

An approach to screen diverse cheese-related bacteria for their ability to produce aroma compounds

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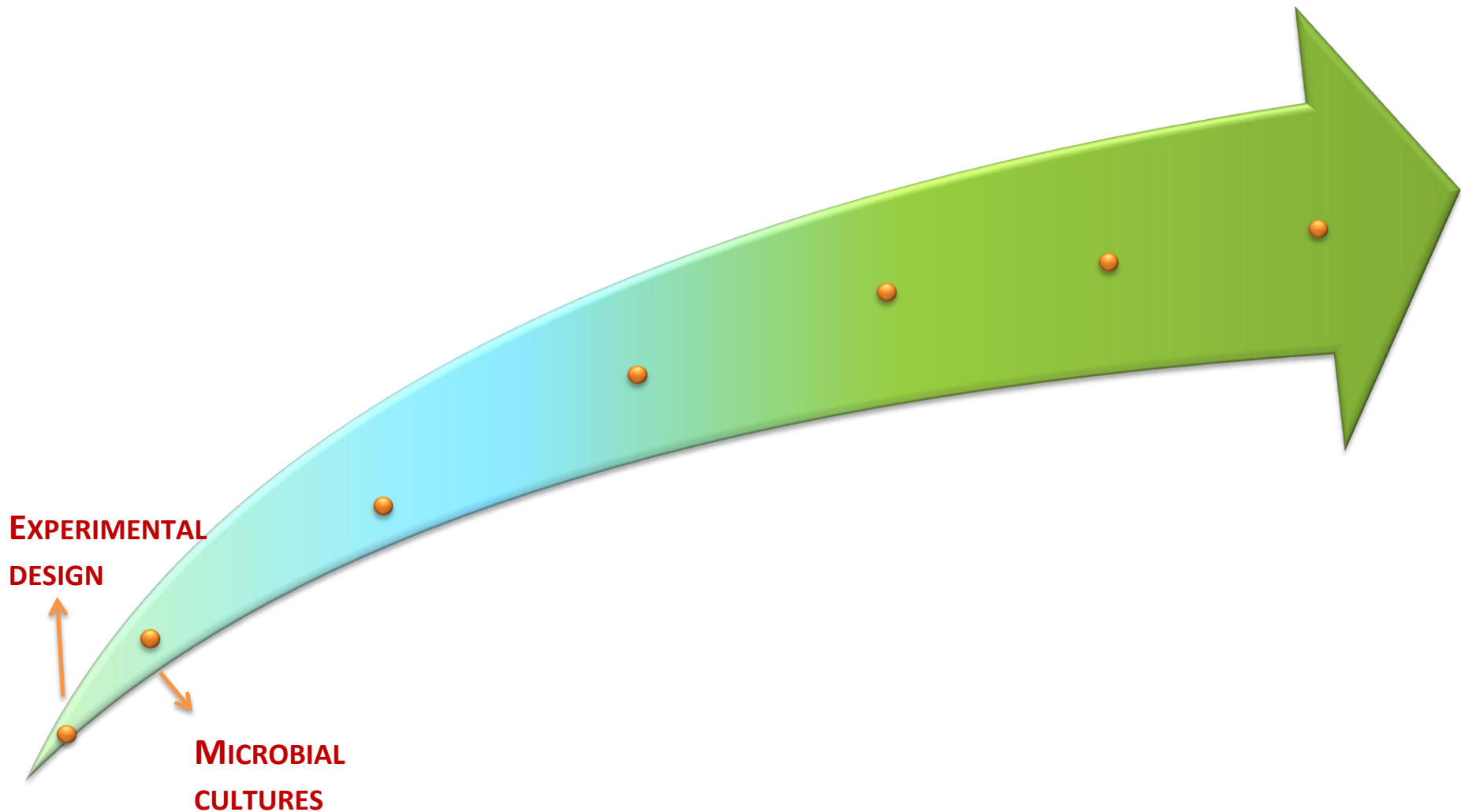
Why?

Flavour...

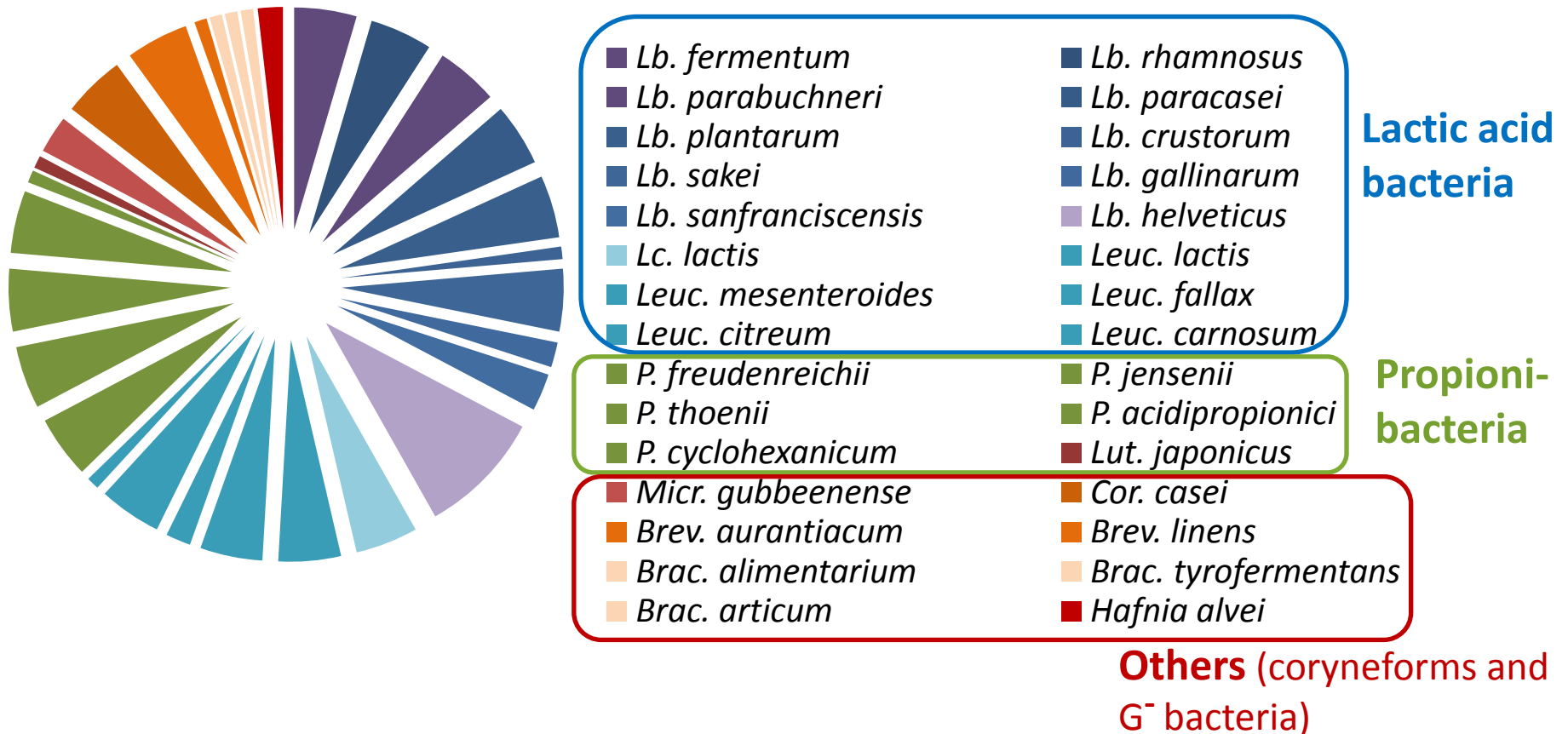
- ✓ an important property for consumers choosing food products
- ✓ is the result of the correct balance and concentration of a wide variety of volatile flavour compounds
 - Mulder (1952)*
 - Kosikowski and Mocquot (1958)*
- ✓ Flavour of fermented food products, including cheese, largely results from the production of aroma compounds by microorganisms
- ✓ The production of flavour compounds by micro-organisms is highly strain-dependent, thus requiring screening studies



A metabolomics-based approach: first steps

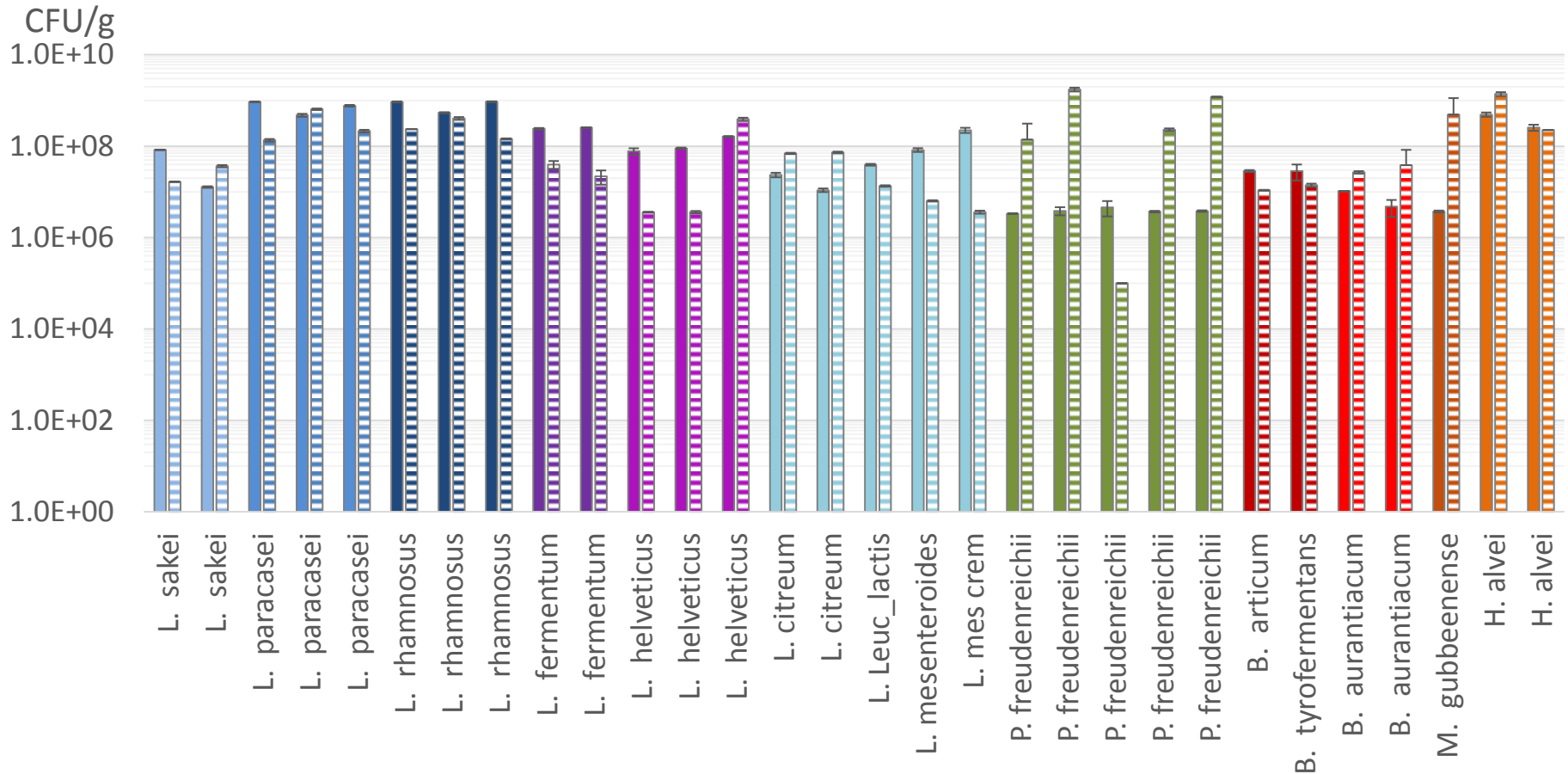


~100 strains tested: 10 genera, 30 species



Growth of bacterial strains

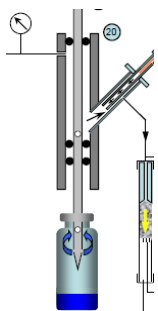
Culturable population, enumerated at 24 h and after 3 weeks incubation at 15°C



maximal populations > 4.10⁷ CFU/g for most strains

SAMPLE EXTRACTION AND ANALYSIS

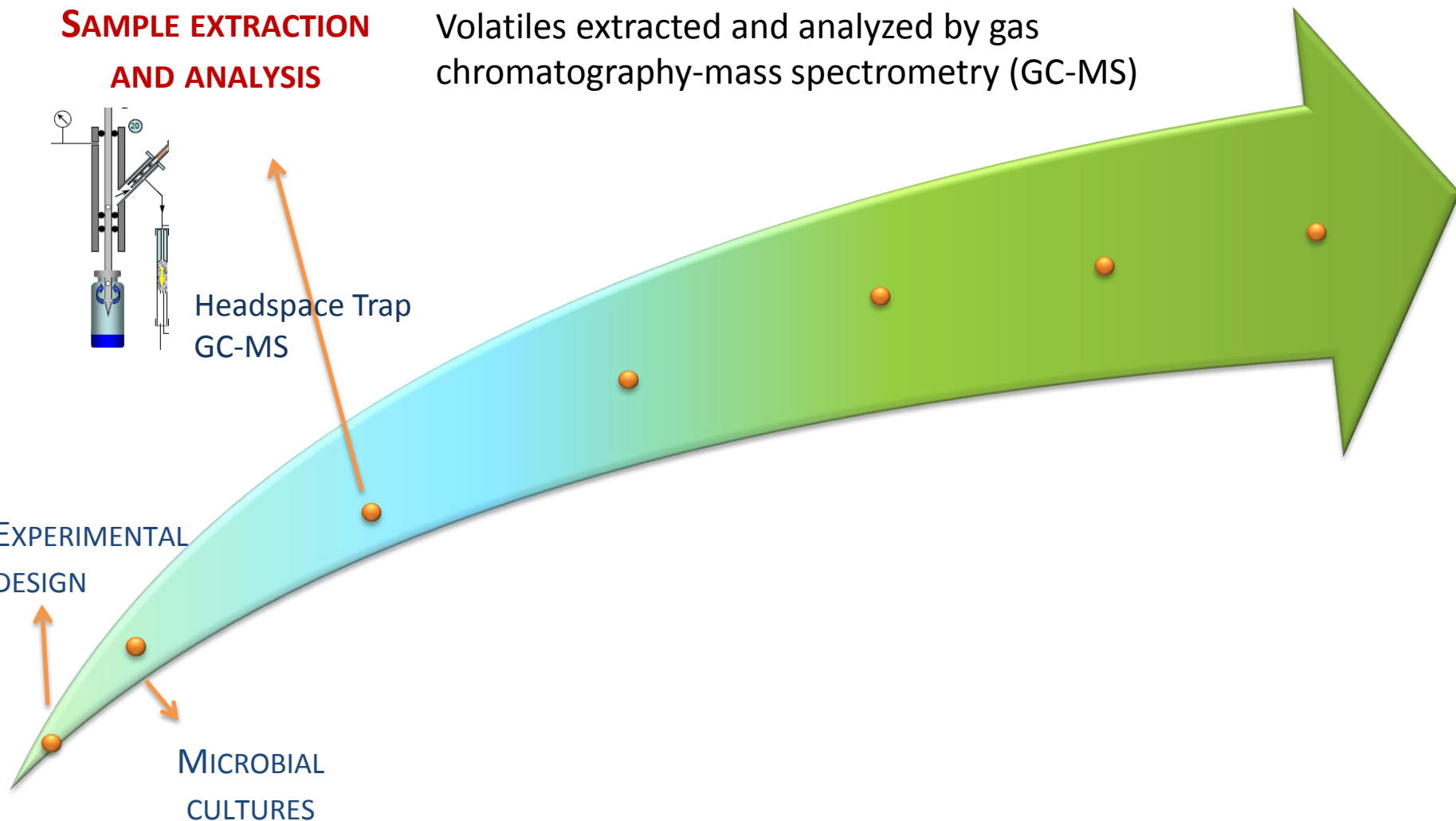
Volatiles extracted and analyzed by gas chromatography-mass spectrometry (GC-MS)



Headspace Trap
GC-MS

EXPERIMENTAL
DESIGN

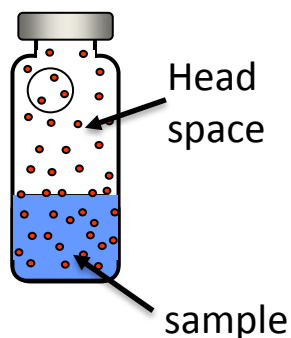
MICROBIAL
CULTURES



SAMPLE EXTRACTION: Head space methods

- ✓ Extraction is a key step !
- ✓ Head space-related methods widely used for study of food flavor

static headspace



- 😊 reliable
- 😞 poor sensitivity

Other head space-related methods

- Solid Phase Micro-Extraction (SPME):

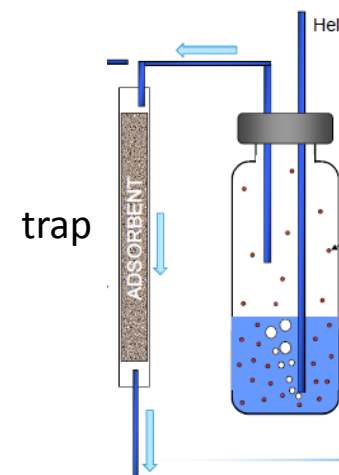
the most widely used for food flavour

- 😞 Small trap capacity
→ risk of competition between the volatiles

- ...

- 📁 Head Space Trap (Perkin Elmer)

dynamic headspace

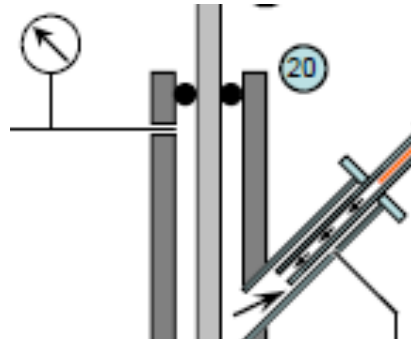


- 😊 sensitive
- 😞 automation

SAMPLE PREPARATION EXTRACTION AND ANALYSIS: Head Space Trap-GC-MS



Headspace Trap (Perkin Elmer patent)



Optimization: four parameters tested

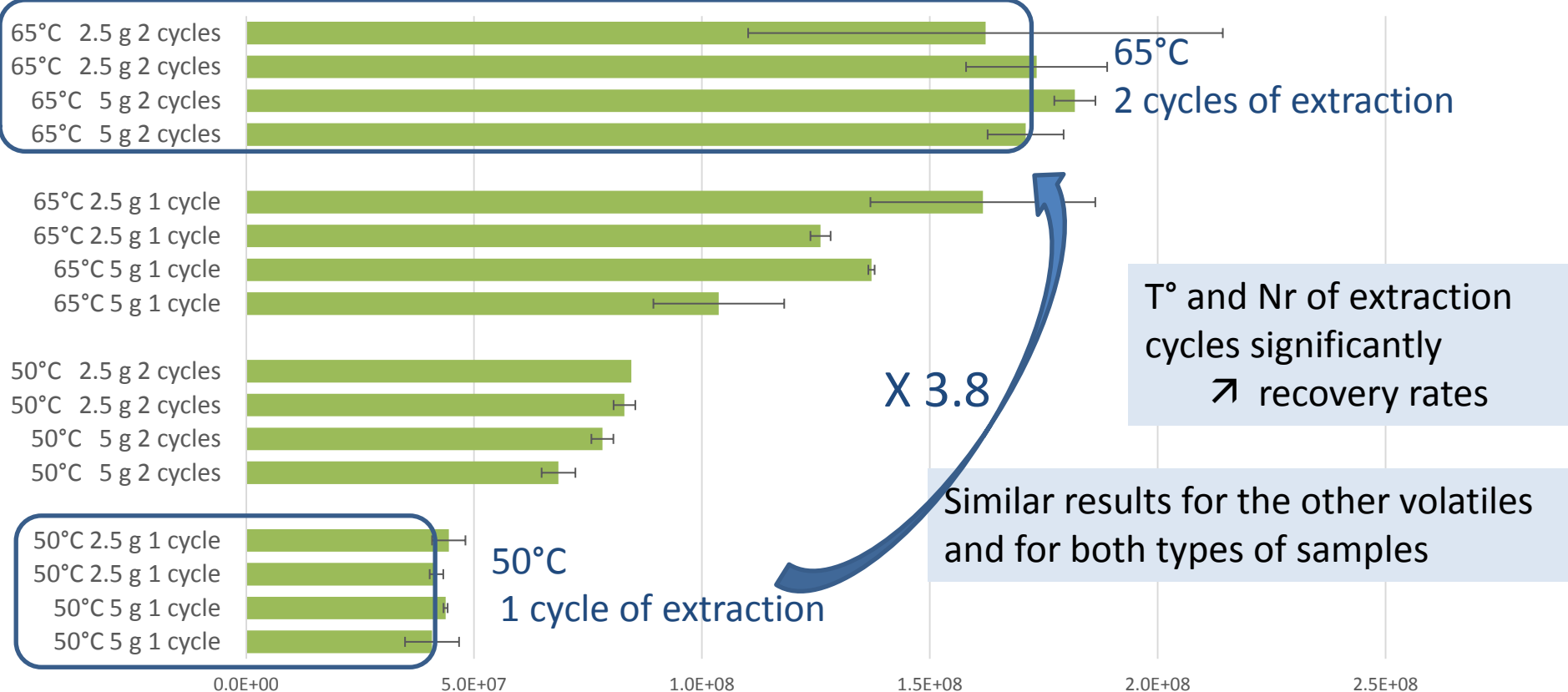
- **T°**: 50-60°C
- **Time**: 15-30 min
- **Nr** of repeated extractions: 1-2
- **Sample size**: 2.5-5 g/vial

for two types of samples: cheese and a mixture of standard compounds

☞ example for the extraction of diacetyl from cheese

Volatile extraction: optimisation of extraction

Abundance of diacetyl as function of the extraction parameters

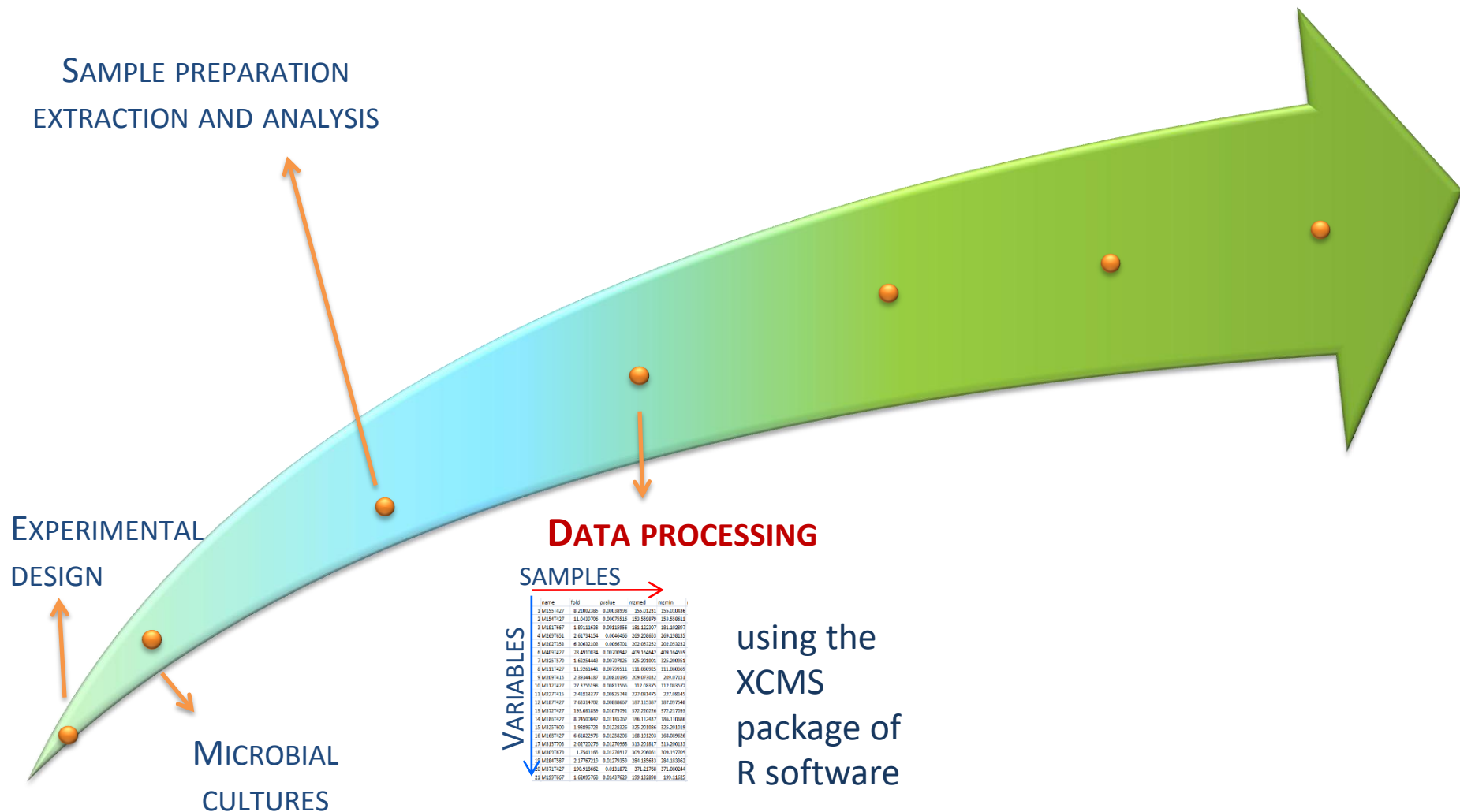


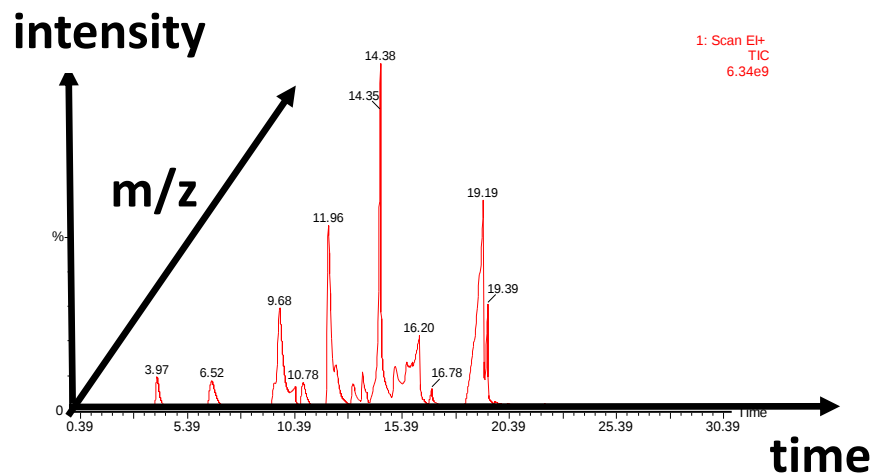
⇒ Limit of quantification : 15 ng/g neutral volatiles, 200 ng/g for acids

⇒ Linearity range: 0-1000 ng/g

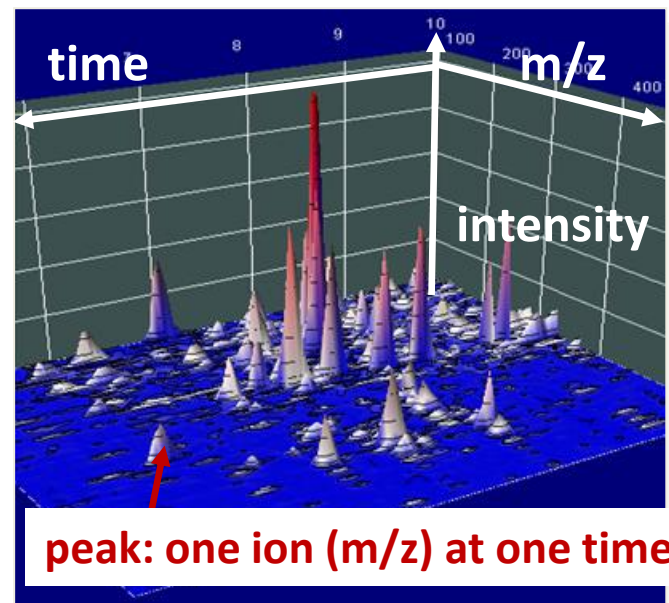
Pogacic et al, *Food Microbiology*, 2015

A metabolomics-based approach: data processing





GC-MS chromatogram



GC-MS data are 3-D files containing the abundance of all the ions detected at each retention time

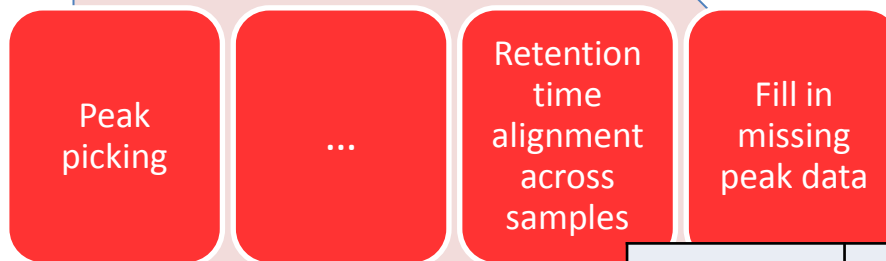
The “manual” extraction of the information (integration of each peak) from GC-MS data is very time-consuming

GC-MS DATA PROCESSING

Input:
Series of GC-MS
raw files
(format .CDF)

- Several softwares developed to facilitate data analysis
- convert the raw data to time- and mass-aligned chromatographic peaks

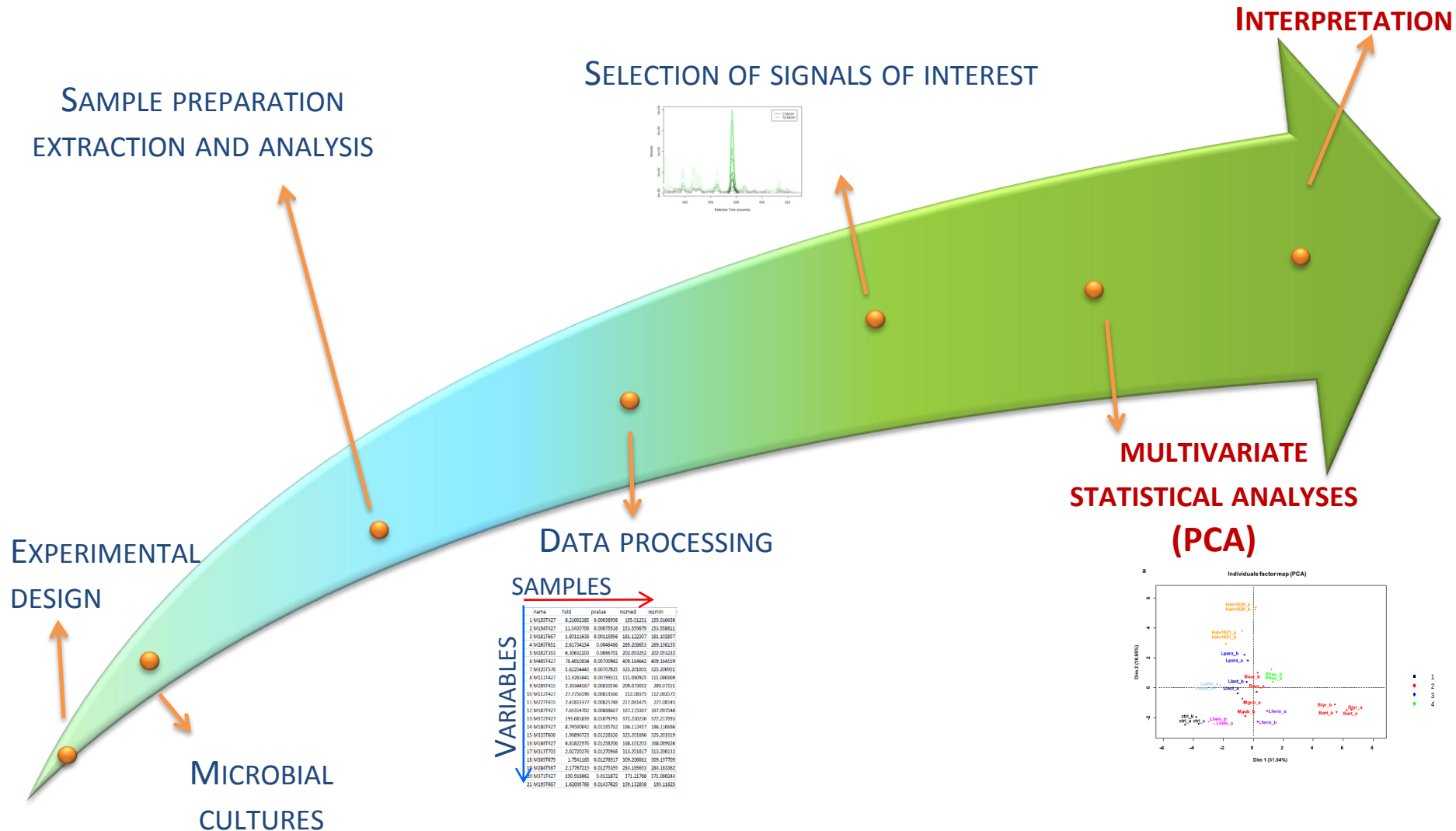
Output:
A unique table for
all samples with
the abundance of
each ion detected
at each time



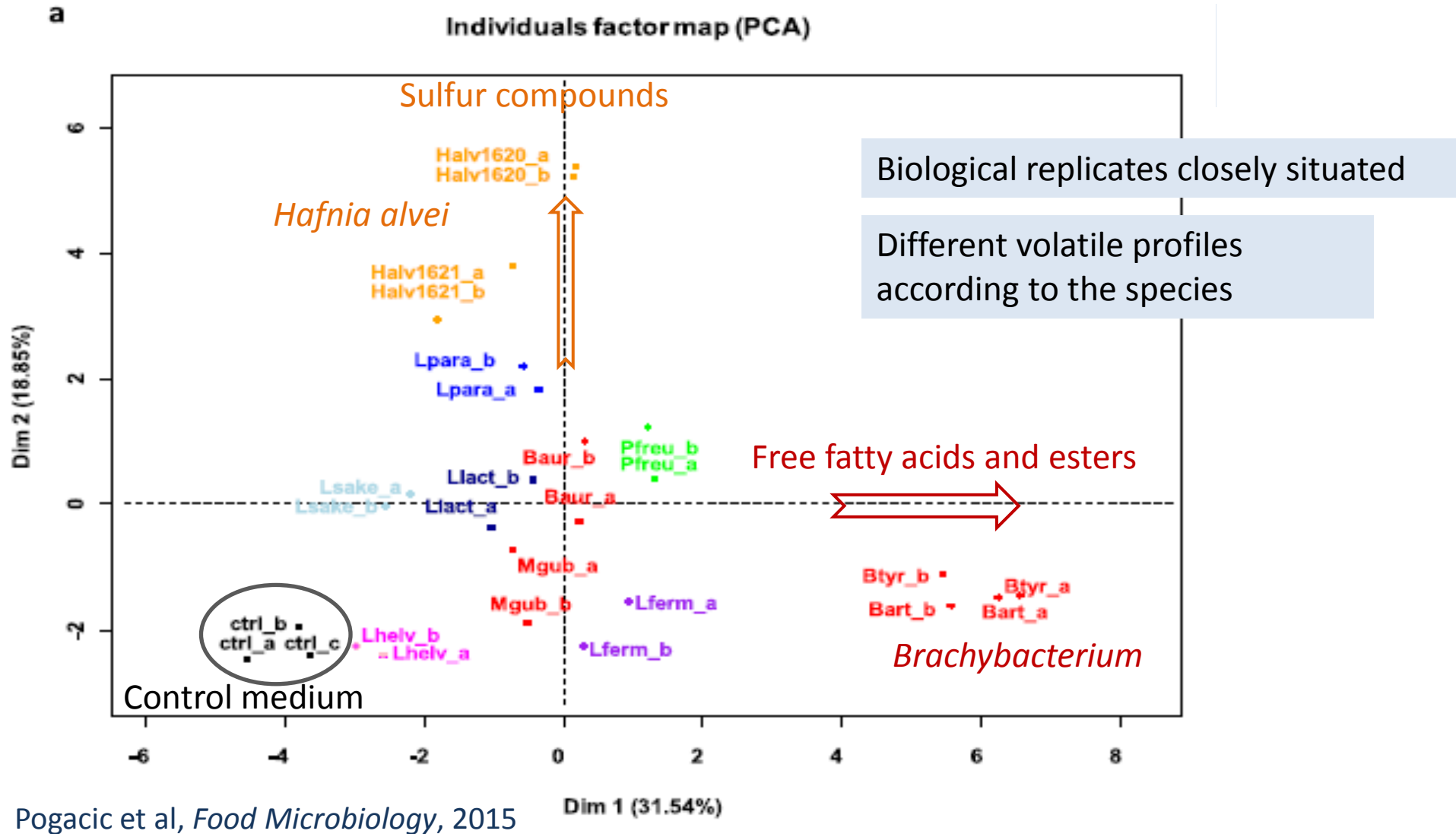
Use of the open source
package XCMS of R
software (Smith et al.,
2006) initially developed
for LC-MS metabolomics

name	sample 1	sample 2	sample 3	sample 4	...
ion 1 time 1					
ion 2 time 1					
ion 3 time 1	~2500 lines → ~ 50-80 volatile compounds				
...					
ion 1 time 2					
ion 2 time 2					
ion 3 time 2					
...					

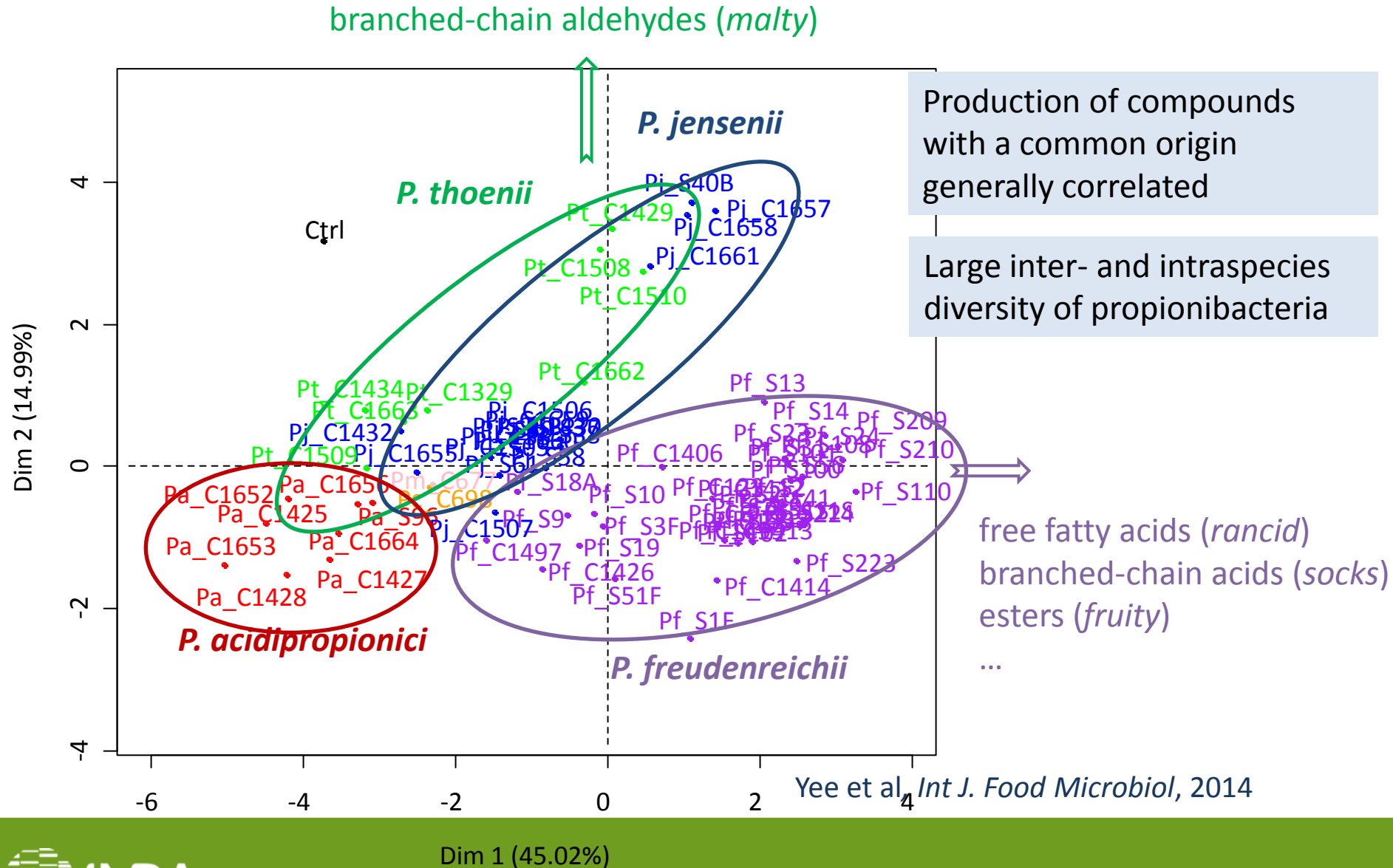
A metabolomics-based approach: examples of results



First example of results: 11 cheese-related bacterial species



Second example of results : 78 propionibacteria strains



Take-home messages

- ✓ The metabolomics-based workflow (data processing using XCMS followed by multivariate analyses) **facilitates data analysis**
- ✓ The HS-trap method used is an **efficient tool for volatile extraction** and an alternative to SPME
- ✓ This approach **can be applied with different objectives**
 - to evaluate very different genera of cheese-associated bacteria for their ability to produce aroma volatile compounds in pure and mixed cultures
 - to determine the volatile profile (“volatilome”) of spoilage micro-organisms
 - to analyze cheeses manufactured under different conditions or with different starters/adjuncts

Thanks to

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Centre of Biological Ressources



THANK YOU FOR YOUR ATTENTION