

## BEER FERMENTATION PROCESSES AND MALDI-TOF MS FOR BREWING MICROBIOLOGY



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### BEER FERMENTATION PROCESSES

#### The 4 fermentation processes

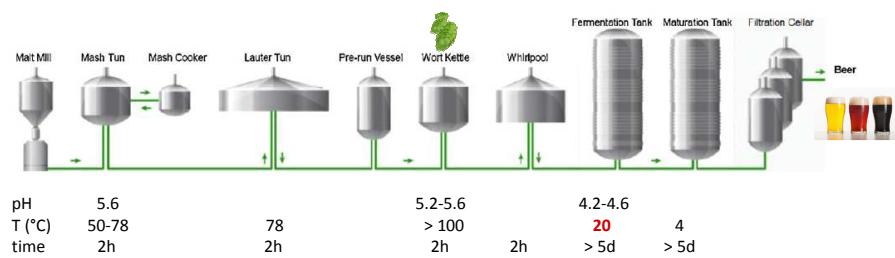


Spontaneous fermentation    Top fermentation  
Mixed fermentation

Bottom fermentation

## BEER FERMENTATION PROCESSES

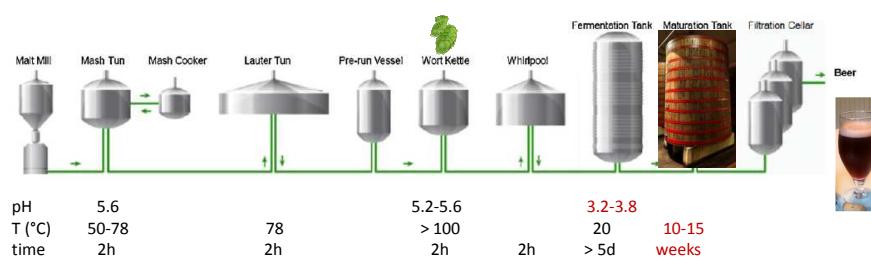
**Top and Bottom fermentation**  
Micro-organisms: pure brewers yeast



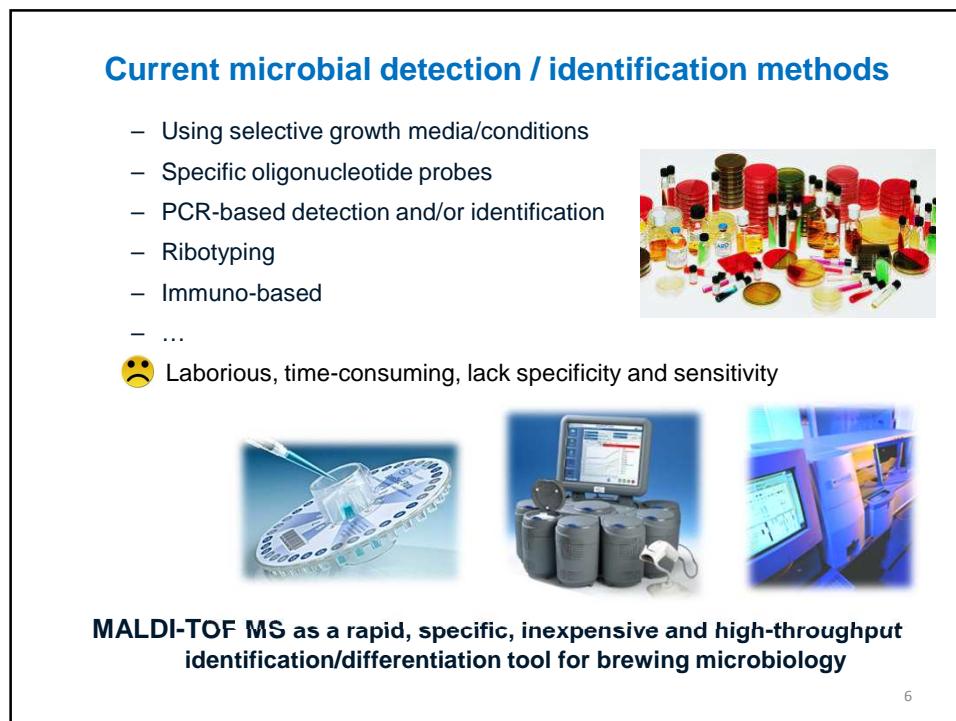
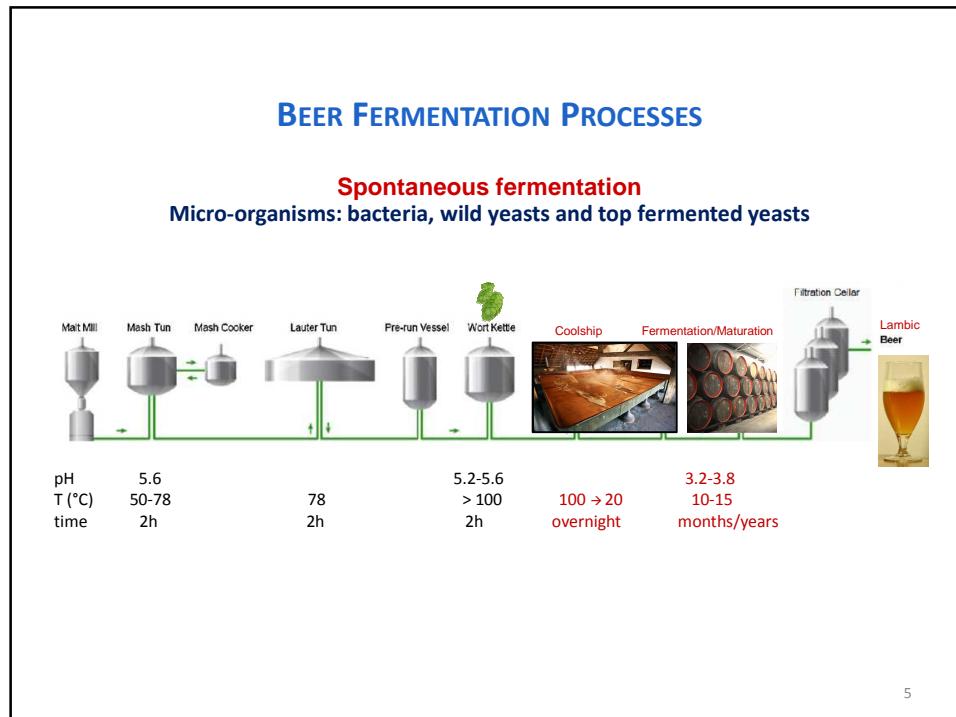
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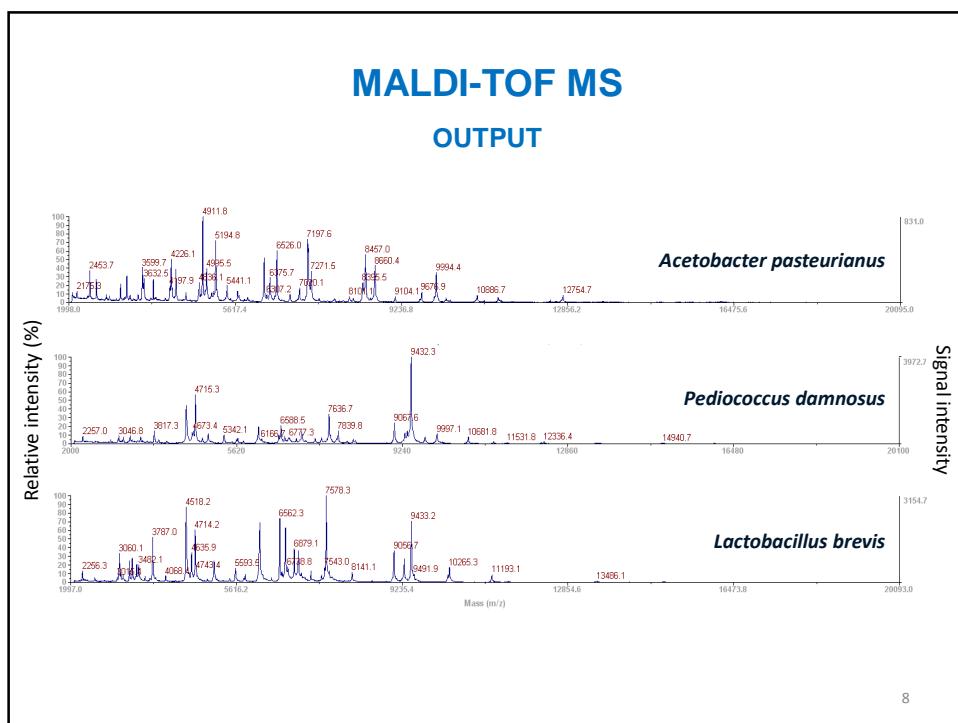
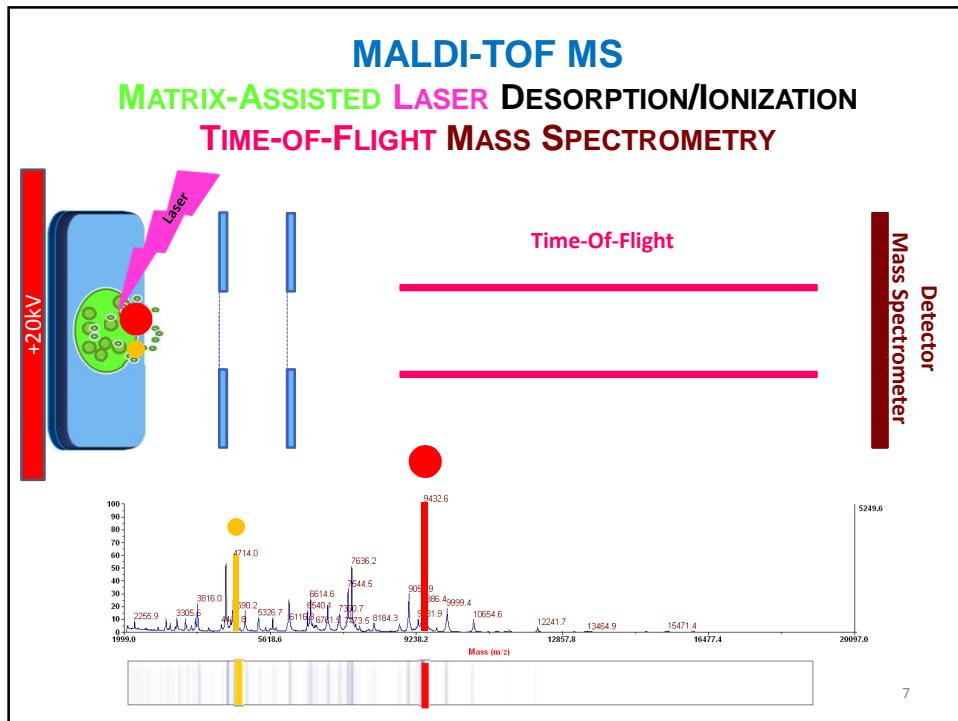
## BEER FERMENTATION PROCESSES

**Mixed fermentation**  
Micro-organisms: bacteria and top fermented yeasts

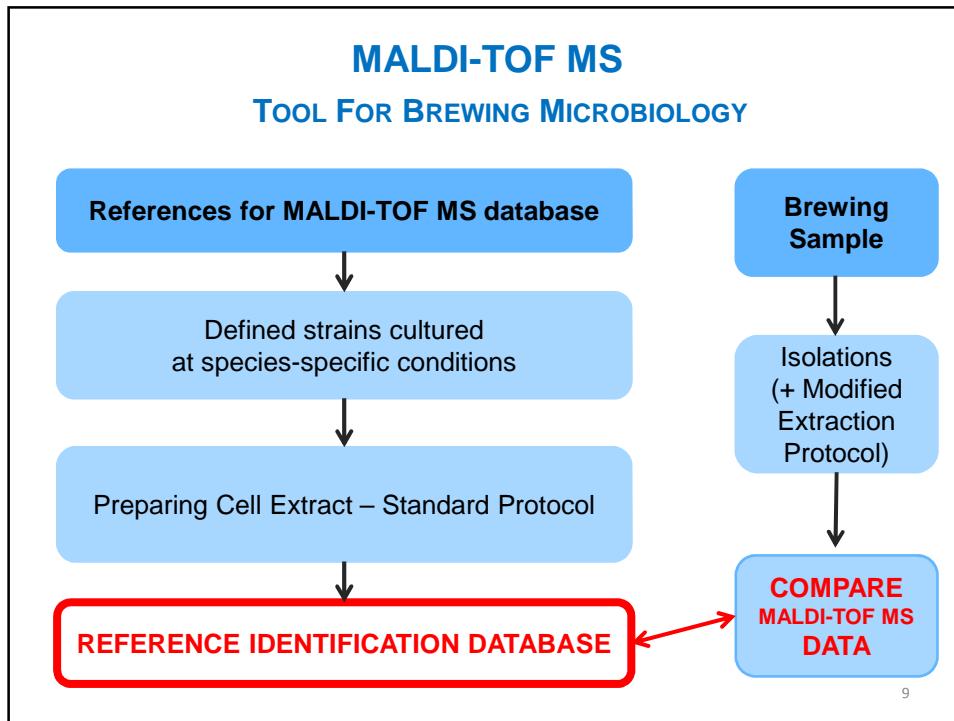


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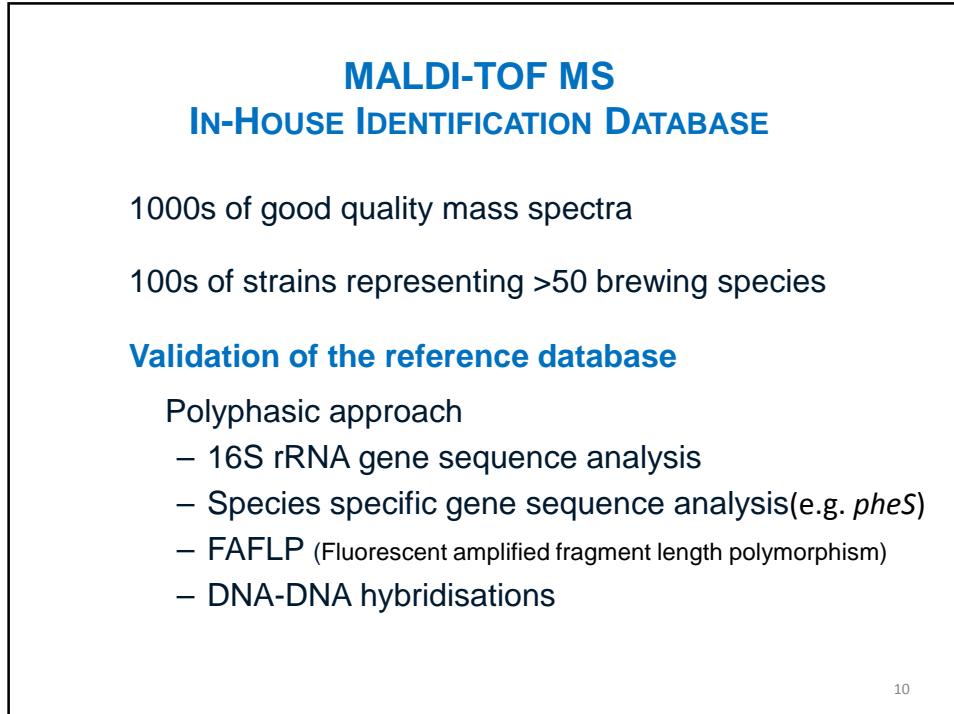




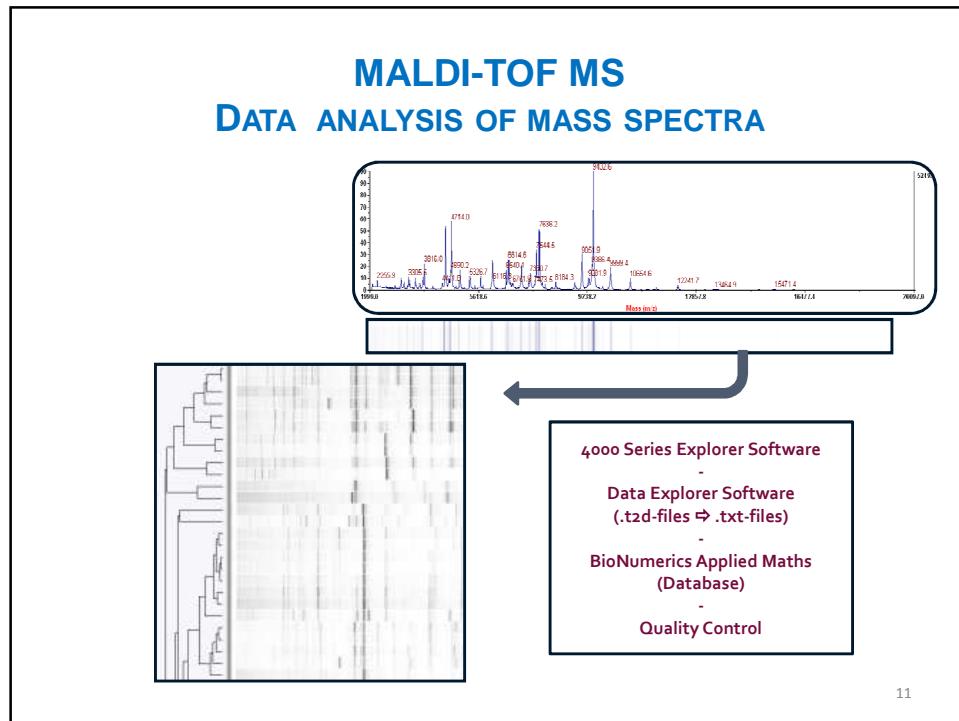
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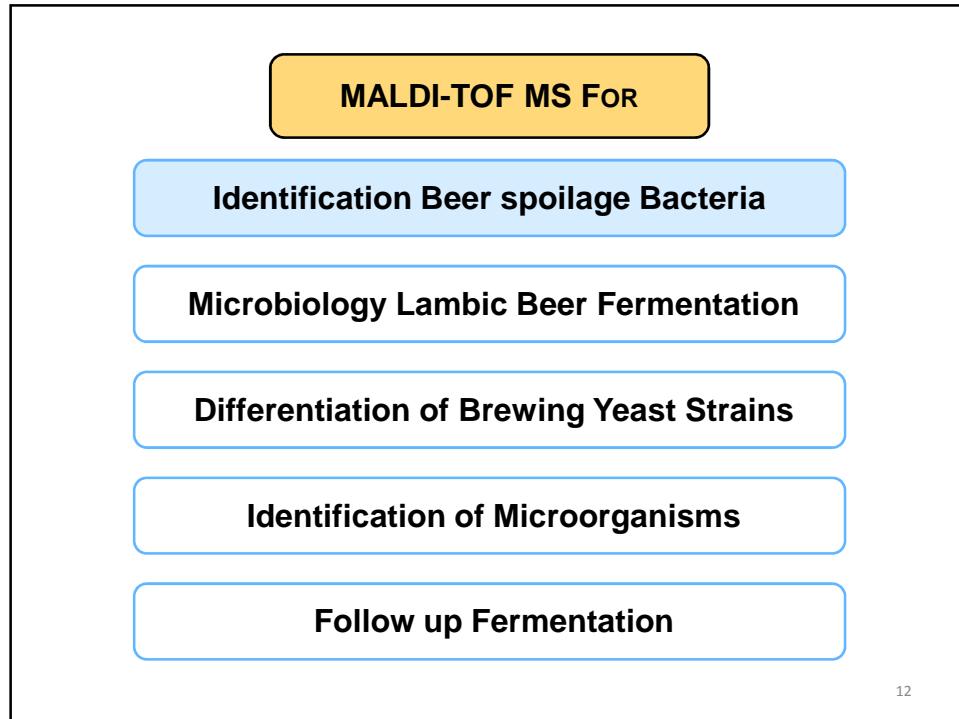
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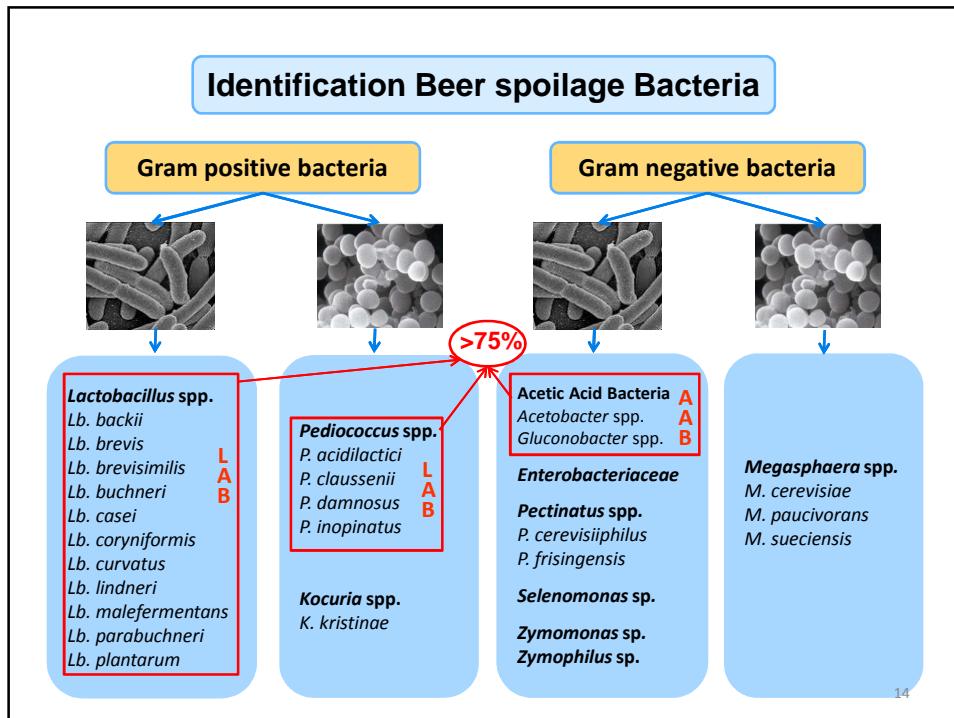
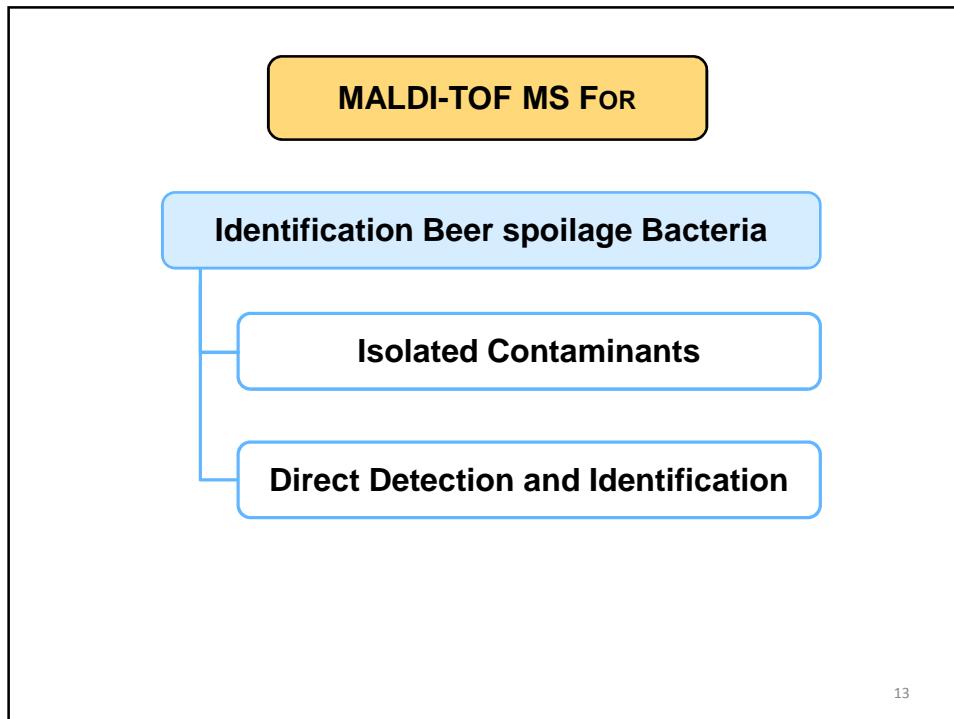
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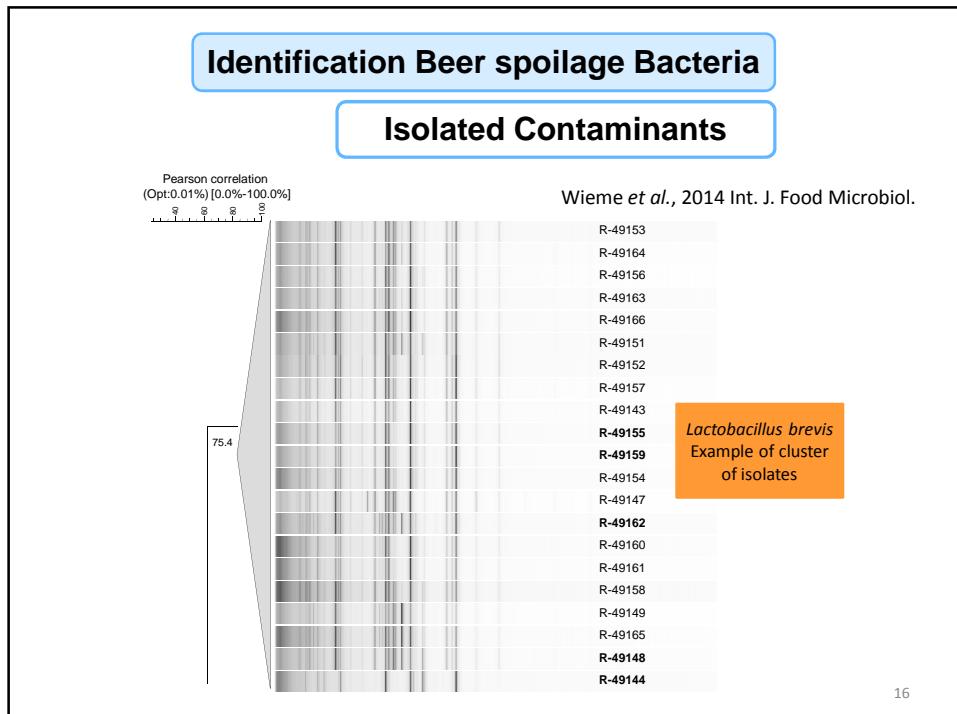
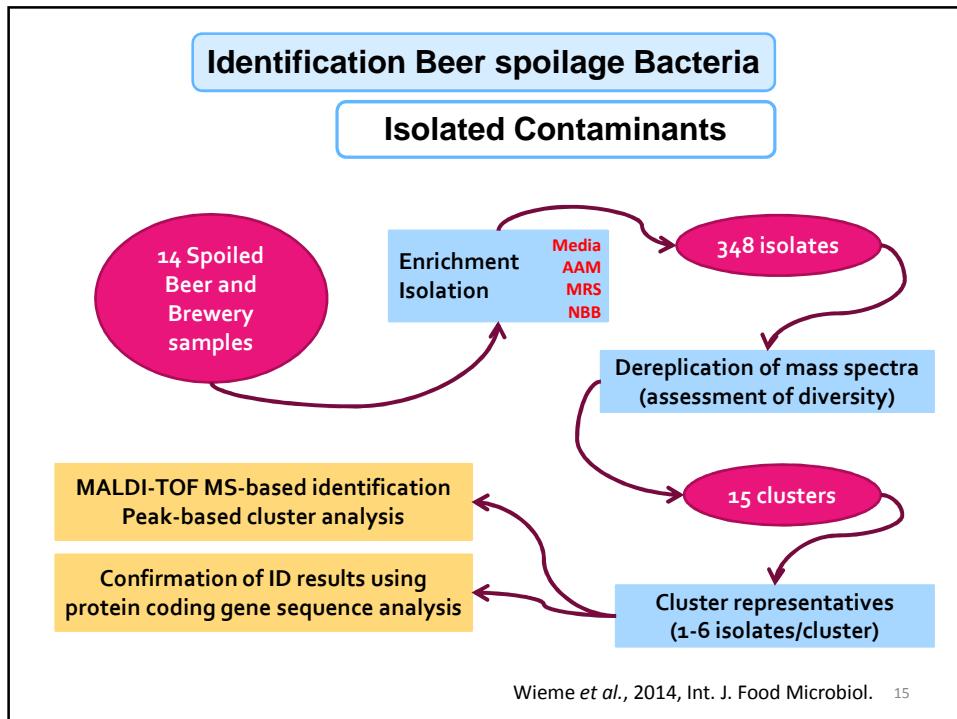


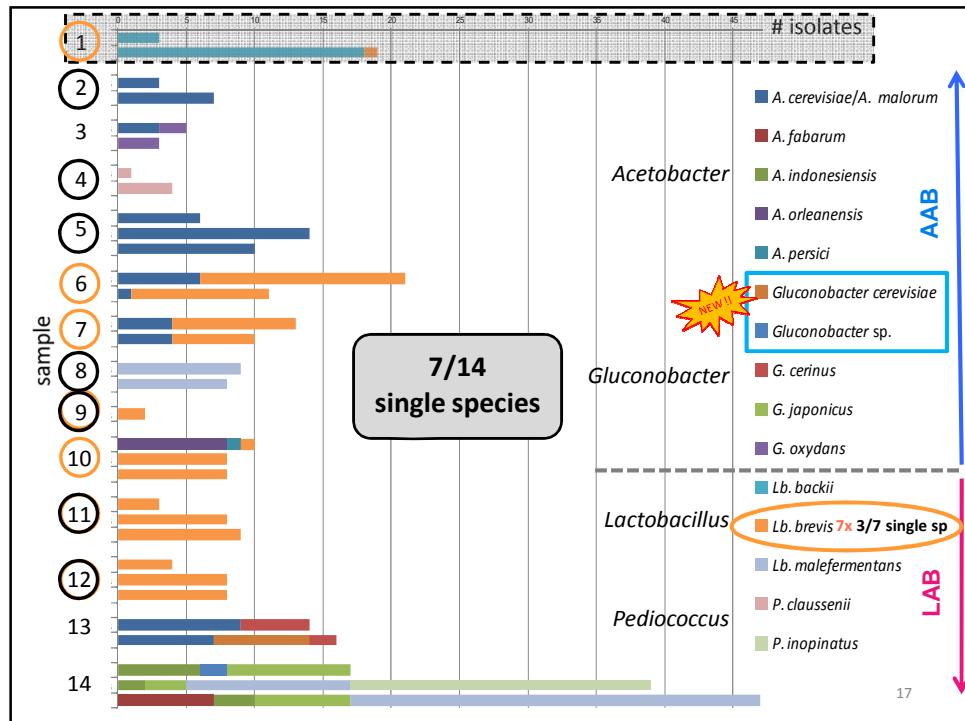
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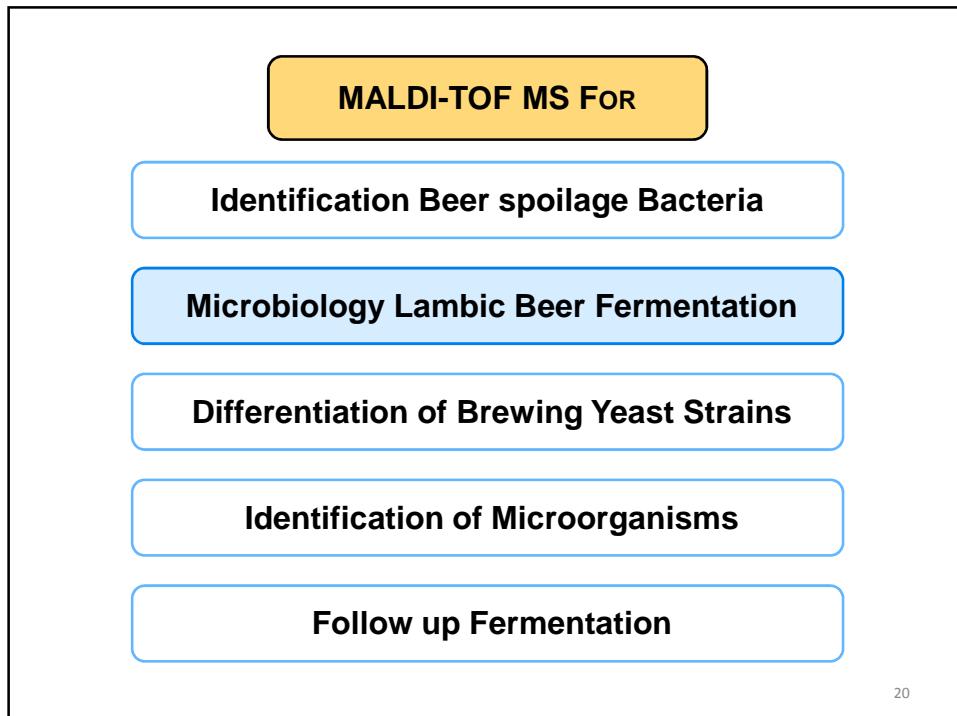
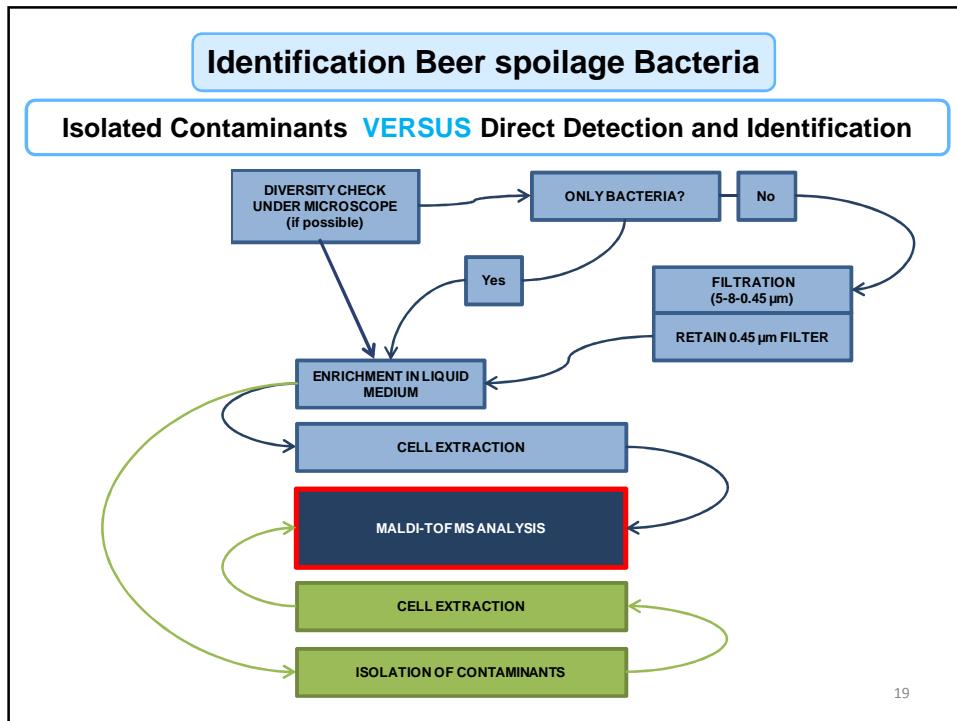


### Identification Beer spoilage Bacteria

#### Direct Detection and Identification

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

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### Microbiology Lambic Beer Fermentation

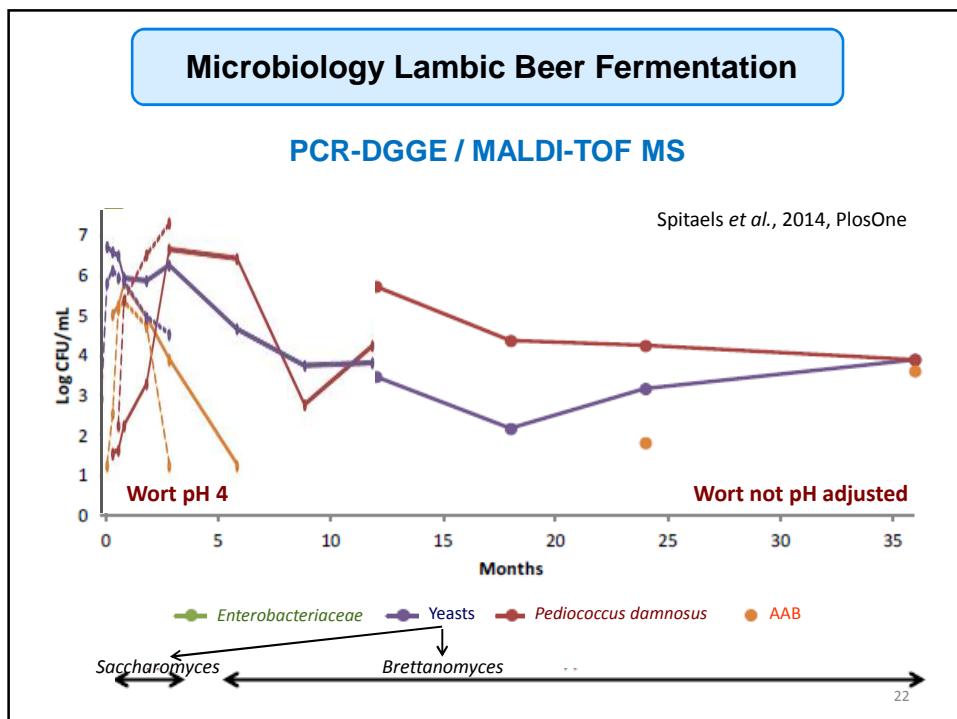
- Beers of spontaneous fermentation
- Spontaneous = no starter cultures
- Acidic beverage
- Basis for other beers e.g. Gueuze, Kriek
- Malted barley, wheat, old hop bells and water
- Traditional, artisan product:
  - Aged in wooden barrels for 2 to 3 years
  - production "only" in Senne river valley







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**MALDI-TOF MS FOR**

**Identification Beer spoilage Bacteria**

**Microbiology Lambic Beer Fermentation**

**Differentiation of Brewing Yeast Strains**

**Identification of Microorganisms**

**Follow up Fermentation**

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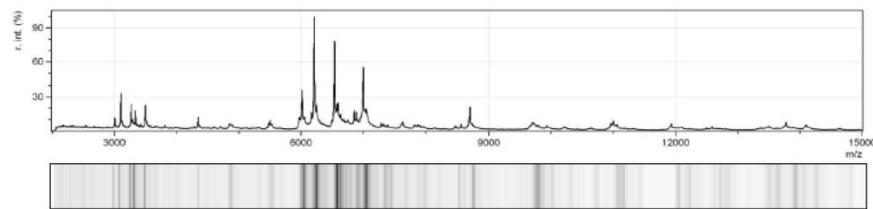
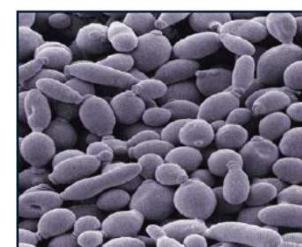
**Differentiation of Brewing Yeast Strains**

**Case study:**

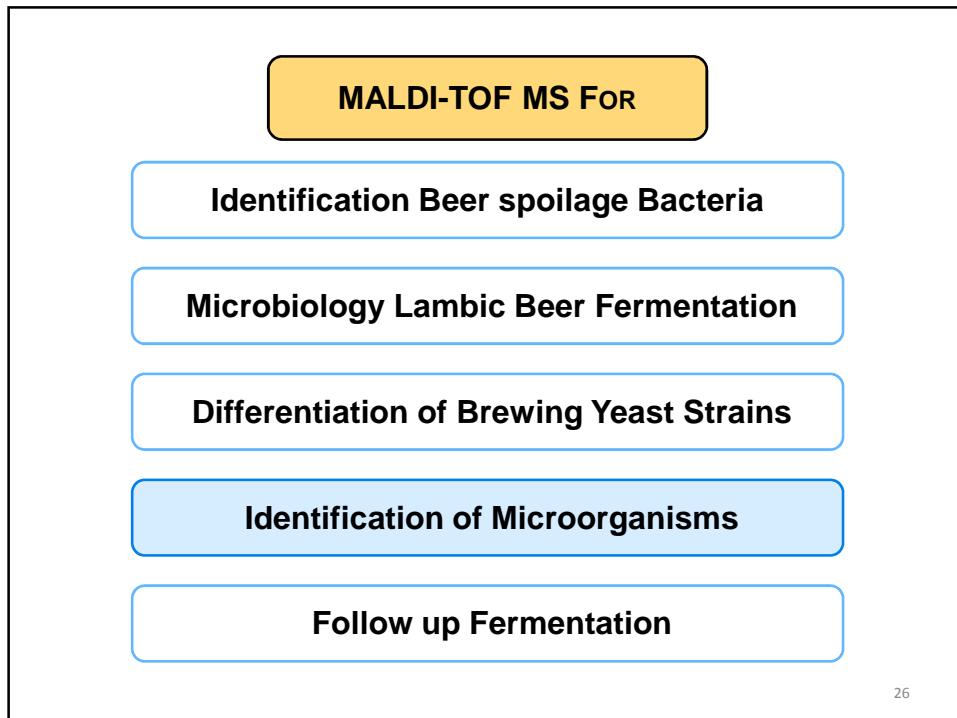
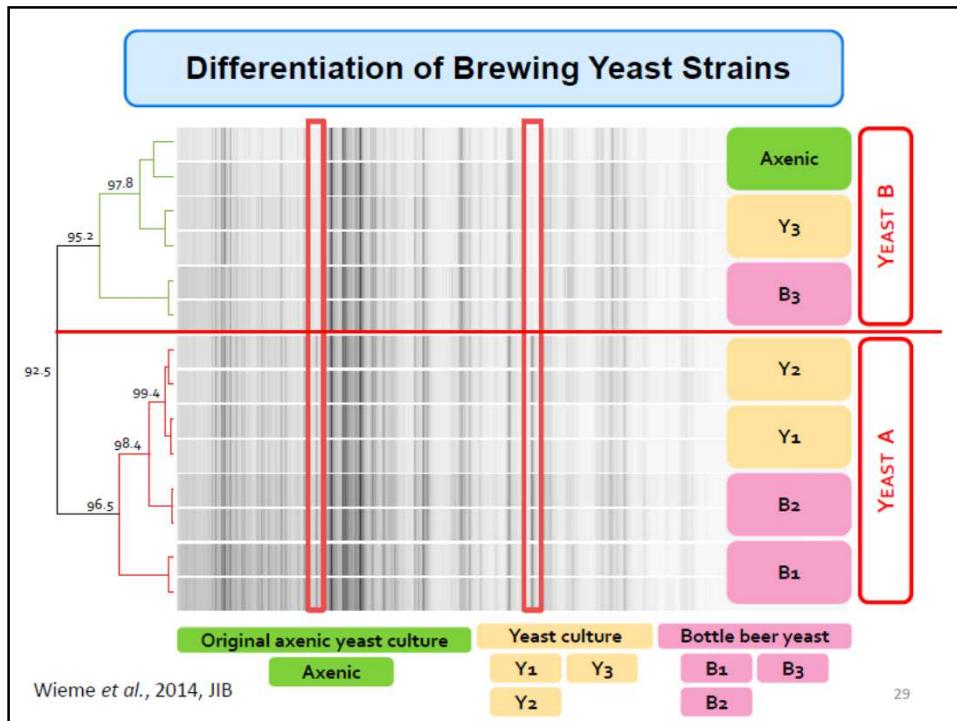
Brewers yeast with deviating  
fermentation properties

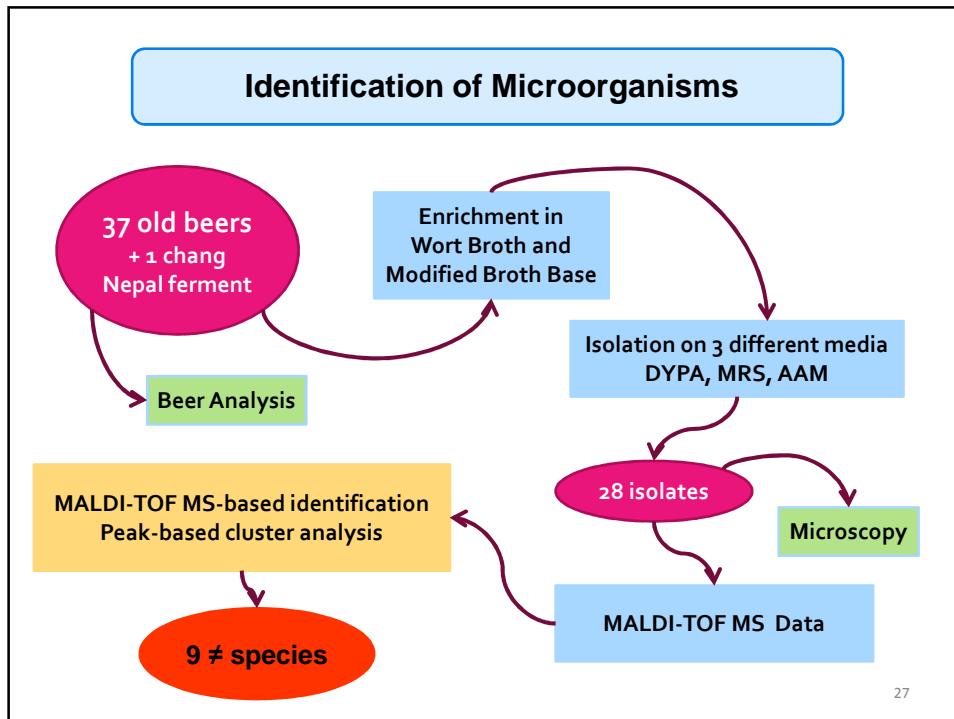
Microbial contamination?  
Bacteria? Yeast?

→ MALDI-TOF MS OF ISOLATES



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### Identification of Microorganisms

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

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### 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

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