



WORLD BREWING CONGRESS

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#ElevateBeer



MALDI-TOF MS for Brewing Microbiology

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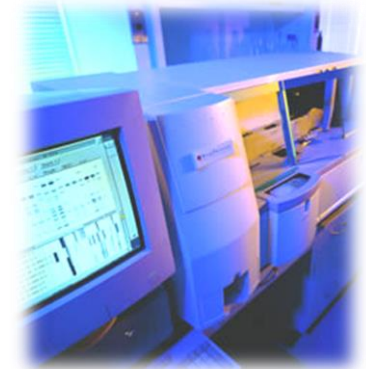


Current microbial detection / identification methods

- Using selective growth media/conditions
- Specific oligonucleotide probes
- PCR-based detection and/or identification
- Ribotyping
- Immuno-based
- ...



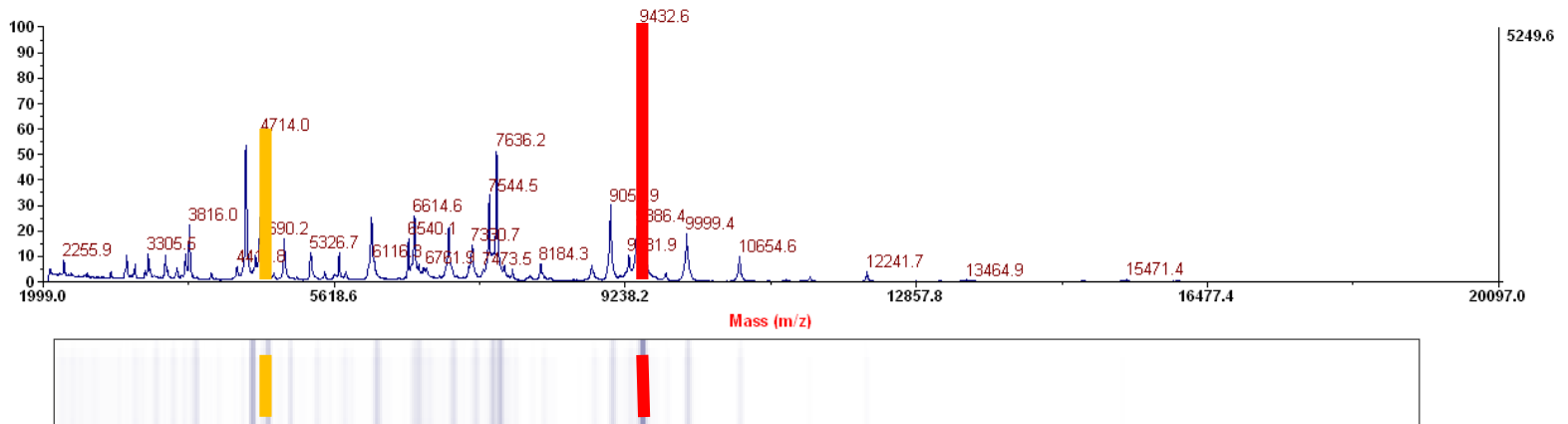
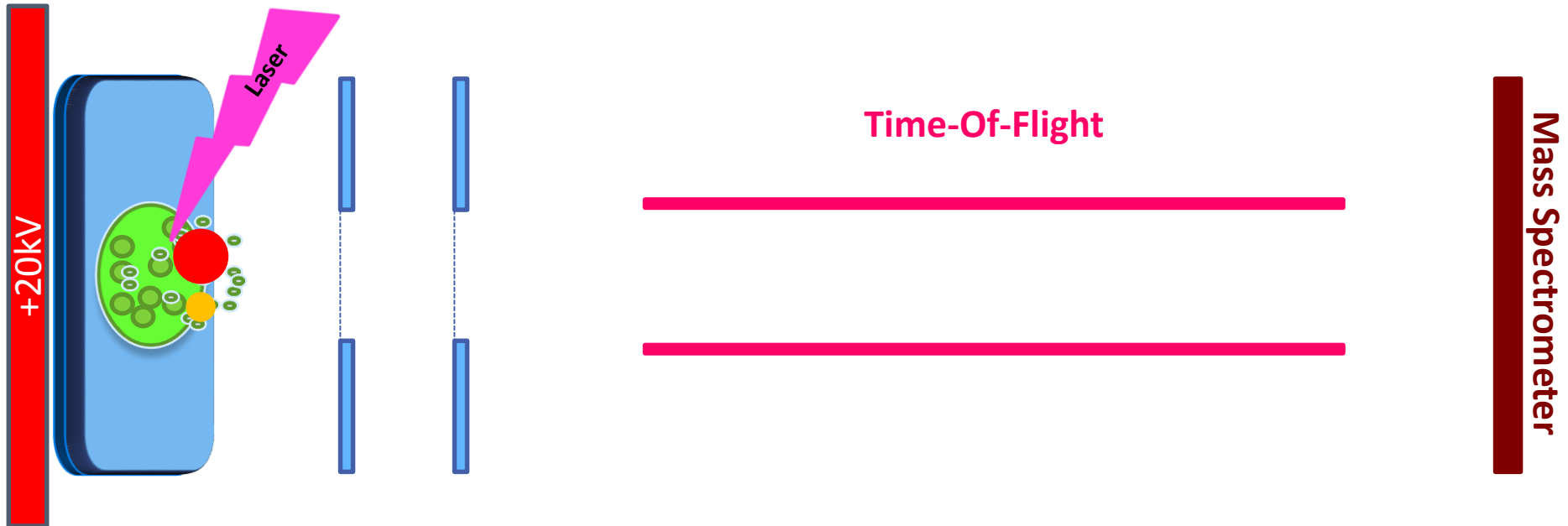
☹️ Laborious, time-consuming, lack specificity and sensitivity



MALDI-TOF MS as a rapid, specific, inexpensive and high-throughput identification/differentiation tool for brewing microbiology

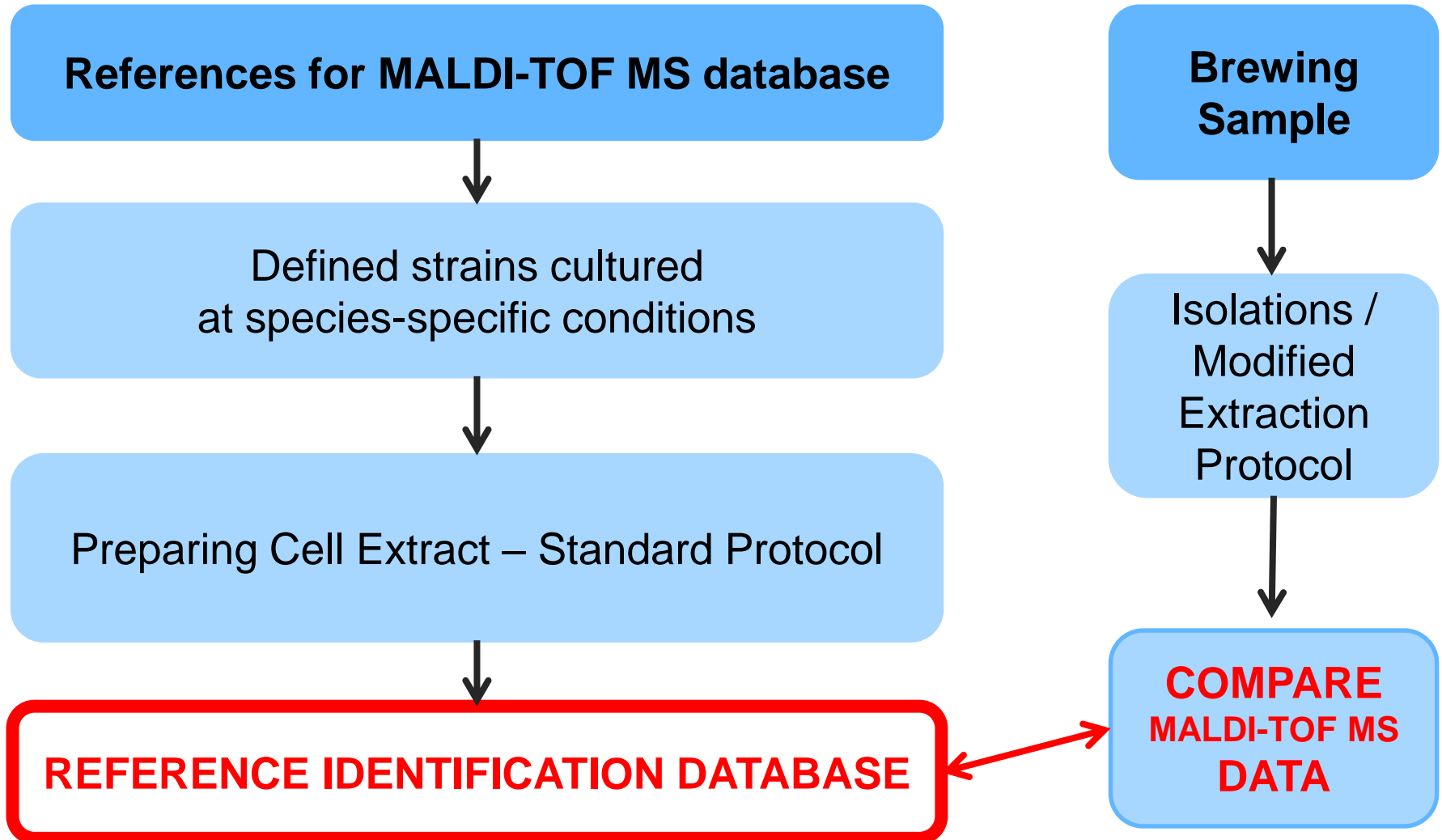
MALDI-TOF MS

MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY



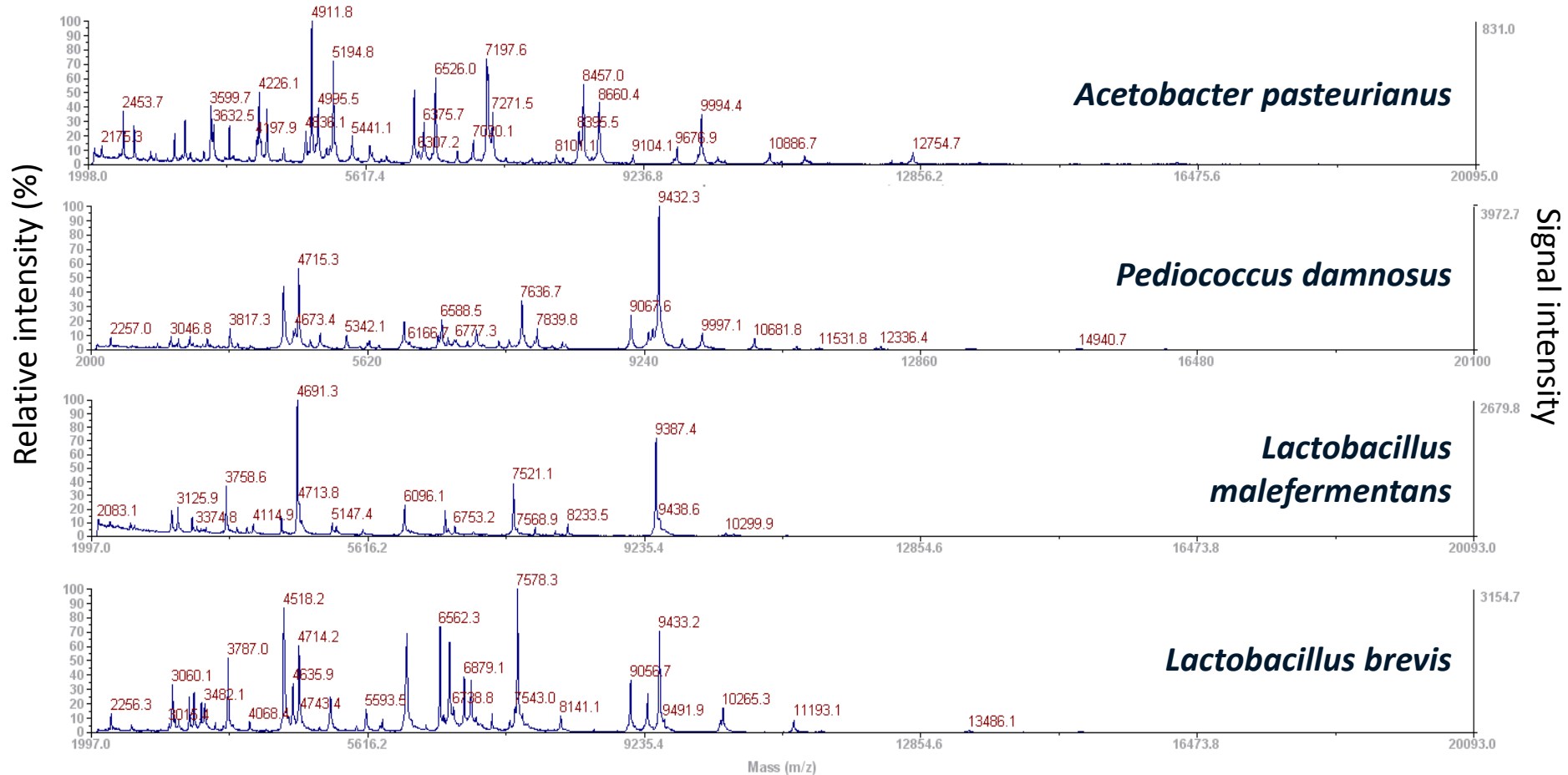
MALDI-TOF MS

TOOL FOR BREWING MICROBIOLOGY



MALDI-TOF MS

IN-HOUSE IDENTIFICATION DATABASE



MALDI-TOF MS

IN-HOUSE IDENTIFICATION DATABASE

1000s of good quality mass spectra

100s of strains representing many species

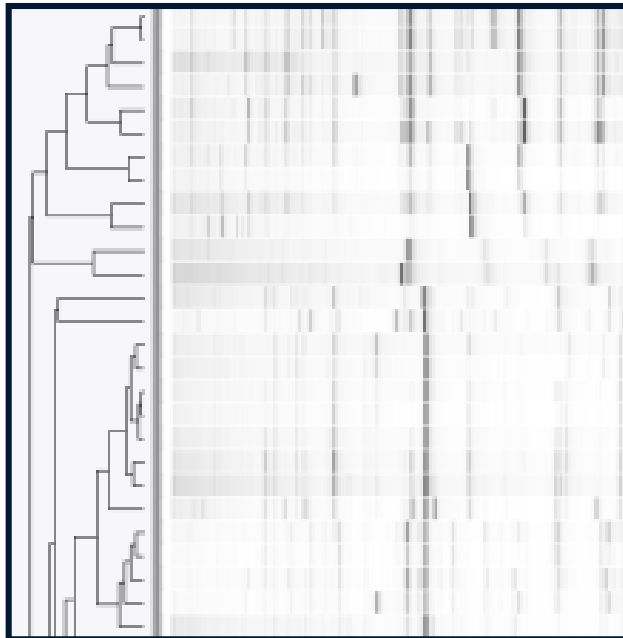
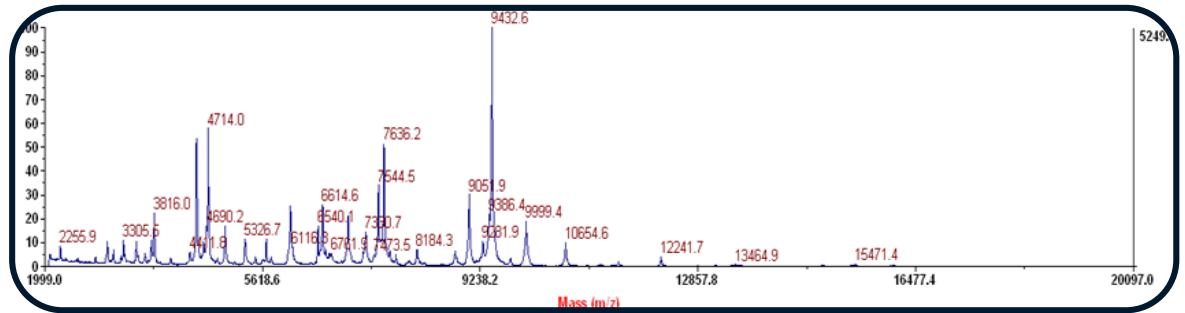
Validation of the reference database

Polyphasic approach

- 16S rRNA gene sequence analysis
- Species specific gene sequence analysis
- FAFLP (Fluorescent amplified fragment length polymorphism)
- DNA-DNA hybridisations

MALDI-TOF MS

DATA ANALYSIS OF MASS SPECTRA



4000 Series Explorer Software 3.5

-

Data Explorer Software 4.6
(.t2d-files ⇌ .txt-files)

-

BioNumerics 7 Applied Maths
(Database)

-

Quality Control

MALDI-TOF MS FOR

IDENTIFICATION BEER SPOILAGE BACTERIA

Microbiology Lambic Beer Fermentation

Differentiation of Brewing Yeast Strains

Identification of Microorganisms

Follow up Fermentation

MALDI-TOF MS FOR

IDENTIFICATION BEER SPOILAGE BACTERIA

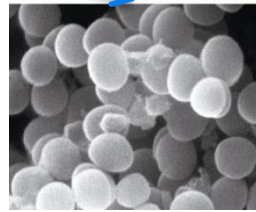
Isolates of Contaminants

**Direct Detection and Identification
Enrichment Cultures**

MALDI-TOF MS

IDENTIFICATION BEER SPOILAGE BACTERIA

Gram positive bacteria



***Lactobacillus* spp.**

Lb. backii
Lb. brevis
Lb. brevisimilis
Lb. buchneri
Lb. casei
Lb. coryniformis
Lb. curvatus
Lb. lindneri
Lb. malefermentans
Lb. parabuchneri
Lb. plantarum

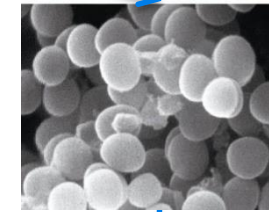
***Pediococcus* spp.**

P. acidilactici
P. claussenii
P. damnosus
P. inopinatus

***Kocuria* spp.**

K. kristinae

Gram negative bacteria



Acetic Acid Bacteria

Acetobacter spp.
Gluconobacter spp.

Enterobacteriaceae

***Pectinatus* spp.**
P. cerevisiiphilus
P. frisingensis

***Selenomonas* sp.**

***Zymomonas* sp.**
***Zymophilus* sp.**

***Megasphaera* spp.**

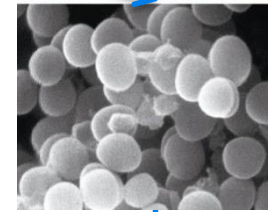
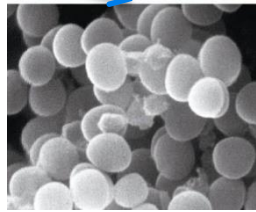
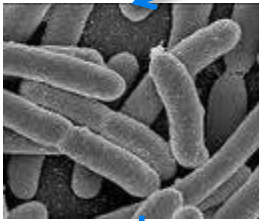
M. cerevisiae
M. paucivorans
M. sueciensis

MALDI-TOF MS

IDENTIFICATION BEER SPOILAGE BACTERIA

Gram positive bacteria

Gram negative bacteria



>75%

Lactobacillus spp.

Lb. backii
Lb. brevis
Lb. brevisimilis
Lb. buchneri
Lb. casei
Lb. coryniformis
Lb. curvatus
Lb. lindneri
Lb. malefermentans
Lb. parabuchneri
Lb. plantarum

L
A
B

Pediococcus spp.

P. acidilactici
P. claussenii
P. damnosus
P. inopinatus

L
A
B

Kocuria spp.

K. kristinae

Acetic Acid Bacteria

Acetobacter spp.
Gluconobacter spp.

A
A
B

Enterobacteriaceae

Pectinatus spp.
P. cerevisiiphilus
P. frisingensis

Selenomonas sp.

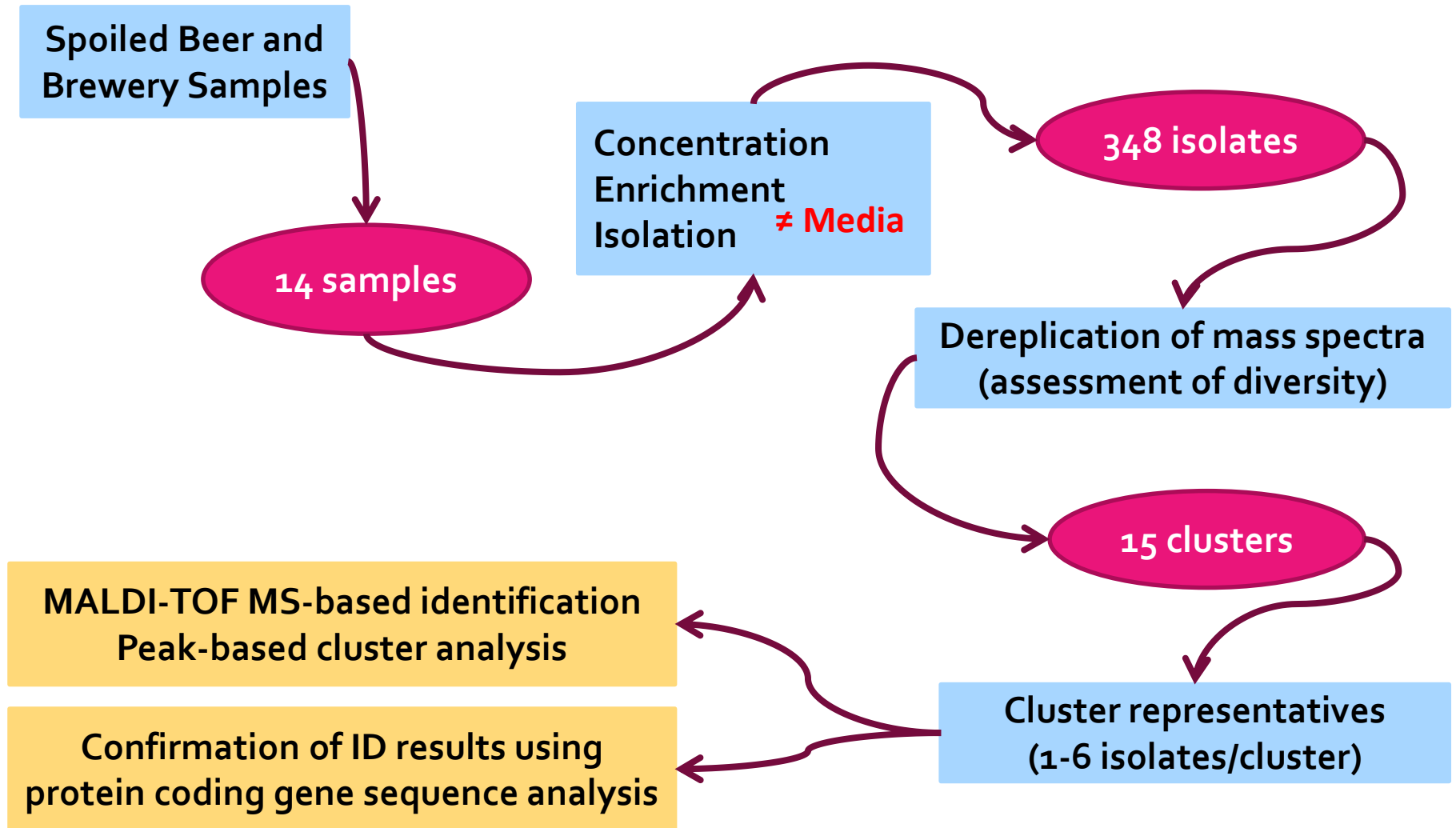
Zymomonas sp.
Zymophilus sp.

Megasphaera spp.

M. cerevisiae
M. paucivorans
M. sueciensis

MALDI-TOF MS

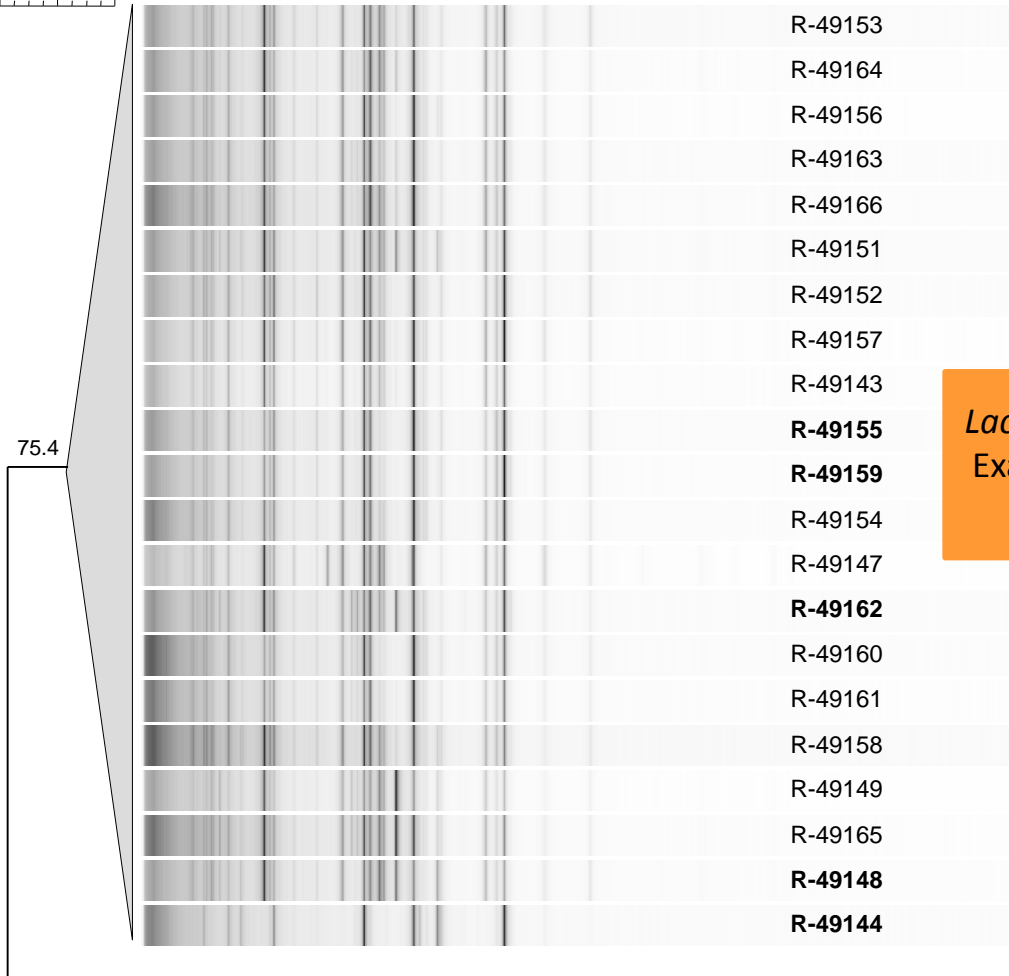
IDENTIFICATION BEER SPOILAGE BACTERIA: ISOLATES



MALDI-TOF MS

IDENTIFICATION BEER SPOILAGE BACTERIA: ISOLATES

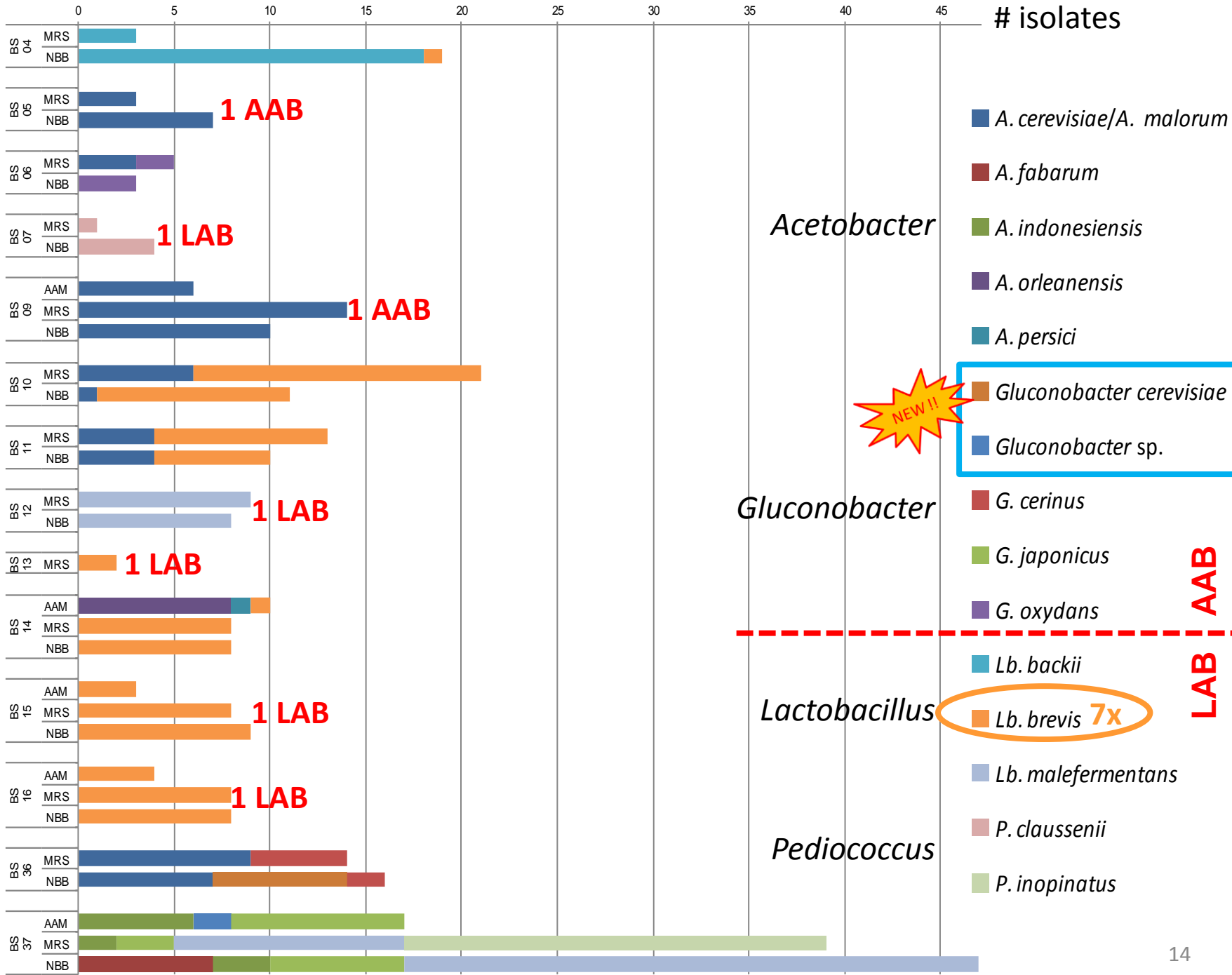
Pearson correlation
(Opt:0.01%) [0.0%-100.0%]



75.4

Lactobacillus brevis
Example of cluster
of isolates

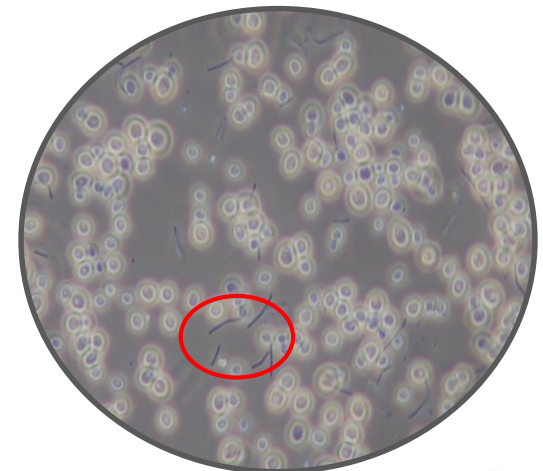
14 samples



MALDI-TOF MS

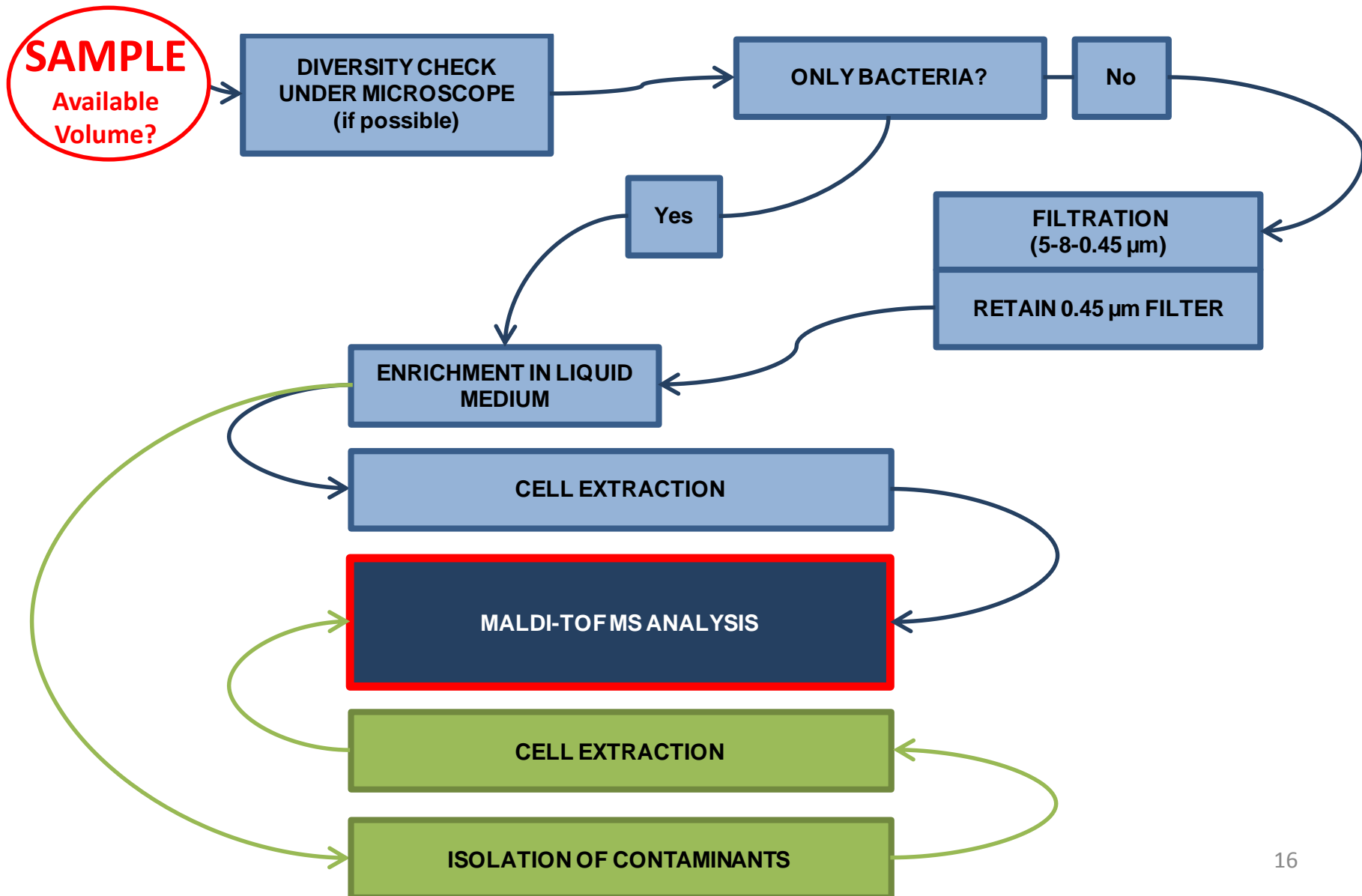
IDENTIFICATION BEER SPOILAGE BACTERIA: DIRECT DETECTION AND IDENTIFICATION ENRICHMENT CULTURES

- ✓ **Beer matrix** → peak suppression effect
 - Easy removal of beer matrix using washing steps
- ✓ **Few cell numbers** present in spoiled samples
 - 1 to 50 CFU/100-250 mL sample
- ✓ Approximately 5×10^7 to 1×10^8 CFU/mL needed for MALDI-TOF MS analysis
- ✓ **Enrichment step necessary**
 - Presence of moulds or yeast cells?
 - Different protocols were tested
 - Filtration step prior to enrichment = best-suited



MALDI-TOF MS

IDENTIFICATION BEER SPOILAGE BACTERIA



MALDI-TOF MS FOR

IDENTIFICATION BEER SPOILAGE BACTERIA

Microbiology Lambic Beer Fermentation

Differentiation of Brewing Yeast Strains

Identification of Microorganisms

Follow up Fermentation

Microbiology lambic beer fermentation

- Beers of spontaneous fermentation
- Spontaneous = no starter cultures
- Acidic beverage
- Basis for other beers e.g. Gueuze, Kriek
- Malted barley, wheat, old hop bells and water
- Traditional, artisan product:

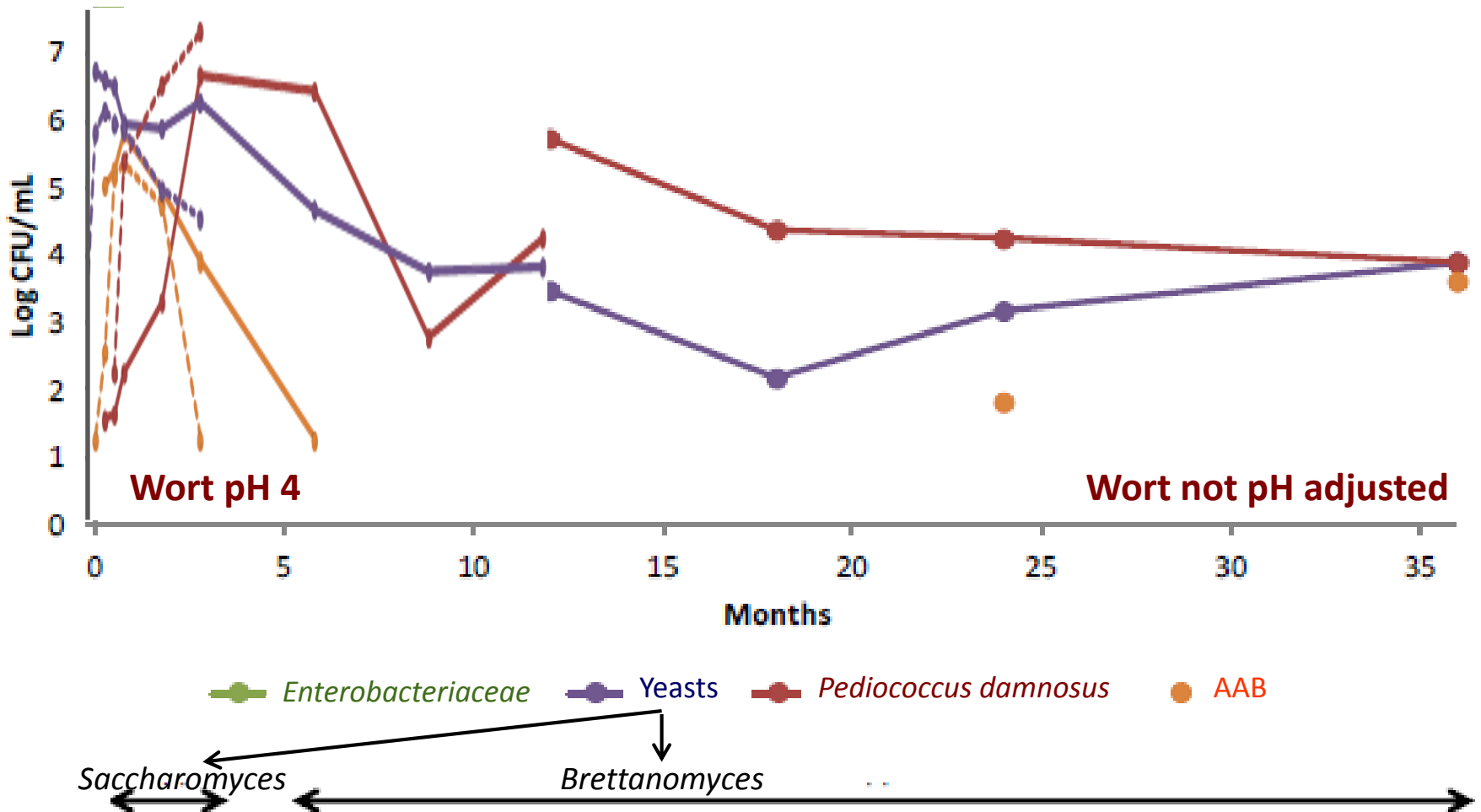


- Aged in wooden barrels for 2 to 3 years
- production "only possible" in Senne river valley



PCR-DGGE / MALDI-TOF MS

Microbiology lambic beer fermentation



MALDI-TOF MS FOR

IDENTIFICATION BEER SPOILAGE BACTERIA

Microbiology Lambic Beer Fermentation

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Identification of Microorganisms

Follow up Fermentation

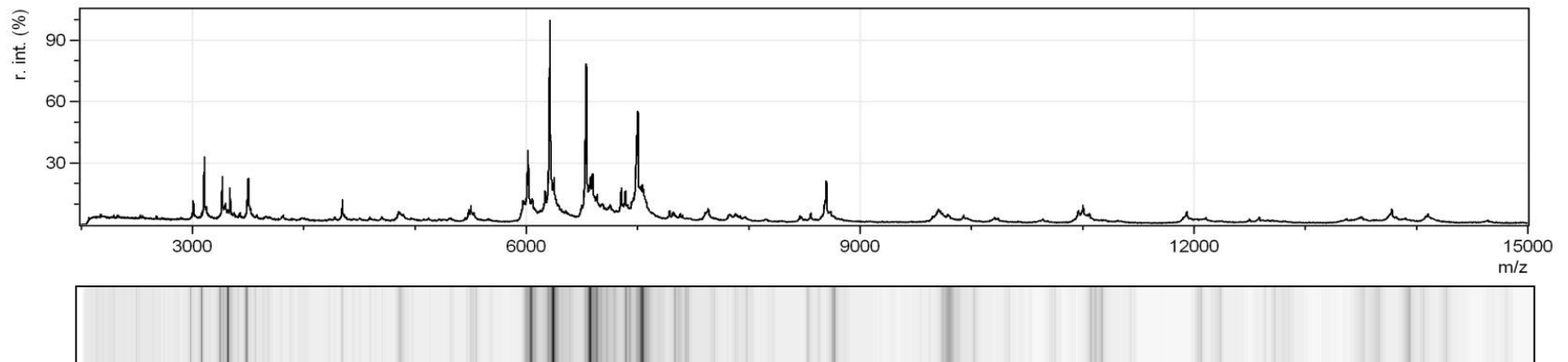
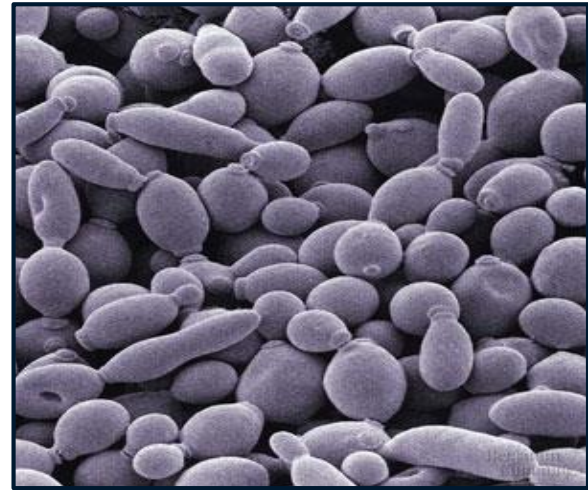
MALDI-TOF MS

DIFFERENTIATION OF BREWING YEAST STRAINS

Case study:
Brewers yeast with deviating
fermentation properties

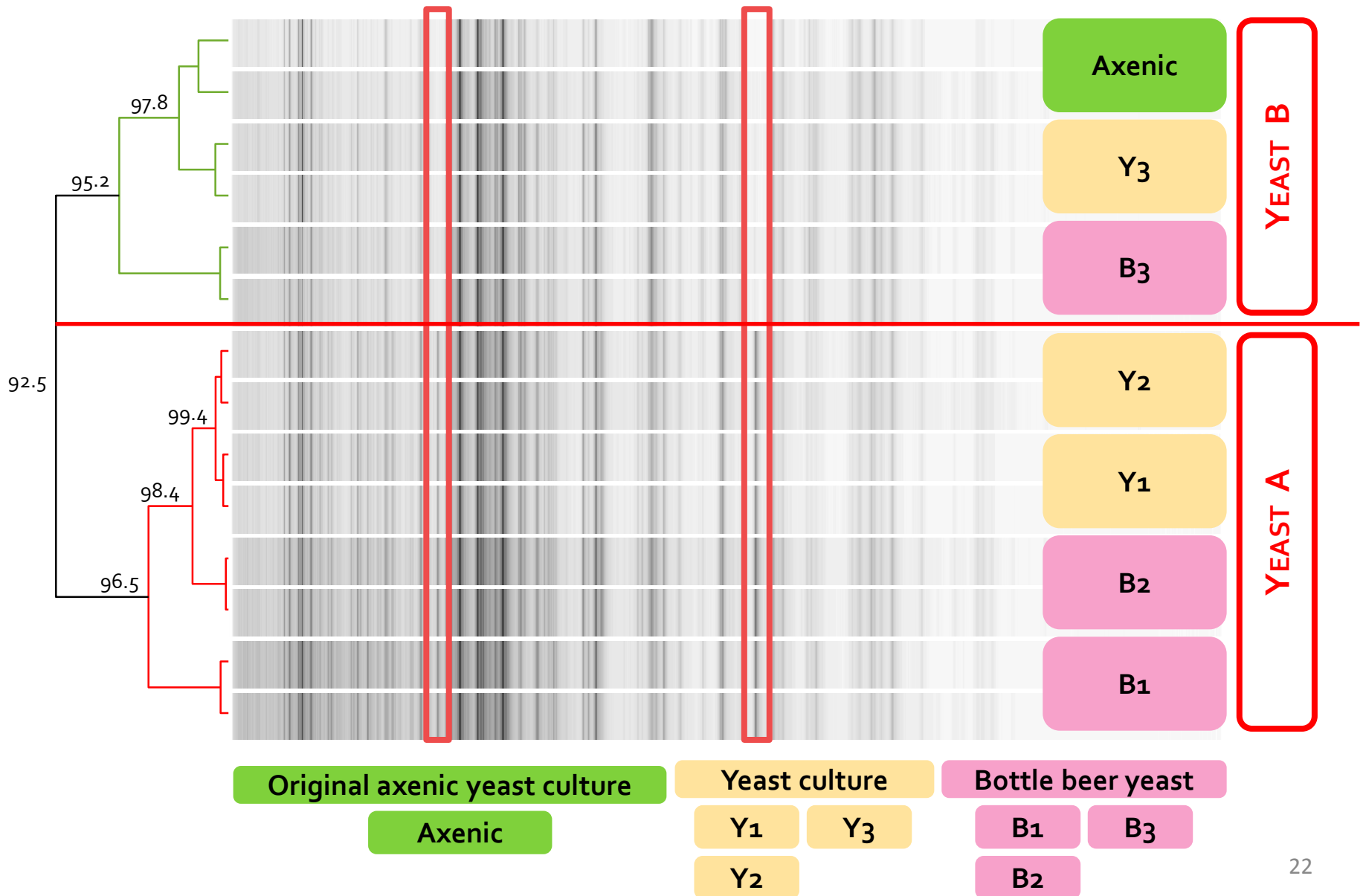
Microbial contamination?
Bacteria? Yeast?

→ MALDI-TOF MS OF ISOLATES



MALDI-TOF MS

DIFFERENTIATION OF BREWING YEAST STRAINS



MALDI-TOF MS FOR

IDENTIFICATION BEER SPOILAGE BACTERIA

Microbiology Lambic Beer Fermentation

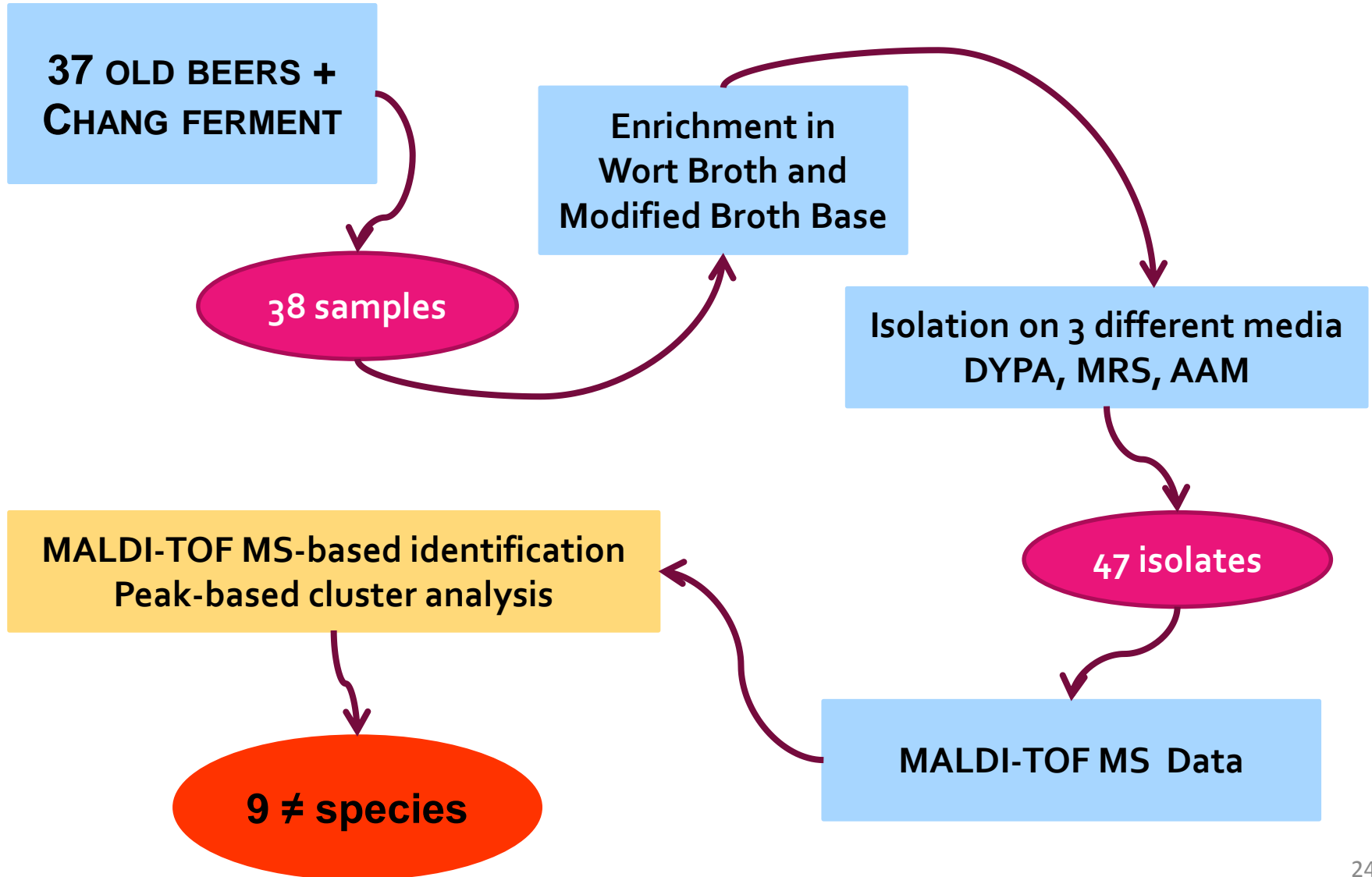
Differentiation of Brewing Yeast Strains

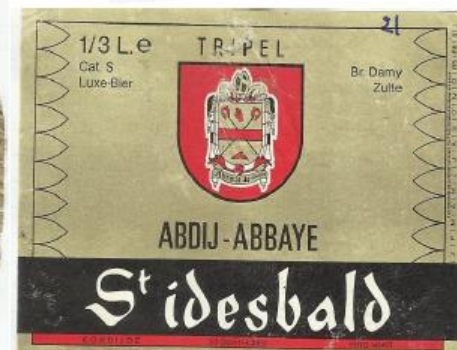
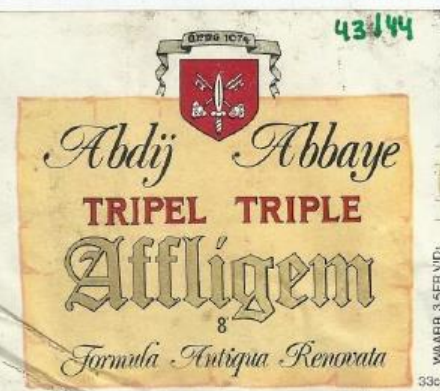
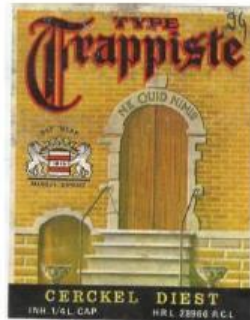
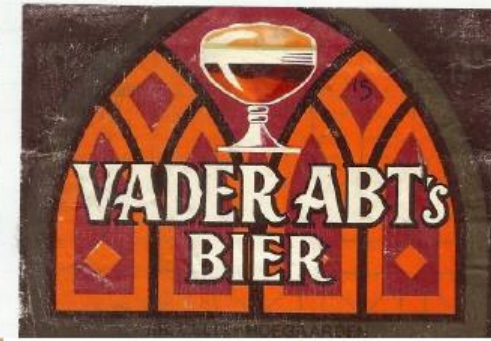
Identification of Microorganisms

Follow up Fermentation

MALDI-TOF MS


IDENTIFICATION OF MICROORGANISMS





MALDI-TOF MS

IDENTIFICATION OF MICROORGANISMS

Isolate(s)	Beer/sample	Microorganism	pH	Alcohol (%V/V)
11f,g 11h 11a,b,c,e 11d	Ladakh (ferment) 	<i>Lactobacillus plantarum</i> <i>Pediococcus acidilactici</i> <i>Pichia sp.</i> <i>Saccharomyces cerevisiae</i>		
7	Oud Hoegaards bier	<i>Pediococcus acidilactici</i>	3.89	4.80
10a 10b	Crombe	<i>Brettanomyces bruxellensis</i> <i>Enterococcus faecium</i>	4.19	5.54
14	Rodenbach	<i>Saccharomyces cerevisiae</i>	3.45	5.63
18a 18b,c	Duvel	<i>Brettanomyces bruxellensis</i> <i>Lactobacillus brevis</i>	3.81	6.59

MALDI-TOF MS

IDENTIFICATION OF MICROORGANISMS

Isolate(s)	Beer/sample	Microorganism	pH	Alcohol (%V/V)
19	Liefmans	<i>Lactobacillus brevis</i>	3.45	4.46
20a,b 20c	Tripel Affligem	<i>Lactobacillus brevis</i> <i>Pichia sp.</i>	4.17	8.30
21	Tripel St. Idesbald	<i>Saccharomyces cerevisiae</i>	3.50	8.12
22	BIOS	<i>Lactobacillus brevis</i>	3.27	6.18
24a,b	Trappiste	<i>Yeast not identified</i>	4.38	7.48
26	Diesters bier	<i>Lactobacillus brevis</i>	3.52	7.80
29	Abdij Affligem	<i>Acetobacter sp.</i>	4.09	7.00
33	Witkap pater	<i>Brettanomyces bruxellensis</i>	3.54	5.53
37b 37a	ID	<i>Lactobacillus brevis</i> <i>Brettanomyces anomala</i>	3.63	8.42

MALDI-TOF MS FOR

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4 time points fermentation

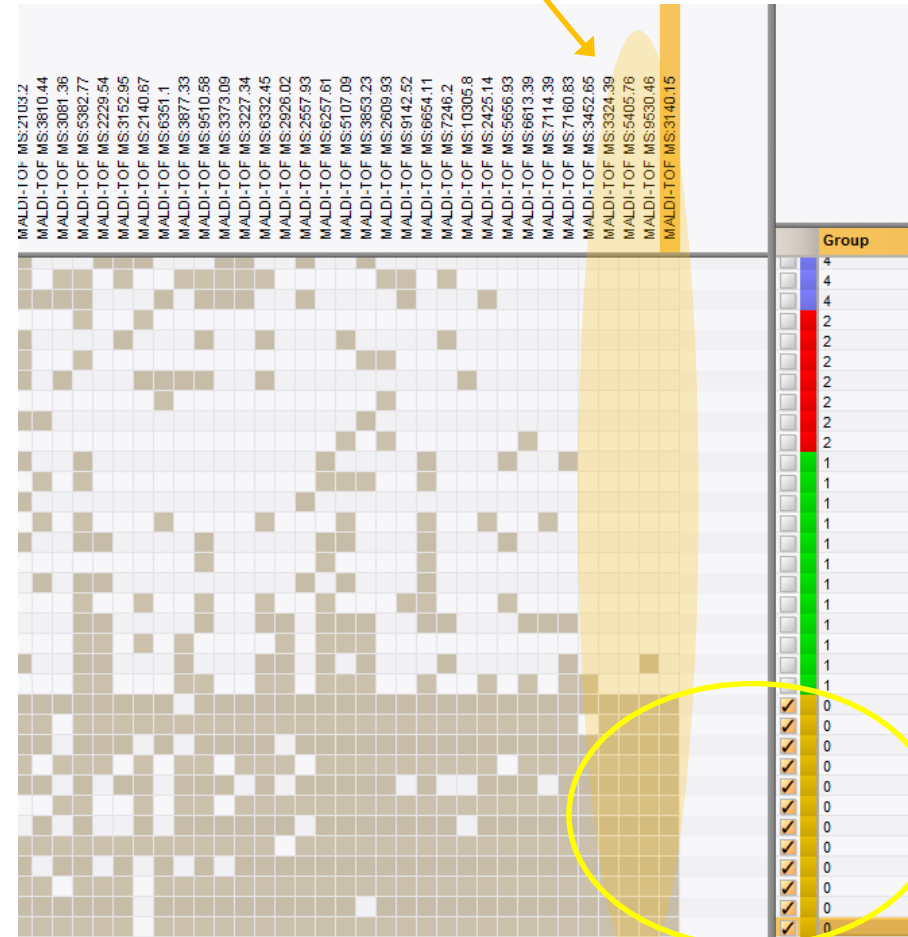
→ 36 spectra/time point

1 day

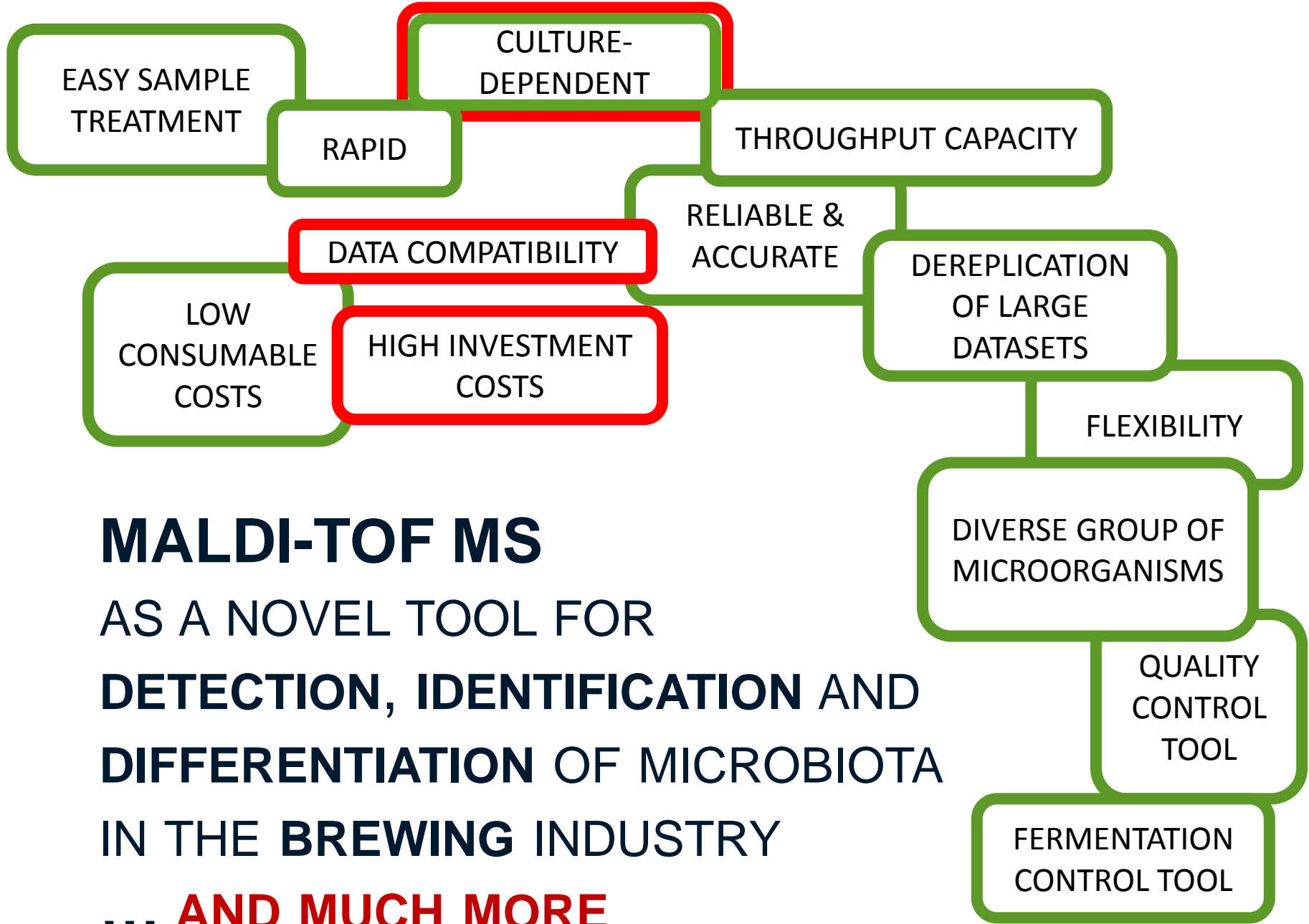
t = 0

2 days

4 days



Discriminant analysis



MALDI-TOF MS
AS A NOVEL TOOL FOR
DETECTION, IDENTIFICATION AND
DIFFERENTIATION OF MICROBIOTA
IN THE **BREWING INDUSTRY**
... AND MUCH MORE

Thanks to:

Dr. Anneleen Wieme, Dr. Freek Spitaels,
ing. Jolien Koek, ing. Freya Van Alboom

Dr. Koenraad Van Hoorde & Prof. Dr. Peter Vandamme

