



Rapid Detection

Chasing *Salmonella* and *Listeria* in Food: Rapid detection and strain discrimination

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Bruker

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Proteomics Technology: MALDI-TOF MS - you can utilize it for...

...confirmation of:

Salmonella

Cronobacter

Campylobacter

L. monocytogenes

...identification of:

Coliforms

Lactic acid bacteria

Yeasts & Molds

Filamentous Fungi



A typical customer application: Confirmation of *Salmonella*

Sampling

- Quality control of ingredients
- End product check
- Environmental monitoring

ISO 6579: *Salmonella* Screening

DAY 1
Liquid enrichment

DAY 2
Transfer to selective agars: Streak on XLD & Chromogenic Agar - Incubation: 37.0°C, 24h



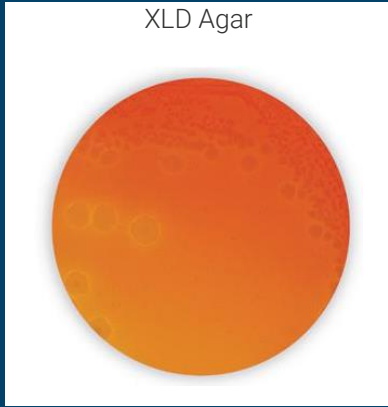
DAY 3
Presumptive *Salmonella*, needs confirmation



ISO-confirmation

DAY 4 & 5 (normal workflow):
Confirmation includes plating on non-selective Agar plates for 24h and biochemical tests.

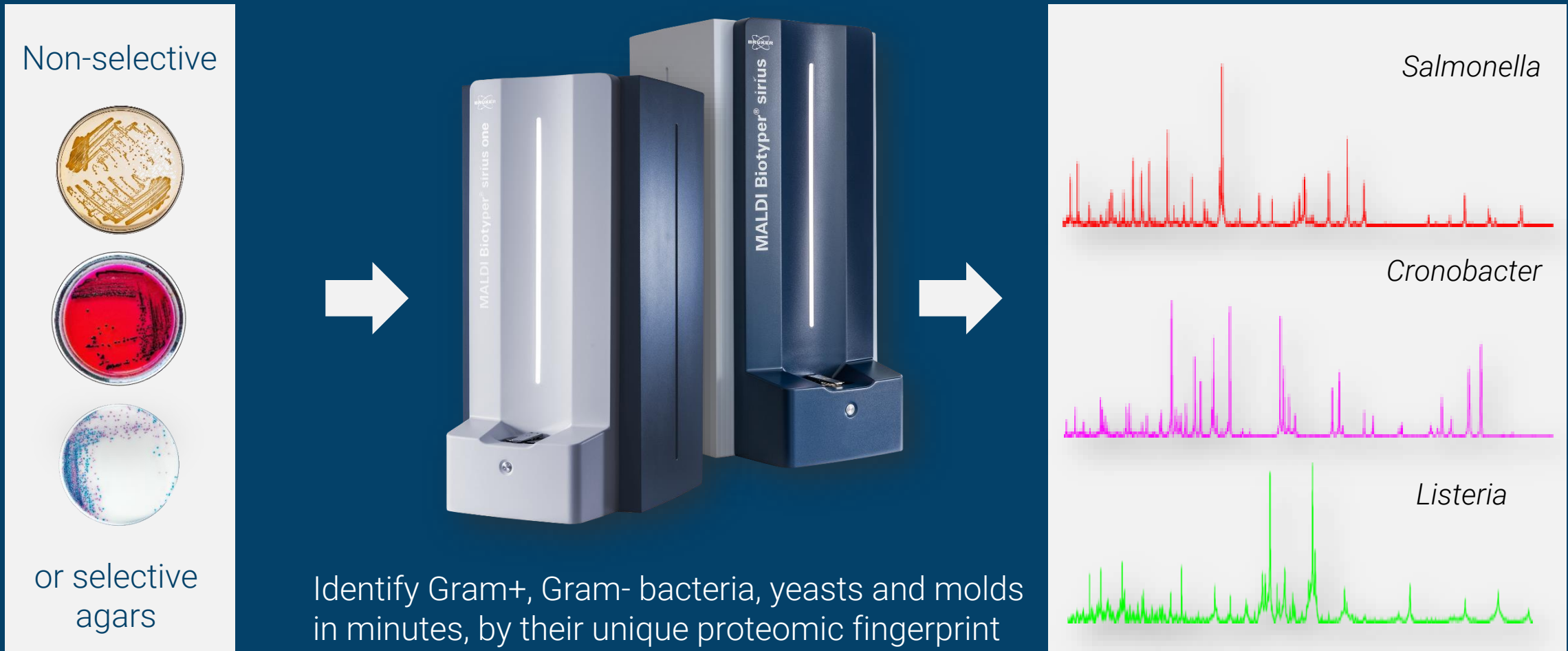
In many cases followed by *Salmonella* serology (subtyping).



Confirmation by MALDI Biotyper®

SAVE workload and Time-to-result

MALDI Biotyper[®] principle



MALDI Biotyper® • ISO 16140 validated, plus AOAC-OMA method and method of USDA-FSIS laboratory guidebook

Four MICROVAL certificates

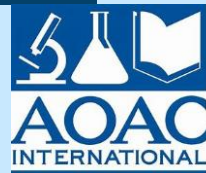
- *Salmonella*
- *Listeria* incl. *L. monocytogenes*
- *Campylobacter*
- *Cronobacter*

Reports of the ISO 16140-part 6 validation studies are available.



Two AOAC OMA methods

- AOAC OMA #2017.09: Confirmation and Identification of *Salmonella* spp., *Cronobacter* spp., *Campylobacter* spp., and other gram-negative organisms
- AOAC OMA #2017.10: Confirmation and Identification of *Listeria monocytogenes* and *Listeria* spp., and other gram-positive organisms



MALDI Biotyper® has been successfully tested by the USDA-Food Safety and Inspection Service (FSIS).

A flow chart specific for FSIS Laboratory Isolation and Identification of *Salmonella* is available (issued 2021):

- Test on Bruker MALDI Biotyper®, starting from isolated colonies, allows final confirmation
- Bruker's MALDI Biotyper® can replace other identification technologies like VITEK®2 Compact



MALDI Biotyper® Library (RUO/GP) and our software: Easy result interpretation for food labs

Range	Interpretation	Symbols	Color
2.00 - 3.00	High-confidence species identification	(+++)	green
1.70 - 1.99	Low-confidence species identification	(+)	yellow
0.00 - 1.69	No Organism Identification Possible	(-)	red

Unknown microorganism is matched against each Main Spectrum in the library

Calculation of a matching score

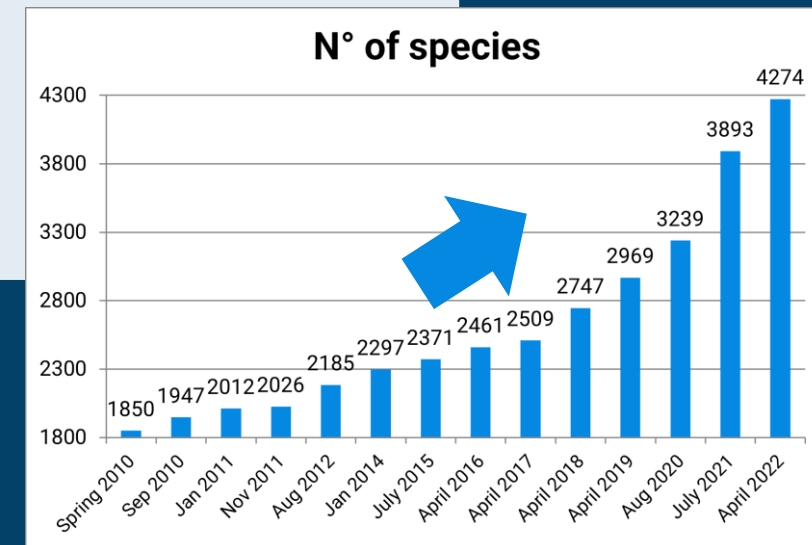
Easy result presentation by „traffic light“ display

Green = high-confidence species ID

Export of raw data possible

Bruker MBT Compass Library (version 2023)

- ✓ 4,274 different species
 - ✓ 2,365 gram-positives
 - ✓ 1,648 gram-negatives
 - ✓ 220 yeasts
 - ✓ 41 filamentous fungi



Product contaminations are rapidly identified with the same workflow

1. Yogurt can be contaminated e.g., with *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Aflatoxin producer!)



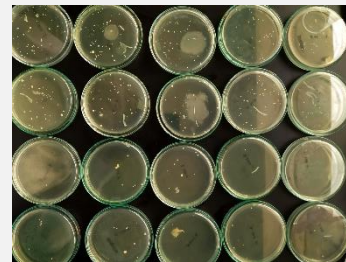
2. Some bacteria (*Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera*) or fungi (*Fusarium*) can generate off-flavors, turbidity and acidity in beer



In-house workflow

Growth and counting

Characterization of the dominant bacteria / yeast / mold



Characterization of the dominant bacteria / yeast / mold

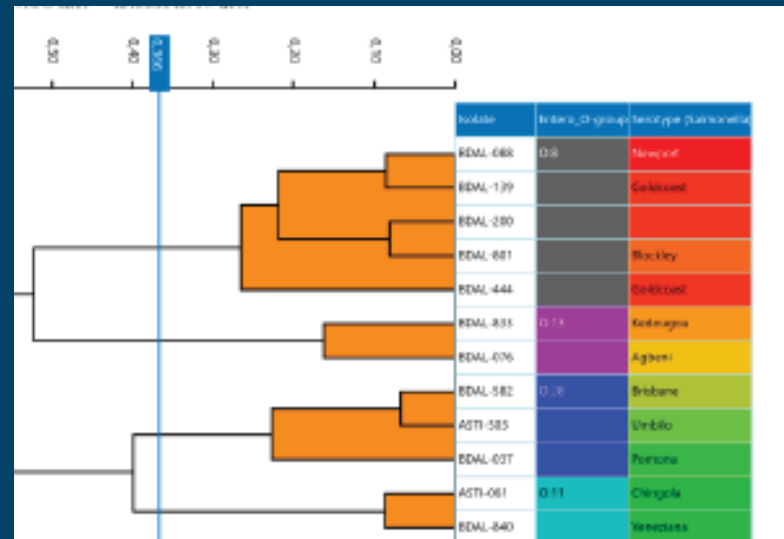
No matter which product or microorganism type, the MALDI Biotyper enables rapid identification with one workflow for all.



Source tracking and strain comparison needed?

Salmonella O-serotypes
(classifier)

Dendrogram for clear visualization of strain discrimination for *Salmonella* O-groups and serotypes



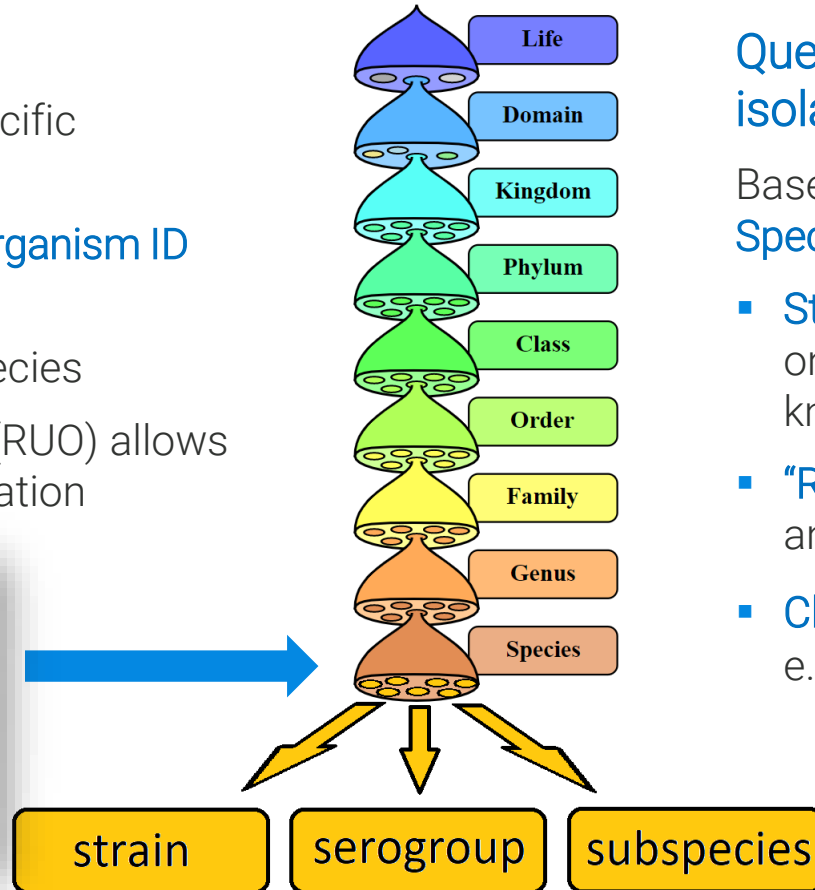
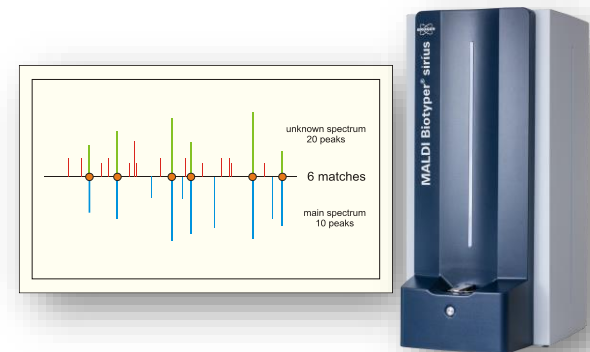
MALDI Biotyper® & IR Biotyper® for actionable insights

Why the Duo?

MALDI Biotyper®

Question: What is it?

- Based on **MALDI-MS** and specific peptide/protein fingerprints
- Gold standard in fast **Microorganism ID** on species level
- Large database** of > 4600 species
- The MBT Subtyping Module (RUO) allows some (sub)species differentiation



IR Biotyper®

Question: Is there a difference between these isolates? Or is it the same?

Based on “cell phenotyping” by **FT-Infrared Spectroscopy (FT-IR)**

- Strain typing method**, allows in-depth differentiation on isolate/strain level, below species (when the ID is known e.g. from MBT)
- “**Real Time**” **Analysis: Used in Infection Control**, analysis of transmission routes
- Classification** based on machine learning (e.g. ANN) e.g. for serogrouping/subspecies determination



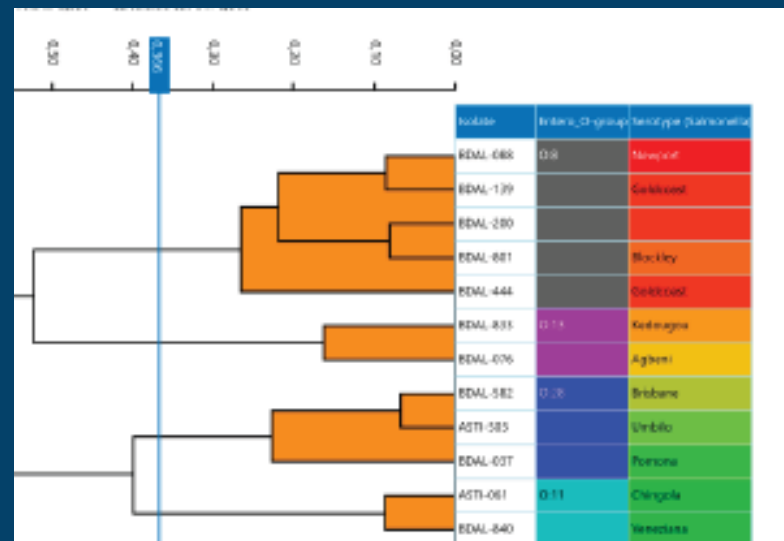
Source tracking and strain comparison needed?

Salmonella O-serotypes
(classifier)

Local discrimination possible, **customized solutions** (no classifiers)
e.g., for

S. enterica serovar
Typhimurium,
S. enteritidis,
S. gallinarum,
S. kentucky

Dendrogram for clear visualization of strain discrimination for *Salmonella* O-groups and serotypes



E. Coli O157, O104:H4,
more, customized

Important gram-positive bacteria

Listeria & Listeria monocytogenes

Found in milk powder, fruits, nuts, meat, seafood

Staphylococcus aureus

food-borne pathogen

Listeria in meat, seafood, vegetables, frozen food



news digest #016

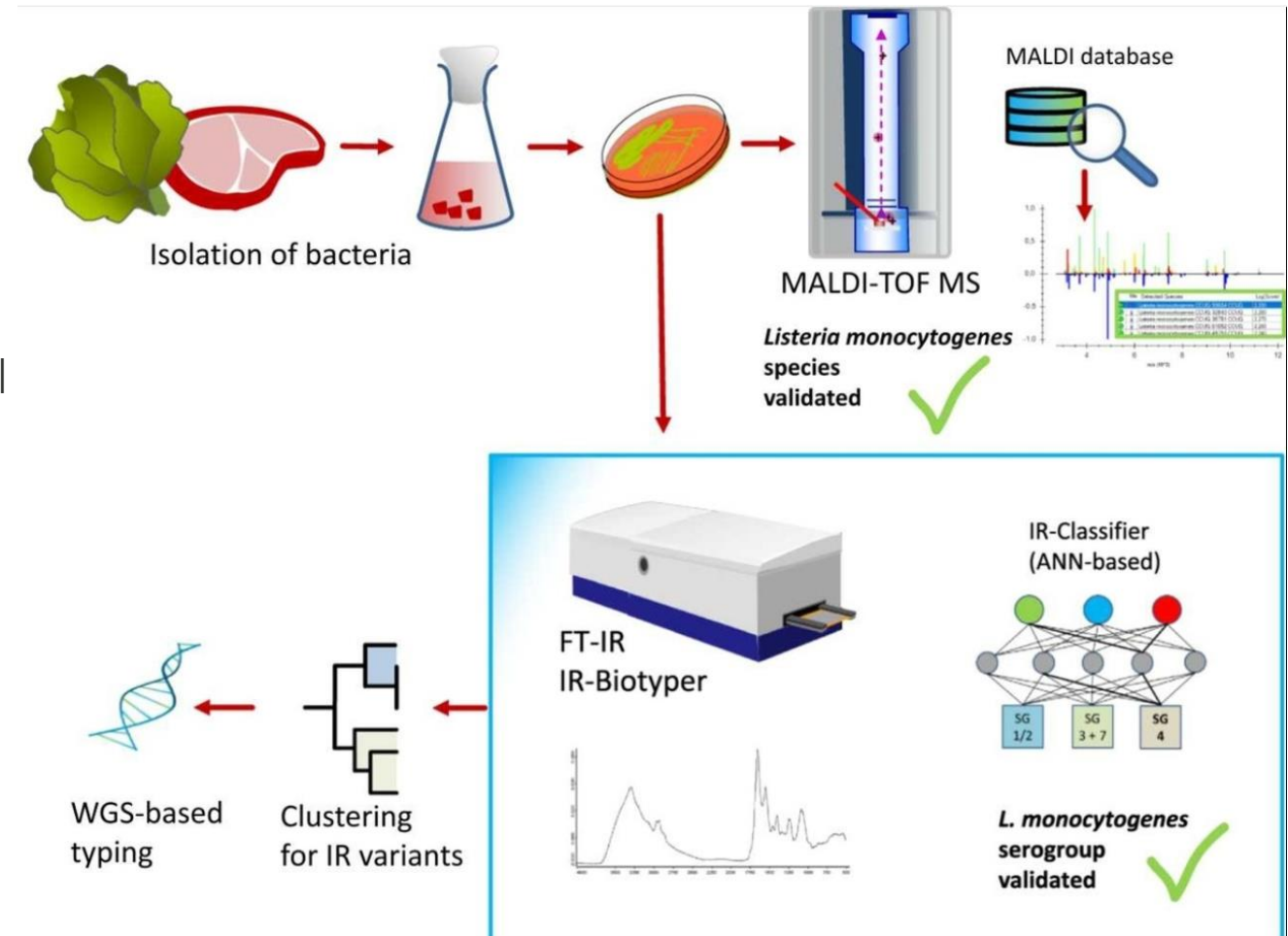
**Chasing *Listeria* in Food and Water:
Advancements in detection and
strain discrimination**

Single lab-validation for *Listeria* classifier in 2023

A validated IR Biotyper method (1) has been integrated into the accredited workflow for *L. monocytogenes* analysis in food samples according to ISO 11290:

- MALDI-TOF MS confirmation on the species level
- subsequent serogrouping and pre-selection by FTIR spectroscopy for Whole Genome Sequencing (WGS)

(1) Source, In: Clinical Spectroscopy, Vol 5, December 2023: Validated differentiation of *Listeria monocytogenes* serogroups by FTIR spectroscopy using an Artificial Neural Network based classifier in an accredited official food control laboratory.
<https://doi.org/10.1016/j.clispe.2023.100030>



Publication on Food-borne outbreak *S. aureus*

DOI: 10.1111/zph.13046

ORIGINAL ARTICLE

WILEY

Investigation of a *Staphylococcus aureus* sequence type 72 food poisoning outbreak associated with food-handler contamination in Italy

Federica Savini¹ | Angelo Romano² | Federica Giacometti¹ | Valentina Indio¹ |
 Monica Pitti² | Lucia Decastelli² | Pietro Luigi Devalle³ | Ilaria Silvia Rossella Gorrasi³ |
 Sergio Miaglia³ | Andrea Serraino¹

that they belonged to Sequence Type 72. Fourier transform infrared spectroscopy (FTIR) was used in parallel to single nucleotide polymorphisms and whole genome sequencing for the determination of the degree of relatedness of the isolates. The results of the FTIR showed the same clustering obtained with single nucleotide polymorphisms and whole genome sequencing and revealed the source of infection. This study underlines the importance of both laboratory evidence and epidemiological data for outbreak investigation and further confirms that FTIR is a suitable support for the short-term epidemiological investigation on source attribution in case of a *S. aureus* infection.

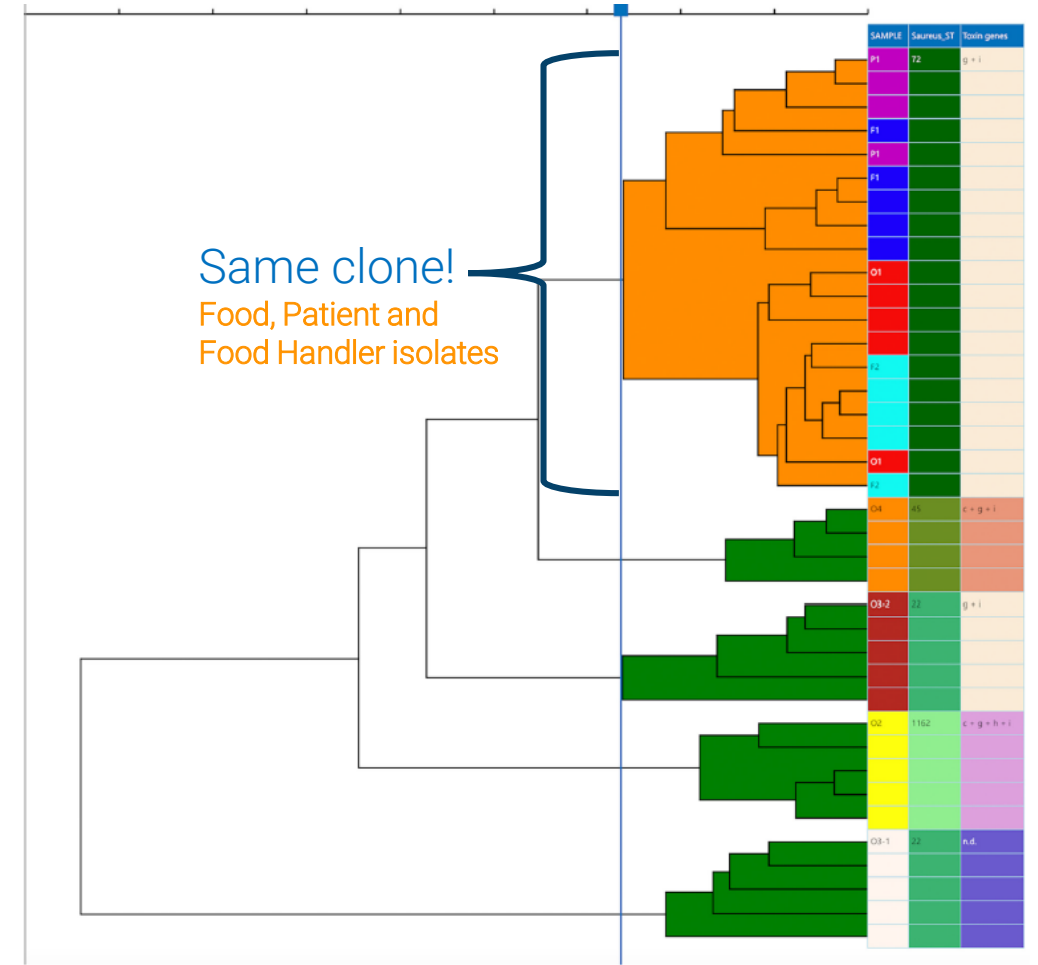


FIGURE 2 Dendrogram delivered by IRBT clustering analysis (WN: 1300-800/cm; Dim. reduction: PCA (7PCs/95.2% variance); Averaging: None; Metric/linkage: Euclidean/Average; Cophenetic correlation coefficient: 0.949; Calculated cut-off: 0.119). On the right side of the figure, the columns show the samples spectra (each line represents a technical replicate). The upper axis shows the cut-off distance that is calculated by the algorithm: Simpson's Diversity Index (SDI) x mean Coherence (mC).

- IR Biotyper (confirmed by WGS) was able to reveal the source of infection
- IR Biotyper is suitable for fast and low-cost epidemiological analysis of source attribution for *S. aureus*

Benefits of the combination MALDI Biotyper® and IR Biotyper®

MALDI-TOF MS technology based on profiling of proteins starting from colony material

- Very fast identification on species level
- One workflow for all microorganisms (bacteria, yeast, molds)
- Ease of use, only short training needed
- ISO 16140-6 certified /AOAC-OMA / USDA-FSIS method for pathogens
- Annual library updates
- Own customer libraries possible



FT-IR-based strain comparison in light of phenotypic aspects starting from colony material

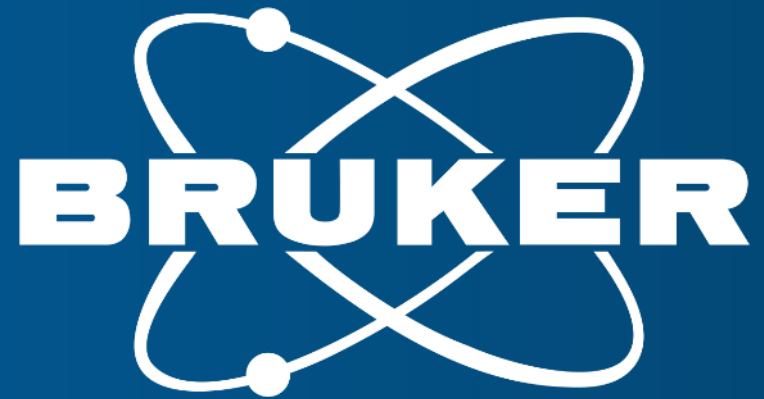
- Very fast strain discrimination
- Easy software for Cluster analysis by a variety of statistical methods
- Dedicated classifiers for targeted questions: Salmonella O-serogroups, Legionella pneumophila SG1

Outlook: MALDI Biotyper® for food fraud testing

- Broadening the research applications for food authenticity testing
- MBT allows e.g., the analysis of meat or insect species
- Bruker is focusing on microbiology validations but is supporting new applications from collaboration partners
- Publicly available collection of sample preparation protocols for MALDI-TOF MS based identification of meat, dairy products, fish and insects published and available from CVUA



Dyk M et al. 2020. Aspects of Food Control and Animal Health, Vol: 13, Pages: 1-13, ISSN: 2196-3460.



Innovation with Integrity