%)
- 1,91% No Identification (Biochemical: 2,34%)
- 0,69% False-Positive Identification (Biochemical: 4,42%)



MALDI biotyper

- Microorganism Identification and Classification

The MALDI Biotyper



A complete and dedicated solution for fast and accurate identification of microorganisms.

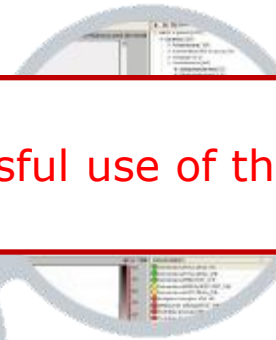
SOP

- Bacteria, Yeast and Fungi
- Solid and liquid media



Library

- Ready-to-use
- Extendable



Regulatory considerations

- Security and traceability
- Manufacturing QC

One-stop-shop solution: to assure successful use of the application

Hardware

- Benchtop
- Self Cleaning Ion Source

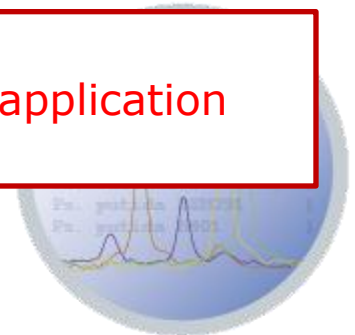


Support

- Application
- Service
- Training

Software

- Automated data processing
- Traceability



MALDI Biotyper - Pr One Reference



Pseudozyma aphidis

**MBT
Coverage**

Aureimonas altamirensis

Myroides odoratimimus

Clinical Relevant

MBT library comprises all you need & all what you (maybe) don't need!

Wohlfahrtimonas chitiniclastica

No. of species →

Robustness



- MALDI-TOF needs to reliably handle between 10-1600 samples per day.
- Created a dedicated MALDI-TOF for microbiology, to provide robustness and reliability for routine analysis.
- **The Microflex.**
 - Long-life time laser
 - Saturation free detector
 - Oil free vacuum system
 - Self cleaning ion source
- Reliable daily use of MALDI-TOF.



MALDI Biotyper – Software



MALDI Biotyper Realtime Classification Project: 2009-11-30

File View Tools Help

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H

Interactive MALDI Validation

| ID | Name | Position | Detected Species | Score | Validation |
|----|------|----------|------------------------------|-------|---|
| 01 | A1 | A1 | Escherichia coli | 2,455 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 02 | A2 | A2 | Acidiphilium acidophilum | 2,693 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 03 | A3 | A3 | Cupriavidus necator | 2,298 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 04 | A4 | A4 | Arthrobacter sulfureus | 2,582 | species <input type="radio"/> genus-only <input checked="" type="radio"/> unknown <input type="radio"/> |
| 05 | A5 | A5 | Microbacterium maritopicum | 2,292 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 06 | A6 | A6 | Proteus mirabilis | 2,422 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 07 | A7 | A7 | not reliable identification | 1,286 | species <input type="radio"/> genus-only <input type="radio"/> unknown <input checked="" type="radio"/> |
| 08 | A8 | A8 | Halomonas halodenitrificans | 1,921 | species <input type="radio"/> genus-only <input checked="" type="radio"/> unknown <input type="radio"/> |
| 09 | A9 | A9 | Lactobacillus rossiae | 2,301 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 10 | A10 | A10 | Methylobacterium rhodesianum | 2,509 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |
| 11 | A11 | A11 | Xanthomonas pisi | 2,383 | species <input type="radio"/> genus-only <input checked="" type="radio"/> unknown <input type="radio"/> |
| 12 | A12 | A12 | Clostridium perfringens | 2,079 | species <input checked="" type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/> |

Ready | Server: kok1 Port: 8080



MALDI Biotyper traceability



Barcodes



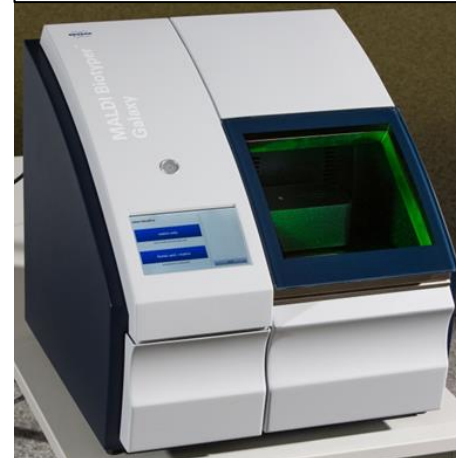
Bench-top Workstations.



Light guided sample preparation



Automated & Quality controlled matrix preparation



MALDI Biotyper – Product

Application support & training



• MALDI Biotyper Basic Operator Training



Training Courses

- MALDI Biotyper

Innovation with Integrity

MALDI-TOF

Participants

All lab members who will potentially work with the MALDI Biotyper are invited to the introductory presentation (optional)

The number of participants should not exceed 4 people for the following training.

Duration

1 day

Objective

The objective of this training is to consolidate the knowledge that the operators have obtained during the first few weeks of operating the instrument.

After the Training, you will be able to undertake sample preparation, to operate the software necessary for microorganism measurement, and to interpret the results reports.

Furthermore, you will be able to train new colleagues based on your acquired knowledge and the sample preparation protocols. You will be able to induct them in sample preparation and MALDI Biotyper software. (only valid for RUO.)

Inhalt

- Introductory Presentation on the theoretical basics (optional)
- Extraction of microorganisms
- Direct Transfer of microorganisms
- Target cleaning
- Calibration of the instrument
- Results interpretation



Bruker Daltonik

• Certificate

We hereby certify that

Mr. Smith

has successfully completed a training course in

MALDI Biotyper Basic Training

Training Topics

- Short introduction in microbiology/Biotyper
- Application training:
 - Chemicals (Matrix preparation, BTS etc.)
 - Direct transfer procedure
 - Extraction procedure
- FlexControl Calibration with Bruker Bacterial Test Standard
- Briefing MALDI Biotyper Software:
 - MALDI Biotyper Automation Control
 - Identification via Biotyper
 - Creation of own database entries, Dendrogram creation
- Interpretation and evaluation of MALDI Biotyper results

Bruker Daltonik GmbH
Bremen, March 6th, 2012

Trainer

Training Manager

think forward

MASS SPECTROMETRY

MALDI Biotyper – Quality Certificates

IVD Registration, ISO 9001 & ISO 13485



Registration of IVD MALDI Biotyper in the EU

We Bruker Daltonik GmbH
(Supplier's name)

Bruker Daltonik GmbH
Fahrenheitstr. 4
28359 Bremen

(Address)

declare under our sole respon

IVD MALDI Biotyper 2.2

(Name, type or model, batch or serial number
to which this declaration relate

Article 10, 98/79/EC (IVD dir
to the

German competent authorit

According to the European Co
registration in all member state
provision in Article 10 of Directiv
to every Member State concern
<http://ec.europa.eu/consumers/vigilance/leudamed/>)

Bremen, 2012-01-03

(Place and date of issue)

I, V.

Jörg Löffelholz
Manager
Quality Assurance

(Name and signature or equivalent marking of

BUREAU VERITAS
Certification

Certificate
awarded to



Bruker Daltonik GmbH

Standort Bremen
Fahrenheitstrasse 4
D-28359 Bremen

Standort Leipzig
Permoserstrasse
D-04318 Leipzig

Bureau Veritas Certification certifies that the Management System
organisation has been assessed and found to be in accord
with the requirements of the standards detailed below.

Standard

DIN EN ISO 9001:2008

Scope of supply

Development, production, sales and service of instruments
system and software solutions for the analytical instrum

Original approval date: 19.04.1998

Date of the audit: 15.04.2010

Date of next certification: 14.0

Subject to the continual satisfactory operation of the organization's Management System, this certifi

Date of certification: 28.05.2010

Valid until: 18.0

To check the certificate validity you may contact Bureau Veritas Certification. Further clarification re
certificate and the applicability of the Management System requirements may be obtained by email

Andrea Leibel

Local Technical Manager

Date: 01.06.2010

Certificate number: DE10000263

Bureau Veritas Certification Germany GmbH
Vertragsk 1 - D-21079 Hamburg

ZERTIFIKAT CERTIFICATE CERTIFICADO CERTIFICADO CERTIFICAT CERTIFICATE CERTIFICATE

CERTIFICATE

No. Q1N 10 10 64137 003

Holder of Certificate: Bruker Daltonik
Fahrenheitstrasse 4
28359 Bremen
GERMANY

Facility(ies): Bruker Daltonik GmbH
Fahrenheitstrasse 4, 28
Bruker Daltonik GmbH
Permoserstrasse 15, 04

Certification Mark:

Scope of Certificate: Design and develop
sales, distribution,
of instruments and
diagnostic use

Applied Standard(s): EN ISO 13485:2003 / A
Medizinprodukte - Quali
Anforderungen für regul
Medical Devices - Quali
Requirements for regul

The Certification Body of TÜV SÜD Product Service GmbH
above has established and is maintaining a quality system
listed standard(s). See also notes overleaf.

Report No.: 71374537

Valid until: 2013-11-30

Date: 2010-12-03

H. Heiner

Hans-Heiner Jurker

Page 1 of 1

TÜV SÜD Product Service GmbH
Zertifizierungsstelle
Ridlerstr. 65 - 80339 München
Germany



Product Service



Robustness and Ruggedness of the IVD MALDI Biotyper and the MALDI Biotyper (RUO)

Bruker did and does perform several tests concerning robustness and ruggedness of the MALDI Biotyper. Apart from various stability tests Bruker especially did perform a performance evaluation study with the participation of two well-known clinical laboratories. Based on this performance evaluation study Bruker performs a in-house validation before updating the mass spectra data base and/or the software.

1. External Performance Evaluation Study

Before introducing the IVD MALDI Biotyper into the European market Bruker performed a performance evaluation study in 2009 to check for potential risks and flaws in the practical use. For this purpose the IVD MBT 2.1 was tested in two well-known clinical laboratories.

For this study 20 representative microorganisms covering all 5 different groups of relevant microorganisms were selected from DSMZ (one of the largest biological resource centers worldwide which is often quoted by ATCC):

| Microorganism type | number |
|---------------------------------------|--------|
| Non-fermenting gram-negative bacteria | 2 |
| Enterobacteriaceae | 3 |
| Other gram-negative bacteria | 3 |
| Gram-positive bacteria | 5 |
| Yeasts | 7 |

Table 1: Number of examples studied from the respective microorganism type.

The minimum number of samples per site was defined for the identification accuracy to be:

| Microorganism type | number |
|---------------------------------------|--------|
| Non-fermenting gram-negative bacteria | 100 |
| Enterobacteriaceae | 100 |
| Other gram-negative bacteria | 100 |
| Gram-positive bacteria | 100 |
| Yeasts | 100 |

Table 2: Number of examples analyzed from the respective microorganism type.

The following percentages of the microorganisms were identified:

| Microorganism type | IVD-MBT ID accur. |
|---------------------------------------|----------------------|
| Non-fermenting gram-negative bacteria | 93.89% |
| Enterobacteriaceae | 99.25% |
| Other gram-negative bacteria | 95.59% |
| Gram-positive bacteria | 97.39% |
| Yeasts | 97.33% |
| Average: | 96.69% |

Table 3: Percent accurately identified for the respective microorganism type.

Innovation with Integrity

Service & Support: Ongoing Assistance



Bruker Daltonics services are performed by well trained and certified engineers:

- DIN EN ISO 9001:2000
- DIN EN ISO 13485
- IQ and OQ/PV with support for 21 CFR 11 compliance

Following services can be supplied:

- System service & maintenance
- Spares & consumables
- Service & maintenance contracts
- Application support
- Software support
- Training and user meetings
- WebEx sessions

Field experience



Bruker Daltonics



MALDI biotyper

- Microorganism Identification and Classification

Bruker serves demands of microbiological labs:



Faster, Better, Cost-Effective!

Quality

- Highly accurate results, comparable to sequencing.
- Single technique for bacteria, yeast and fungi
- Hardly any fals positive results.
- No misidentification by external influences (media, temperature, operators)

Significant pressure to reduce overall costs

- Low consumable costs
- Reduced # of culture plates
- Reduced # of chromogenic & selective media
- Less double tests

Faster:

- No pre-selection test.
- Quick result, < 10 minutes for 1 sample.
- Real-time

The Netherlands



 45 MBT systems (Februari 2014)

Febr. 2014: >1100 MBT users. Examples of Industrial references.



• **Food Safety**

- Nestlé, Switzerland
- Eurofins, USA
- Danisco, Denmark
- SGS, Germany
- Asahi Softdrinks, Japan
- Green Lines, Russia
- Fonterra, New Zealand
- BML, Germany
- CLF (Danone) Germany
- Suntory Business Expert Limited, Japan
- Maeil Dairies Co., Ltd., South
- Kibun Foods, Japan
- Nissin Foods, Quality Assurance, Tokyo, Japan
- Vitens, NL
- Waterlaboratorium Noord, NL
- Het Waterlaboratorium, NL
- Aqualab Zuid
- Gezondheidsdienst voor Dieren, NL

• **Pharma Industry**

- Pfizer, USA
- Abbott Biologicals, NL
- MSD, NL
- Sanofi, Germany
- Similisan AG, Switzerland
- Vaxxinova, Germany
- Boehringer Ingelheim, Germany
- Lohmann Therapiesysteme, Germany
- Astellas, Japan
- Otsuka Pharmaceutical, Japan

Local Authorities

- AGES, Graz and Vienne, Austria (**2** MALDIs)
- LAVES, Oldenburg, Germany
- CVUA, Krefeld, Germany
- CVUAs, BaWü, Germany (**4** MALDIs)
- LALLV, Rostock, Germany
- TLLV, Bad Langensalza, Germany
- LGL, Munich, Germany
- Chinese Academy of Inspection and Quarantine, China
- Bundesamt für Umwelt (Bafu), Switzerland
- RIVM, NL
- Centraal Veterinair Institute
- Wageningen Universiteit, department of Food Microbiology

Consumer/Packaging Industry

- Henkel, Germany
- Tetrapak, Germany
- Procter & Gamble, USA

Service Suppliers

- Accugenix, USA & Germany (**3** MALDIs)
- MIDI Labs, USA
- Eden Biodesign, UK
- Quotient Bioresearch, UK
- Confarma, France
- Genematrix, South Korea
- Biobeheer, NL

Campylobacter sp. as example for the differentiation power of MALDI-TOF MS fingerprinting



- 24 blinded isolates (*Campylobacter* and related bacteria) were tested

Result Overview

| Analyte Name | Analyte ID | Organism (best match) | Score Value | Organism (second best match) | Score Value |
|-----------------------------|-------------|--------------------------------------|-------------|--------------------------------------|-------------|
| A1 (+++) | Cb_14166_02 | Campylobacter jejuni ATCC 29428 THL | 2.43 | Campylobacter jejuni MB_5195_05 THL | 2.392 |
| A2 (+++) | H48 | Campylobacter jejuni ATCC 29428 THL | 2.115 | Campylobacter jejuni MB_4738_05 THL | 1.962 |
| A3 (+++) | Cb_464_99 | Campylobacter upsaliensis 412_01 NVU | 2.219 | Campylobacter upsaliensis 451_01 NVU | 2.197 |
| A4 (+++) | Cb_221_99 | Campylobacter lari 227_99 NVU | 2.458 | Campylobacter lari 165_98 NVU | 2.439 |
| A5 (+++) | ZC15 | Campylobacter coli 11167_03 NVU | 2.229 | Campylobacter coli 10090_03 NVU | 2.012 |
| A6 (+++) | Cb_180C_98 | Campylobacter jejuni ATCC 29428 THL | 2.338 | Campylobacter jejuni MB_5195_05 THL | 2.326 |
| A7 (-) | ZC27 | Aromatoleum tolulyticus Tol4_MPB | 1.14 | Arthrobacter tumbae DSM 16406T_DSM | 1.137 |
| A8 (+++) | ZC54A | Campylobacter coli 11167_03 NVU | 2.349 | Campylobacter coli 10090_03 NVU | 2.156 |
| A9 (+++) | Sx568 | Arcobacter butzleri 347_98 NVU | 2.396 | Arcobacter butzleri 460_98 NVU | 2.393 |

Strain ZC27 was identified as *Helicobacter canis* after adding its reference spectrum to the Bruker database.

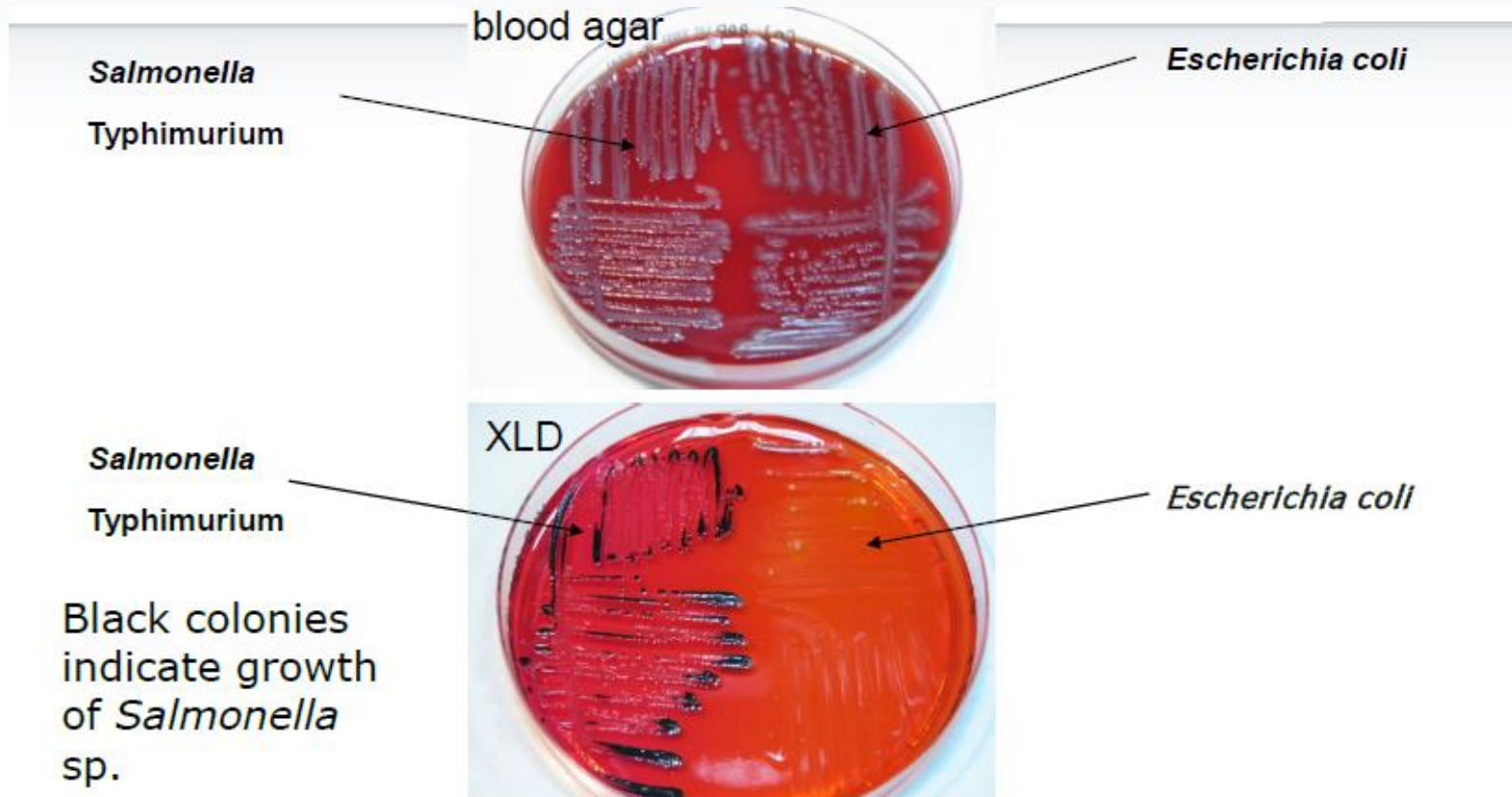
Campylobacter sp. as example for the differentiation power of MALDI-TOF MS fingerprinting



Results

- All isolates could be measured well by MALDI-TOF MS
- 22 strains could be identified correctly as *Campylobacter jejuni*, *C. coli*, *C. upsaliensis*, *C. helveticus*, and *Arcobacter butzleri*, respectively
- The remaining 2 isolates could not be identified initially because of lacking db reference entries.
 - After introduction of the reference spectra into the database they could be identified as *Campylobacter hyointestinalis* and *Helicobacter canis*.
- Even unexpected strains can be identified using a comprehensive reference database (*Bacteroides uniformis* from another set of tested suspected *Campylobacter* sp. isolates)

Salmonella sp. identification from XLD agar



Growth of *Salmonella* Typhimurium and *Escherichia coli* on Columbia Blood Agar and XLD

Salmonella sp. identification from XLD agar



| Analyte Name | Analyte ID | Organism (best match) | Score Value |
|---|--------------------------------------|--|-----------------------|
| Escherichia coli DH5alpha Blut (+++) | 7b42d492-6d44-46d0-b5c4-1e97772e5361 | Escherichia coli ATCC 25922 THL | 2.478 |
| Escherichia coli DH5alpha Blut (+++) | 5748d88f-e0cf-422b-a580-898bef223d0c | Escherichia coli ATCC 25922 THL | 2.49 |
| Escherichia coli DH5alpha Blut (+++) | | | 2.442 |
| Escherichia coli DH5alpha Blut (+++) | | | 2.426 |
| Salmonella typhimurium 75 XLD (+++) | c10f0731-9a77-4eb8-9eb3-36be41310d8b | Salmonella sp (enterica serotyp Dublin) Sa05_188 VAB | 2.576 |
| Salmonella typhimurium 75 XLD (+++) | | | 2.565 |
| Salmonella typhimurium 75 XLD (+++) | 314270fc-b996-4bb5-8d53-3bf414bfacea | Salmonella sp (enterica serotyp Dublin) Sa05_188 VAB | 2.62 |

All supposed *Salomella* spp. colonies grown on selective media e.g. XLD, XLT4, Ramach have to be confirmed e.g by an agglutination test with Salmonella antisera.

Doing this by MALDI-TOF fingerprinting has two main advantages:

- saving of antisera (minimal costs of sample preparation and analysis)
- very fast measurement with high reproducibility

Bruker Daltonics MALDI Biotyper:

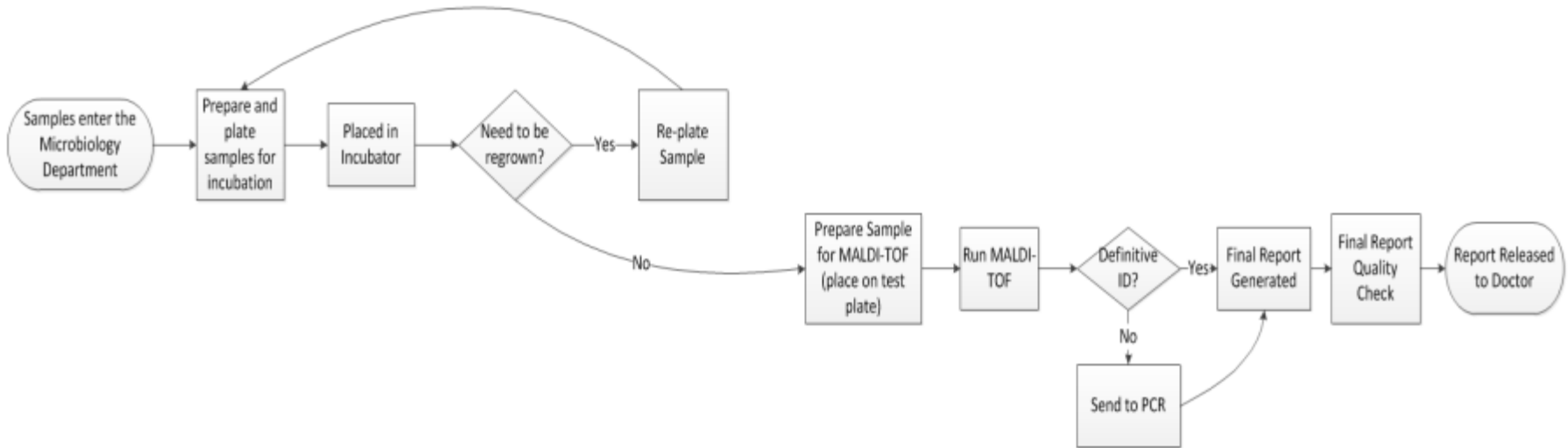
Unquestionable identification regardless of culture medium, no significant differences in log (score) values.

Differentiate between desired and unwanted contaminated lactic acid bacteria.



- Out of 24 isolates investigated, 18 were reliably identified as different species from genus *Lactobacillus* ($\log(\text{score}) \geq 2.0$): *L. fermentum* (n=3), *L. gasseri* (n=2), *L. crispatus* (n=10), *L. delbrueckii* (n=3).
- The remaining five isolates were identified as: *L. fermentum* (n=3) and *L. jensenii* (n=2) with a less reliable $\log(\text{score}) < 2.0$.
- One isolate was identified as *Bacillus coagulans*. All results were confirmed by 16S rRNA gene sequencing.
- *Data by: Alexandra Borovskaya, Elena Ilina, Vadim Govorun, Andrey Letarov, Alina Isaeva, Thomas Maier, Markus Kostrzewa; poster at the VAAM 2009, Bochum, Germany*

MALDI-TOF + FLOW STATE



**Average:
26.55 Hours**

18 – 24 hours

30 minutes – 24 hours

< 1 hour

- **Identity Confirmation** of micro-organisms is important to determine:
 - Risk level
 - Contamination source
 - Decontamination treatment
 - Comparison of contaminations
- **Quick identification helps to:**
 - Stop production / Close the source earlier=> Prevent further product contamination.
 - Accurate/earlier decontamination treatment. => Quickly pick-up production again.
 - Earlier recall of products => lower exposure.



AGES Selects Bruker's MALDI Biotyper System for Mass Spectrometry-based Molecular Microbial Identification

. May 2010

AGES is responsible for several tasks in regard to public health and food safety for the Austrian government. The organization researches, analyzes and performs inspections according to the policy guidelines of Austrian Food Laws. The agency requires veterinary inspections and dedicates itself to the prevention and control of infectious diseases in the population. Just recently, scientists of the AGES discovered the **source of a listeriosis outbreak** in Austria and Germany that caused **eight deaths** due to contaminated **cheese products**. In order to provide always state of the art microbiological analyses AGES is the first organization in Austria using the IVD-CE marked IVD MALDI Biotyper system that is in accordance with the European Union In Vitro Diagnostic Directive 98/79/EC.

Direct reaction prevented additional deaths and prevented the further spread through cheese production.

MALDI Biotyper Options



Objective: Find source of bacterial contamination in production process of facial creme

Water pipes Facial creme Price/sample Time to result

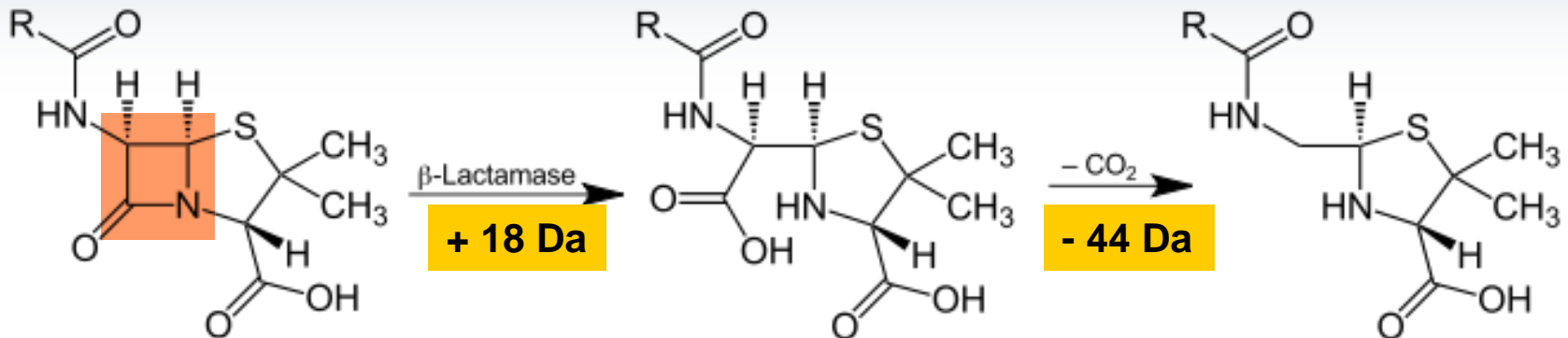
Different taxonomic allocation of the isolates from the facial crème and the water supply system clearly indicates that the source of product contamination in this case is **not related to the biofilms** inside of the water pipes. This conclusion was made as a result of the whole microbial cells MALDI-TOF MS spectra profiles data mining.

The same results were obtained in the alternative isolate identification using RiboPrinter (DuPont Qualicon) with the difference that MALDI-TOF MS based procedure is much faster and cost efficient. The use of API test (Biomérieux) did not allow to identify any of the strains; Vitek 2 (Biomérieux) was efficient in a case of the isolate from the facial crème only.

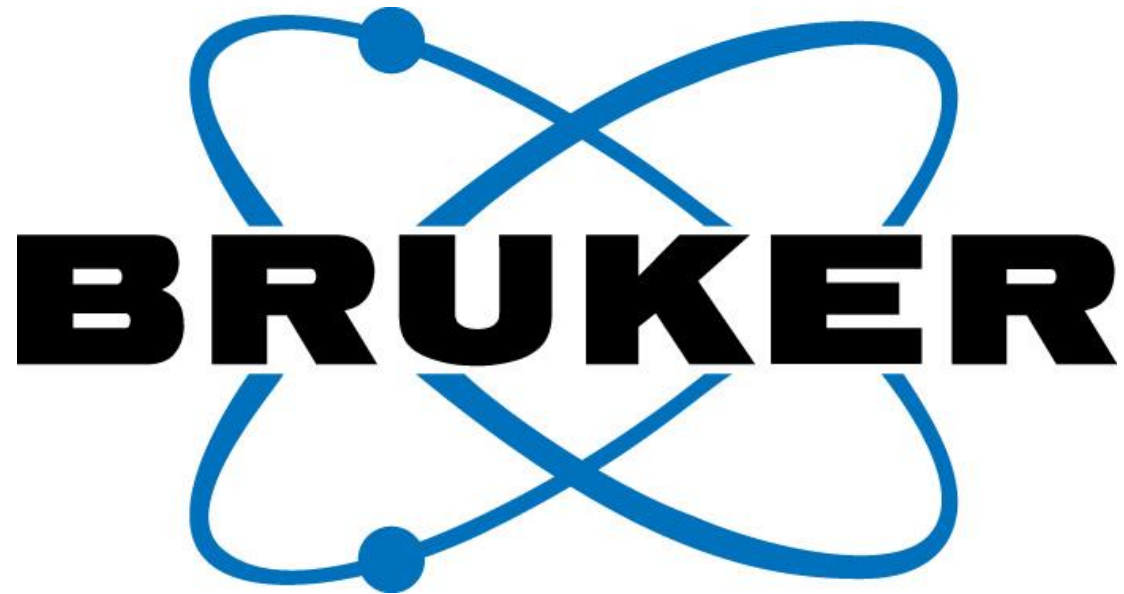
| | | | | |
|---------|---|--------------------------|--|--|
| API | - | - | | |
| Vitek 2 | - | Burkholderia cenocepacia | | |

MALDI-TOF MS resistance detection

Functional assay for antibiotics-sensitivity testing



- **Mass shifts by metabolism of antibiotics can be monitored with the MALDI Biotyper!**
- **Penicillins**
 - Ampicillin, plus inhibition by clavulanic acid
 - Piperacillin, plus inhibition by tazobactam
- **3rd generation Cephalosporins**
 - Cefotaxime, plus inhibition by clavulanic acid
 - Ceftazidime, plus inhibition by clavulanic acid
- **Carbapenemes**
 - Ertapenem, plus inhibition by APBA
 - Ertapenem, directly from positive blood culture



www.bdal.com
www.maldibiotyper.com



Product Authenticity Testing



Food Chemistry 135 (2012) 473–478

Contents lists available at SciVerse ScienceDirect



ELSEVIER

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Analytical Methods

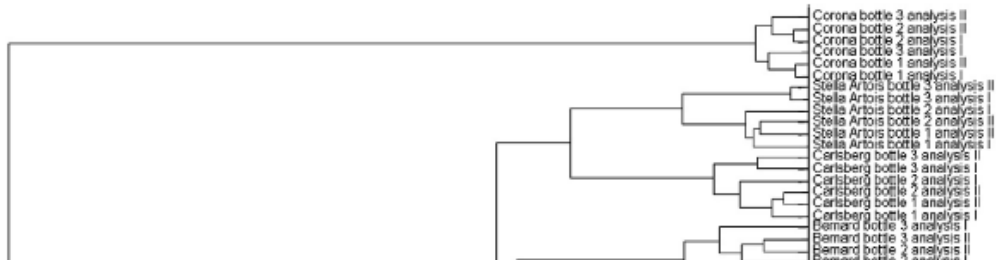
Beer fingerprinting by Matrix-Assisted Laser Desorption-Ionisation-Time of Flight Mass Spectrometry

Ondrej Šedo^a, Ivana Márová^{b,c}, Zbyněk Zdráhal^{a,*}

^aCore Facility – Proteomics, Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

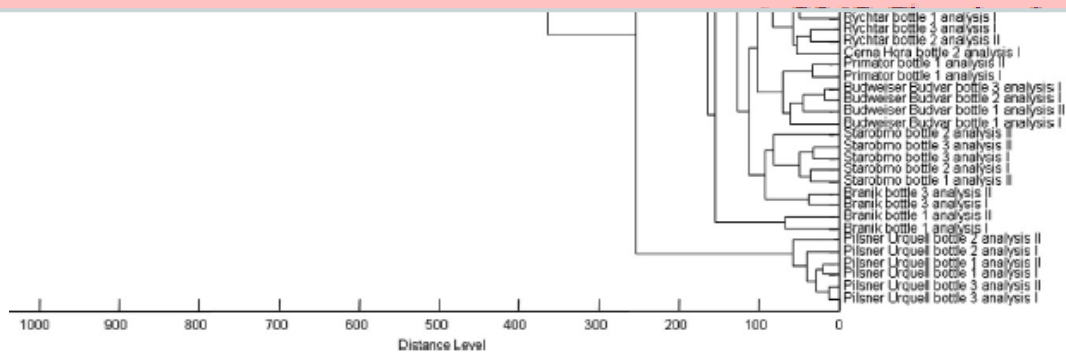
^bDepartment of Food Chemistry and Biotechnology, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic

^cCentres for Materials Research, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic



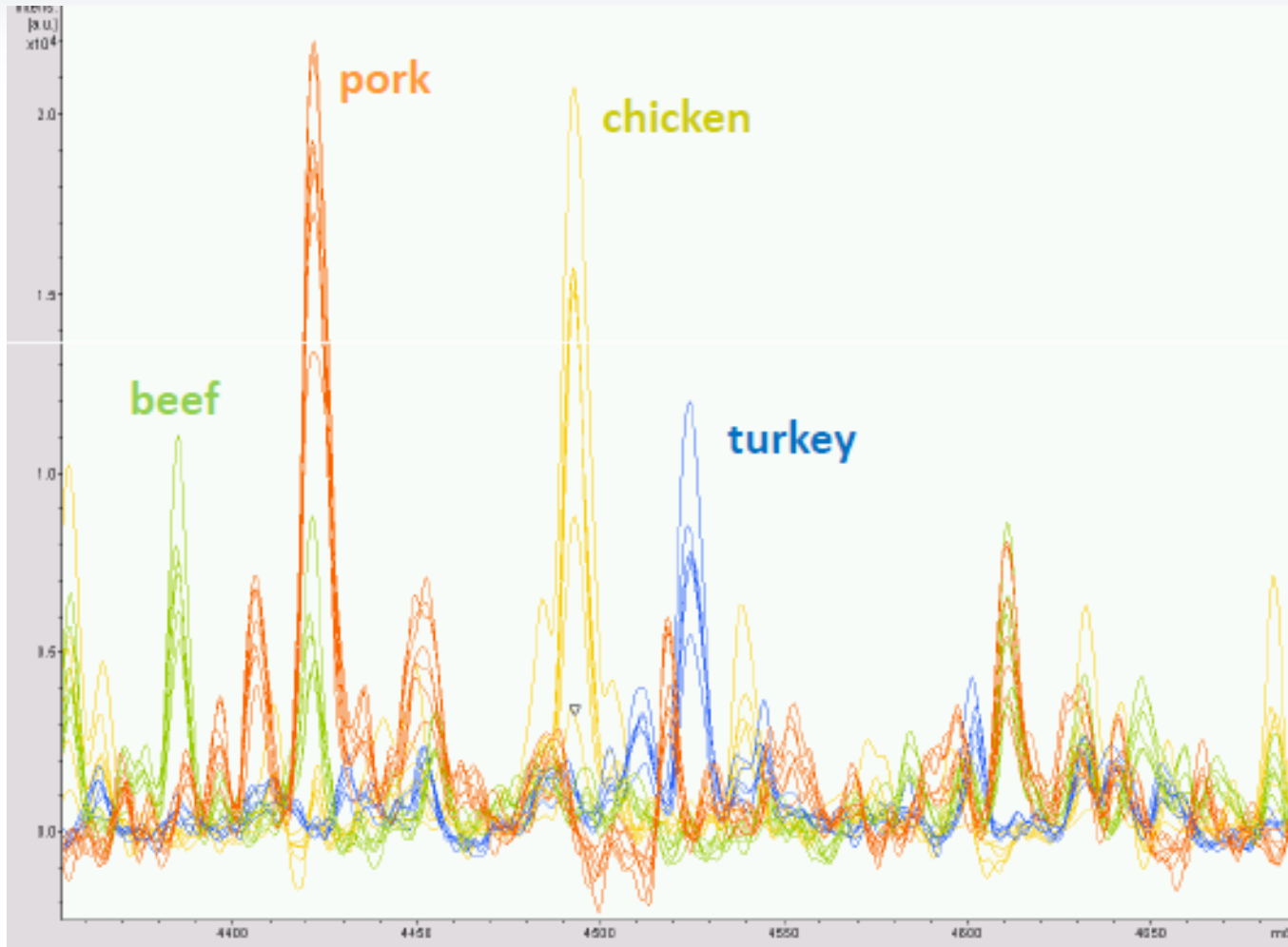
ABSTRACT

A method allowing parallel fingerprinting of proteins and maltooligosaccharides directly from untreated beer samples is presented. These two classes of compounds were detected by Matrix-Assisted Laser Desorption-Ionisation-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) analysis of beer mixed with 2,5-dihydroxybenzoic acid solution. The maltooligosaccharide profiles acquired from the MALDI sample spot center were not found characteristic for beers of different source and technology. On the other hand, according to profiles containing protein signals acquired from crystals formed on the border of the MALDI sample spot, we were able to distinguish beer samples of the same brand produced by different breweries. The discriminatory abilities of the method were further examined on a set of 17 lager beers, where the fingerprints containing protein signals enabled resolution of majority of examined brands. We propose MALDI-TOF-MS profiling as a rapid tool for beer brewing technology process monitoring, quality control, and determination of beer authenticity.





Identification of Meat with the MALDI Biotyper

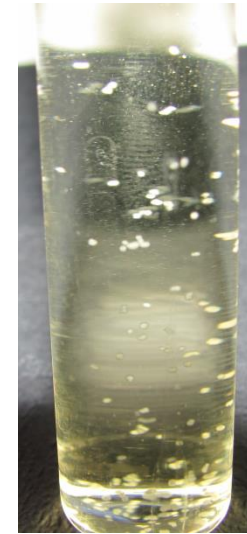
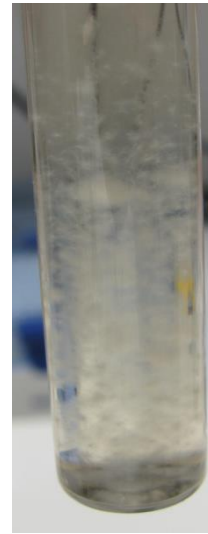
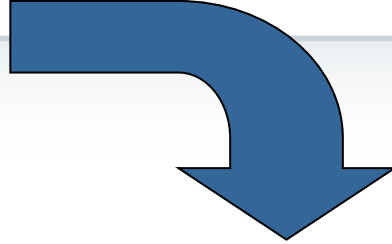
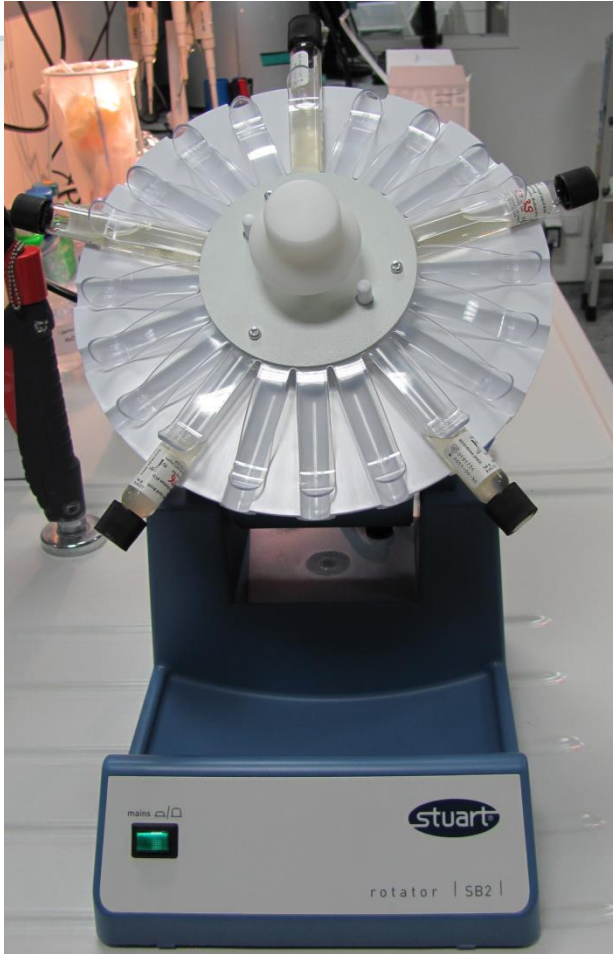


“Filamentous Fungi Consortium”

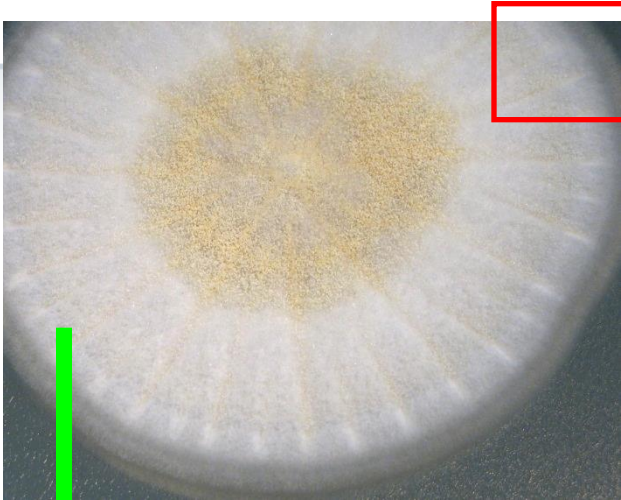


- >20 contributing laboratories (8 countries)
- Goals:
 - reliability test of sample preparation
 - lab-to-lab reproducibility
 - collection of securely identified species
- Project phase:
 - Established sample prep technique
 - Reproducibility test successful (Six strains from three different genera, three different species within one genus, and two strains within one species; ten laboratories)
 - Data and sample exchange ongoing
 - Establishment of extended database

Modified liquid broth cultivation for library generation → new cultivation recommendation



ID Filamentous Fungi - workflow



1. Direct Transfer of “Front Mycelium” (1 min)

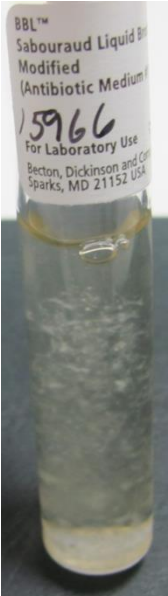
➤ if successful: ID is **FINISHED**

2. Ethanol Extraction of “Front Mycelium” (10 min)

➤ if successful: ID is **FINISHED**

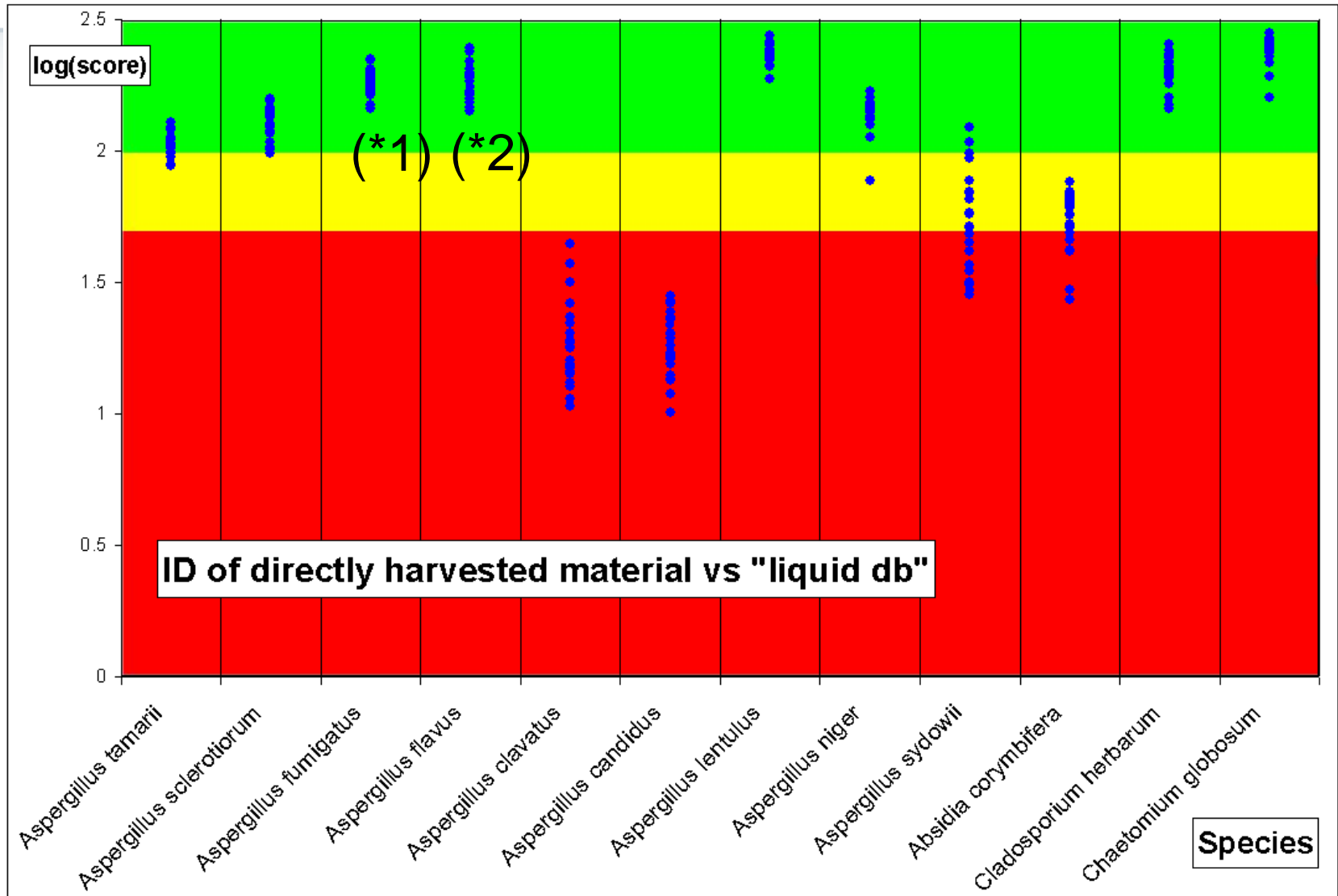
3. Broth Cultivation & extraction (approx. 1 additional day)

ID is possible for agar adhering filamentous fungi
ID is possible for fast sporulating fungi
ID is possible for every kind of filamentous fungi



→ **ALL matches against the SAME Filamentous Fungi DB**

Sample from agar plate vs Fungi DB



New Fungi Database



45 genera, 129 species – 366 strains

| | Genera | | | |
|----------------------------|----------------|-----------------|-----------------|---|
| Absidia coerulea | Absidia | Epidermophyton | Phialophora | a sorghina |
| Acremonium strictum | | | | mucor pusillus |
| Alternaria alternata | | | | ous microsporus |
| Arthrinium phaeospermum | | | | ous oryzae |
| Arthrographis_kalrae[ana] | Acremonium | Fennellia | Phoma | ous stolonifer |
| (Eremomyces_langeronii | | | | |
| Aspergillus candidus | Alternaria | Fusarium | Rhizomucor | sporium prolificans |
| Aspergillus clavatus | | | | sporium_apiospermum[ana] |
| | Arthrinium | Geomyces | Rhizopus | allescheria_boydii[teleo] |
| Aspergillus flavus | | | | phyllum commune |
| Aspergillus fumigatus | Arthrographis | Geosmithia | Rhizopus | lariopsis acremonium |
| Aspergillus glaucus | | | | lariopsis brevicaulis |
| Aspergillus niger | Aspergillus | Kerstesia | Scedosporium | lariopsis brumptii |
| Aspergillus nomius | | | | dium lignicola |
| Aspergillus ochraceus | Aureobasidium | Lecythophora | Schizophyllum | thrix schenckii |
| Aspergillus oryzae | | | | phalastrum racemosum |
| Aspergillus parasiticus | Beauveria | Lichtheimia | Scopulariopsis | tephorus cucumeris |
| Aspergillus sclerotiorum | | | | derma koningii |
| Aspergillus sydowi | Botrytis | Microsporum | Scytalidium | derma longibrachiatum |
| Aspergillus tamarii | | | | phyton eboreum |
| Aspergillus terreus | Chaetomium | Monilinia | Sporothrix | phyton equinum |
| Aspergillus unguis | | | | phyton interdigitale |
| Aspergillus ustus | Chrysosporium | Mucor | Syncephalastrum | phyton rubrum |
| Aspergillus versicolor | | | | phyton tonsurans |
| Aspergillus_amstelodami | Cladosporium | Paecilomyces | Thanatephorus | phyton violaceum |
| Eurotium_amstelodami[te | | | | |
| Aspergillus_nidulans[ana] | Cunninghamella | Penicillium | Trichoderma | phyton_mentagrophytes_var_erinacei[ana] |
| Emericella_nidulans[teleo] | | | | derma benhamiae[teleo] |
| Aureobasidium pullulans | Curvularia | Phaeoacremonium | Trichophyton | rus sp |
| Beauveria bassiana | | | | |
| | Epicoccum | Phialemonium | Trichurus | |



Monitor unintended transportation of microbes by Ballast Water Analysis



OPEN ACCESS Freely available online



Characterization of Bacteria in Ballast Water Using MALDI-TOF Mass Spectrometry

Kaveh Emami^{1*}, Vahid Askari², Matthias Ullrich³, Khwajah Mohinudeen⁴, Arga Chandrashekar Anil⁵, Lidita Khandeparker⁵, J. Grant Burgess⁶, Ehsan Mesbahi⁶

1 School of Biology, Newcastle University, Newcastle upon Tyne, United Kingdom, **2** School of Science and Engineering, Teesside University, Middlesbrough, Tees Valley, United Kingdom, **3** Molecular Life Science Research Center, Jacobs University Bremen, Bremen, Germany, **4** NEPAF Proteome Analysis Facility, Newcastle University, Newcastle upon Tyne, United Kingdom, **5** National Institute of Oceanography, Dona Paula, India, **6** School of Marine Science and Technology, Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

To evaluate a rapid and cost-effective method for monitoring bacteria in ballast water, several marine bacterial isolates were characterized by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Since International Maritime Organization (IMO) regulations are concerned with the unintended transportation of pathogenic bacteria through ballast water, emphasis was placed on detecting species of *Vibrio*, enterococci and coliforms. Seawater samples collected from the North Sea were incubated in steel ballast tanks and the presence of potentially harmful species of *Pseudomonas* was also investigated. At the genus-level, the identification of thirty six isolates using MALDI-TOF MS produced similar results to those obtained by 16S rRNA gene sequencing. No pathogenic species were detected either by 16S rRNA gene analysis or by MALDI-TOF MS except for the opportunistically pathogenic bacterium *Pseudomonas aeruginosa*. In addition, in house software that calculated the correlation coefficient values (CCV) of the mass spectral raw data and their variation was developed and used to allow the rapid and efficient identification of marine bacteria in ballast water for the first time.



MALDI-Biotyper as a tool for polyphasic taxonomy approaches



RESEARCH ARTICLE

A polyphasic approach for the differentiation of environmental *Vibrio* isolates from temperate waters

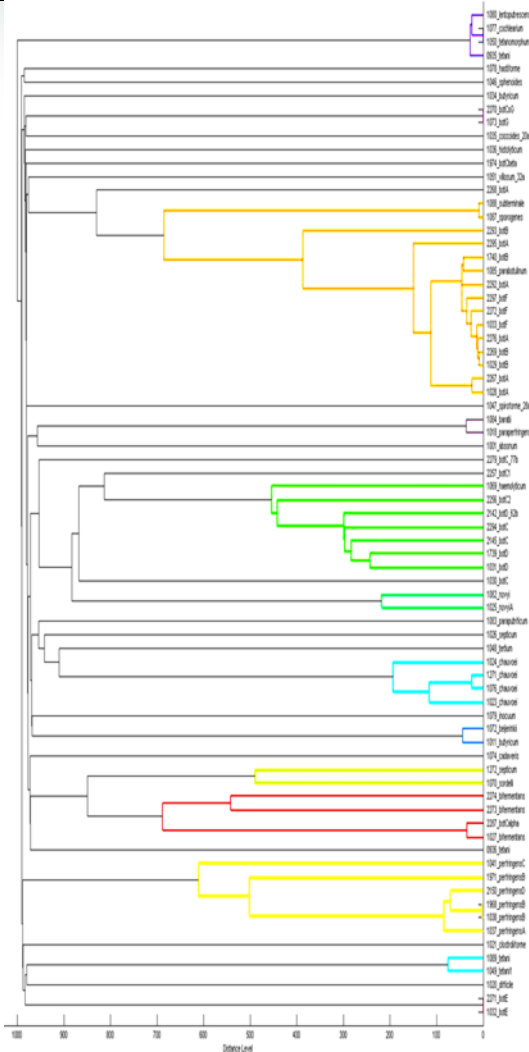
Sonja Oberbeckmann¹, Antje Wichels¹, Thomas Maier², Markus Kostrzewa², Sarah Raffelberg¹ & Gunnar Gerds¹

¹Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Helgoland, Germany; and

²Bruker Daltonik GmbH, Bremen, Germany



Dereplication of identical isolates



- **Even if an environmental isolate is not in the library, the MALDI Biotyper taxonomy tree can find identical species.**
- **Sequencing efforts are reduced.**
- **Large environmental studies are manageable and cost-effective.**



Analysis of difficult organisms, like the algae *Prototheca*



J. Dairy Sci. 95:1–10

<http://dx.doi.org/10.3168/jds.2011-5106>

© American Dairy Science Association®, 2012.

Herd characteristics and cow-level factors associated with *Prototheca* mastitis on dairy farms in Ontario, Canada

L. Pieper,^{*1} A. Godkin,[†] U. Roesler,[‡] A. Polleichtner,[‡] D. Slavic,[§] K. E. Leslie,^{*} and D. F. Kelton^{*}

^{*}Department of Population Medicine, University of Guelph, Ontario, Canada, N1G 2W1

[†]Veterinary Science and Policy Group, Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Guelph, Ontario, Canada, N1G 4Y2

[‡]Institute of Animal Hygiene and Environmental Health, Free University of Berlin, 10115 Berlin, Germany

[§]Animal Health Laboratory, University of Guelph, Ontario, Canada, N1G 6R8



MALDI-TOF MS for subtyping and epidemiology



***Streptococcus equi* subspecies:**

S. equi subsp. *zooepidemicus* – commensal, opportunistic pathogen

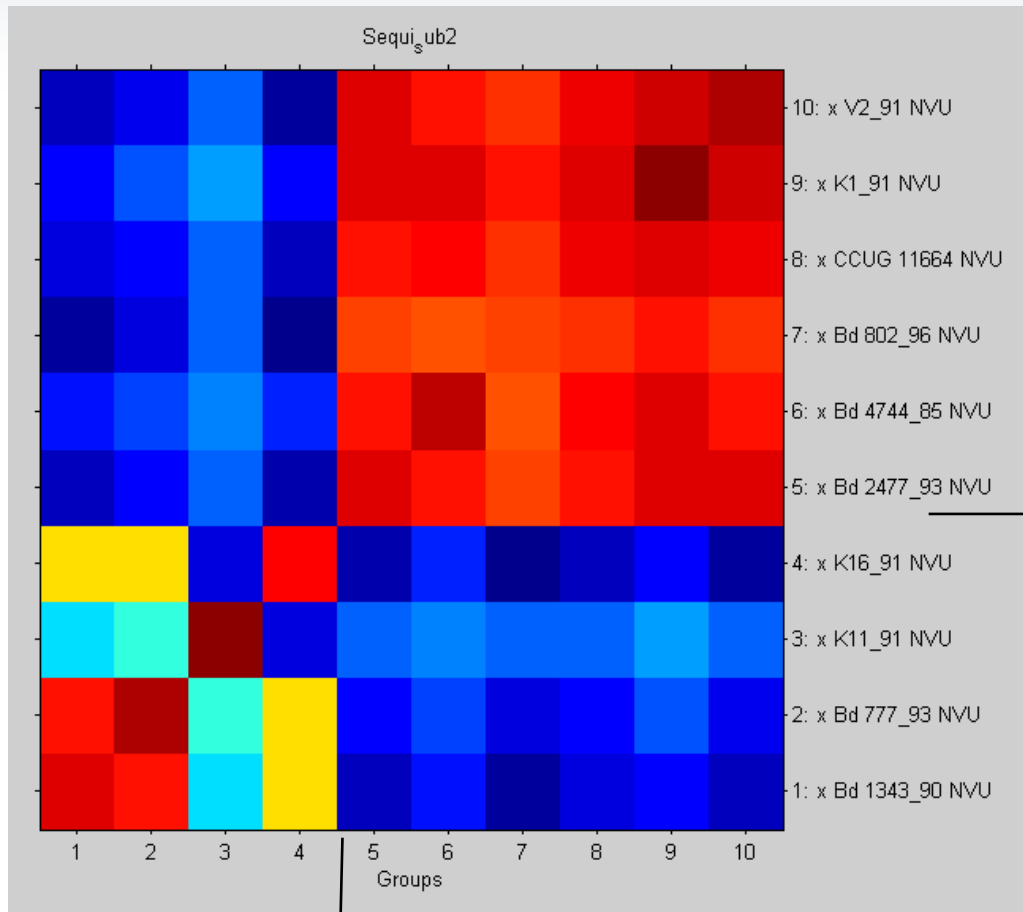
S. equi subsp. *equi* – virulent, dangerous infection (“strangles“)

Can the subspecies be differentiated by MALDI Biotyper analysis?

- MALDI Biotyper analysis of 10 strains (6 *S. equi equi*, 4 *S. equi zooepidemicus*) from national veterinary institute of Sweden (SVA):
- Spectra are very similar, high log(scores) in matches between subspecies (>2.4!)



Correlation analysis



S. equi equi

- very homogenous group

Clear dissimilarity!

S. equi zooep.

- much more heterogenous

S. equi zooep.

S. equi equi

red/high similarity, blue/low similarity



Veterinary Microbiology xxx (2012) xxx–xxx



Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Short communication

MALDI-TOF mass spectrometry confirms clonal lineages of *Gallibacterium anatis* between chicken flocks

Merima Alispahic^{a,*}, Henrik Christensen^b, Claudia Hess^a, Ebrahim Razzazi-Fazeli^c,
Magne Bisgaard^b, Michael Hess^a

^a Clinic for Avian, Reptile and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

^b Department of Veterinary Pathobiology, Faculty of Life Science, University of Copenhagen, Denmark

^c VetOMICS Core Facility for Research/Proteomics and Metabolomics, University of Veterinary Medicine, Vienna, Austria