QUALITY PARTNER

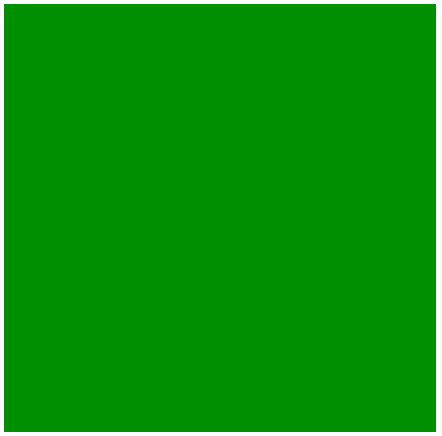
Université de Liège

FARAH

Evaluation of the microbiota of foods with metagenetics

2nd Annual
Food, Nutrition and Agriculture Genomics Congress
29-30 April 2015, London, UK





Agenda

- **Introduction**
  - *The food microbial ecosytem*
  - *-omic technology*
  - *Metagenetics*
- **Case study**
- **Take home message**

- **Objectives**
  - *To give an overview of the omics tools available for food industies*
  - *To present case studies to apply metagentics in food industry*

Structural Interdisciplinary Research Center in *Fundamental and Applied Research for Animals & Health*

Food science of the University of Liege

Analysis , Inspection, Quality, microbiology and technology

Objectives

- Research
- Teaching
- Services

2 spin offs

QUALITY PARTNER — Analysis, certification and inspection

FOOD SAFETY CONSULT — A pragmatical approach to food safety and quality — Consulting and training

Key facts

Notre groupe en bref

Accrédité
BELAC

Agrément AFSCA

BIO

Chiffre d'affaires
6 MILLIONS €

2 sociétés

20 freelances

Trois implantations

B-F-NL-UK-D-L

11 000 audits annuels

250 000 tests/an

Organisme certificateur

ISO 22000

SAC

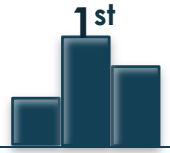
61 employés

XVI %
Croissance

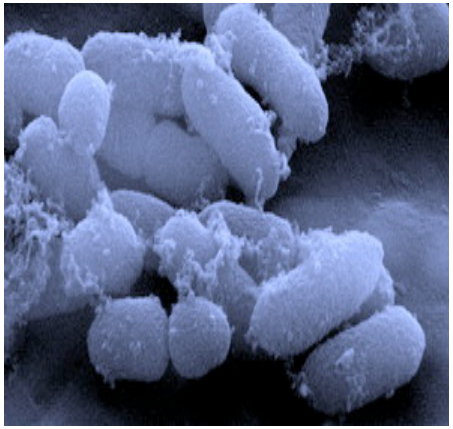
> 4000 clients

400 jours de Coaching par an

ISO 17025 : Labo
ISO 17020 : Inspection
EN 45011 : Products Certification
ISO 17021 : Mgmt Systems Cert

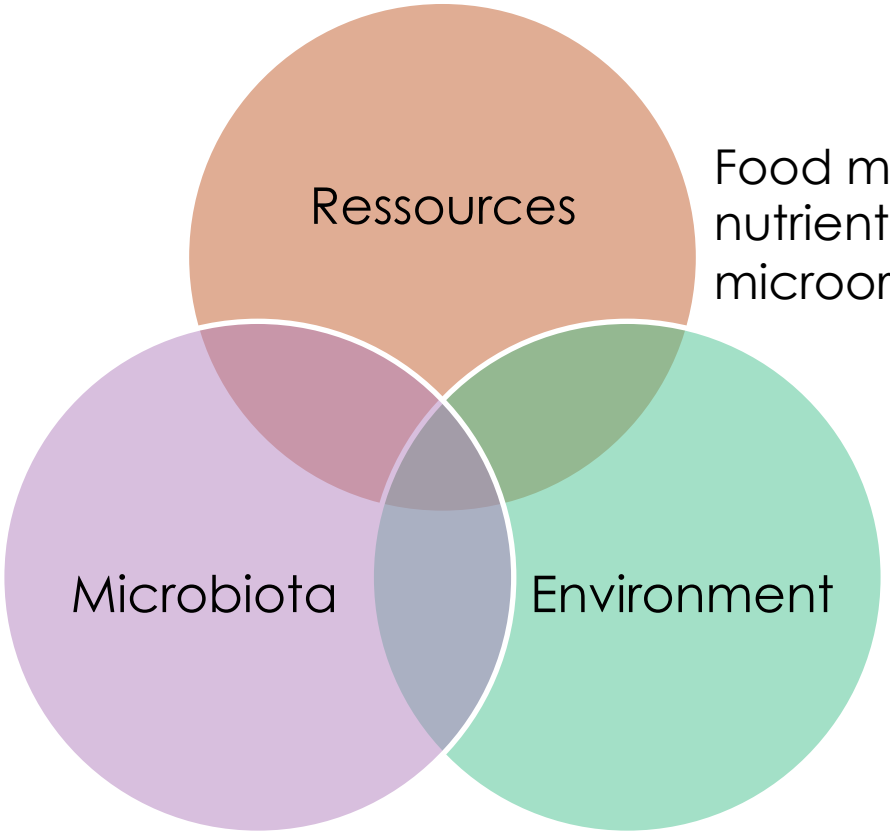


INS Retail
INS Farming
LABO Food microbio



The food microbial ecosystem

The food ecosystem

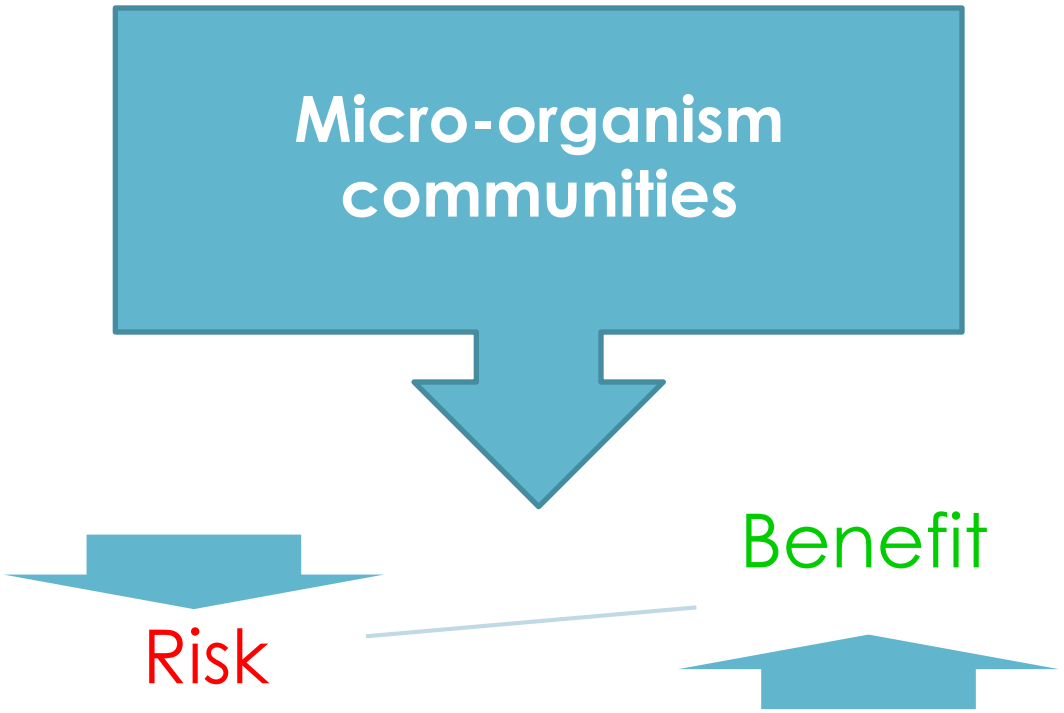


Food matrix:
nutrients for
microorganisms

Raw products/
ingredients,
Environment (handling,
cross contamination,
biofilm)

Process:
temperature, pH,
gaz, additives, …

The food ecosystem



The food ecosystem

Fermented products

> 10^9/g

Raw meat, fish, fruits, vegetables

10^3 to 10^9/g

Dehydrated, high sugar or very fatty foods

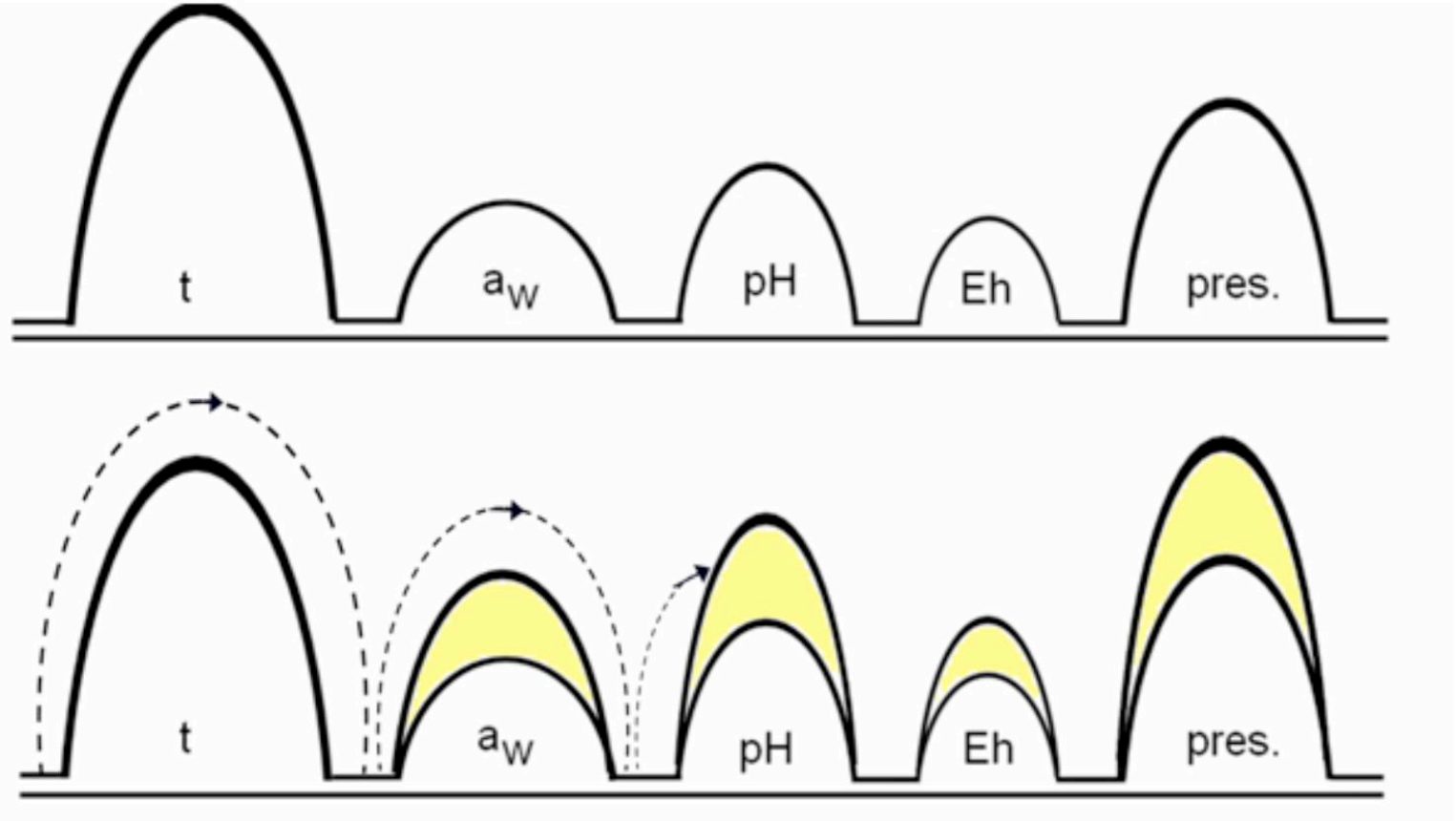
10^2/g

Cooked products, chilled ready meals: 10^2 to 10^9/g

Gut 10^{11}/g

Soil 10^9/g

Hurdle theory

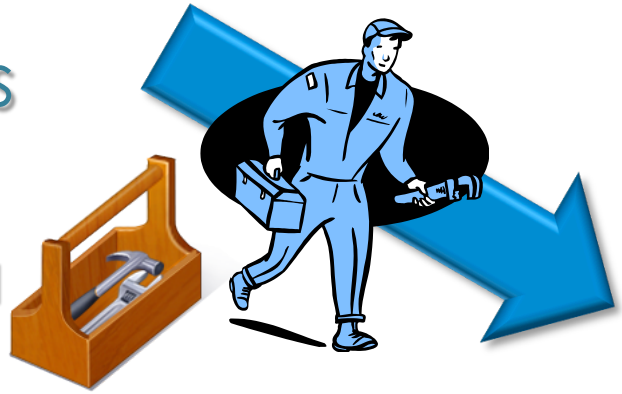


Which challenges?

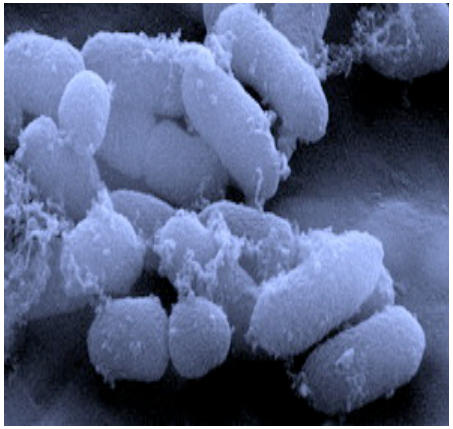
- Moderate complexity of food ecosystem (regarding gut and soil)
- High stringent environment (fermentation/storage)
- Fermentation ? Spoilage? Preservation? safety? Health benefit?

Metagenomics

Culture independent analysis of genetic material of microbial communities



- Characterizing products
- Looking for new functionalities
- Monitoring process
- Selecting strains



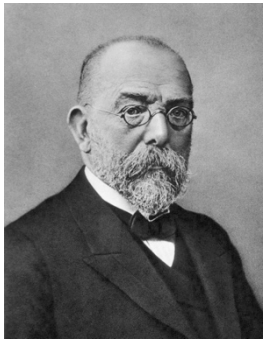
The Metagenomic technologies

How to identify microorganisms?

- What do they look like?
- What do they do, eat or produce?
- Who are they: genetic background?
- Alone, single cells
- All together (ecosystem)

To detect, to identify, to count

Louis Pasteur, 1822-1895



Robert Koch, 1843-1910

Julius Pétri, 1852-1921

Counting

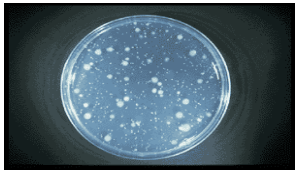
Detection

Culture step

24 h to 5 D

3-7 D

24 h



Culture-independent tools

« Looking large to learn more? »

DNA

16S rDNA/Other genes

Random

RNA

Proteins

All components

Metagenetics*

Metatranscriptomics

Metabolomics

Maldi-TOFF

Metagenomics

Metaproteomics

*Esposito and Kirschberg 2014, FEMS microbial lett **351** 145-146

Culture-independent tools

« Looking large to learn more? »

Sequencing NGS

DNA

16S rDNA/Other genes

Random

RNA

Proteins

All components

Metagenetics*

Metagenomics

Metatranscriptomics

Metaproteomics

Metabolomics

Maldi-TOFF

*Esposito and Kirschberg 2014, fFEMS microbial lett **351** 145-146*

Culture-independent tools

« Looking large to learn more? »

Sequencing NGS

DNA

16S rDNA/Other genes

Random

RNA

Proteins

All components

Metagenetics*

Metatranscriptomics

Metabolomics

Maldi-TOFF

Metagenomics

Metaproteomics

Diversity

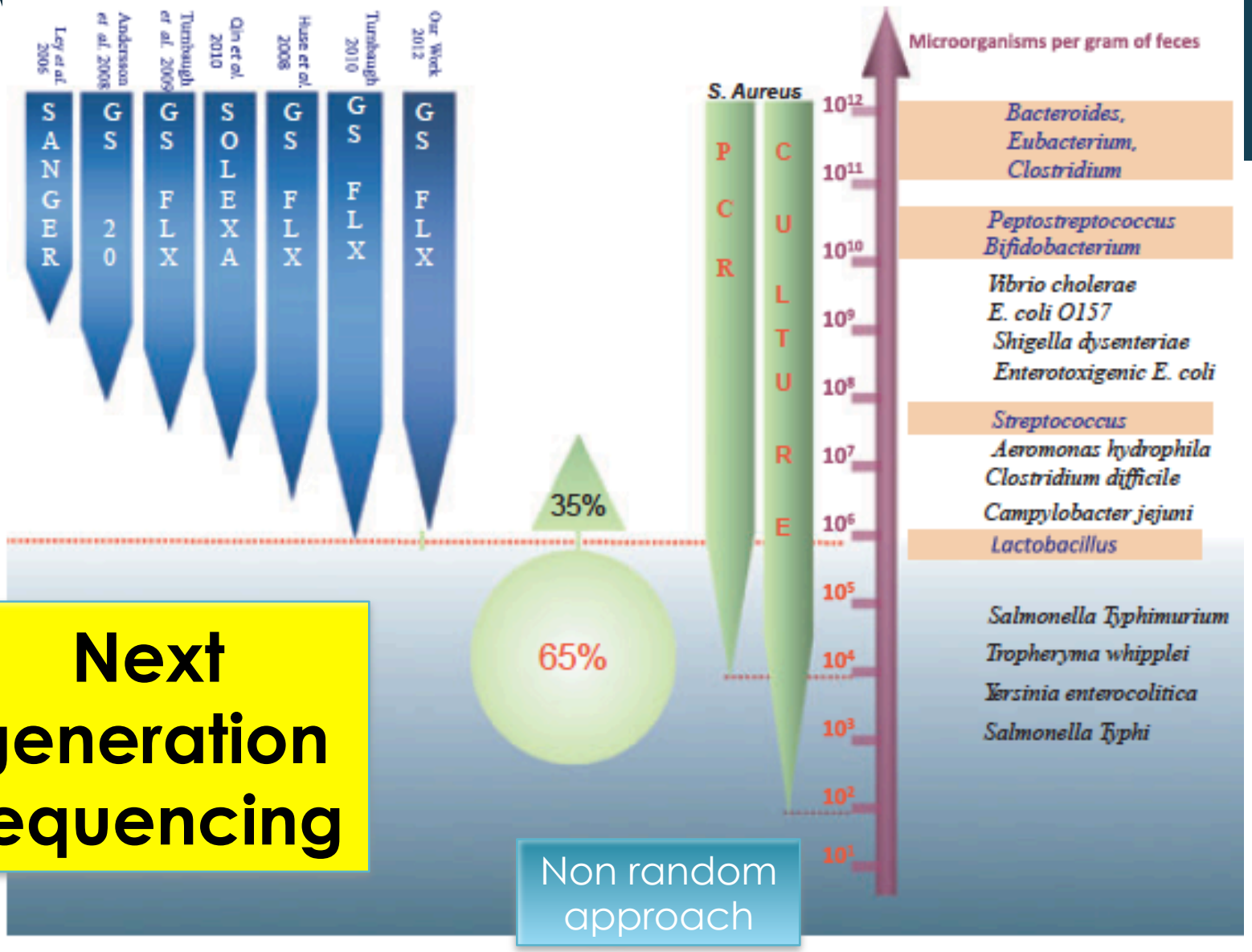
Functionality

Diversity

Diversity

*Esposito and Kirschberg 2014, fFEMS microbial lett **351** 145-146

How does it work?



Next generation sequencing

Non random approach

Metagenetics

A technological breaktrough

Classical approach

Metagenomics

Deposits of 2 european patents: « *Metagenomic Analysis of Samples* »
« *Detection Method* »

Patenten

Exclusive servivces

- European patent n° 13199610.0 deposited the 24 december 2013. **(2013-54 Metagenetic analysis of food samples)**

- European patent n° 13199634.0 deposited the 27 december 2013 **(ref : 2013-55 Detection methods of animal species)**

Bibliography
Quality Partner and ULg

Advancing analytical

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International Journal of Food Microbiology 191 (2014) 157–163

Contents lists available at ScienceDirect

INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY

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Short

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[a] LFMFF
Coupure
[b] Labor
[c] Labor
[d] Qualit

J. Dairy Sci. 97:6046–6056
http://dx.doi.org/10.3168/jds.2014-8225
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J. Dairy Sci. 98:1–6
http://dx.doi.org/10.3168/jds.2014-9065
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Short communication: **Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments**

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Applications

Food	• Quality control • Innovation • R&D, Détermination or extension of the shelf life
Animals	• Feeds • Pre and probiotics • Intestinal tract
Cosmetics and phamaceutics	• Quality control • Innovation • Determination of the shelf life
Human	• Intestinal tract (ex: Crohn disease) • Cohort studies • Pre and probiotics
Environment	• Water • Soils • Plant, seeds

What are the applications for food?

Metagenetics for control quality, innovation and trouble shooting

- To control quality of the final product
- To identify bacteria responsible of food spoilage
- To follow and control process/storage
- To monitor strains
- To monitor fermentation process
- To create new food products
- To extend the shelf life
- To improve washing/disinfection procedure
- Etc

CASE STUDY 1

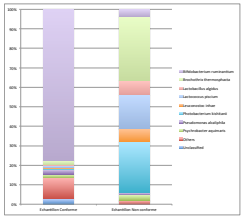
Incident management

Solving problems

Non conform

€ ↘

No solution



Solution

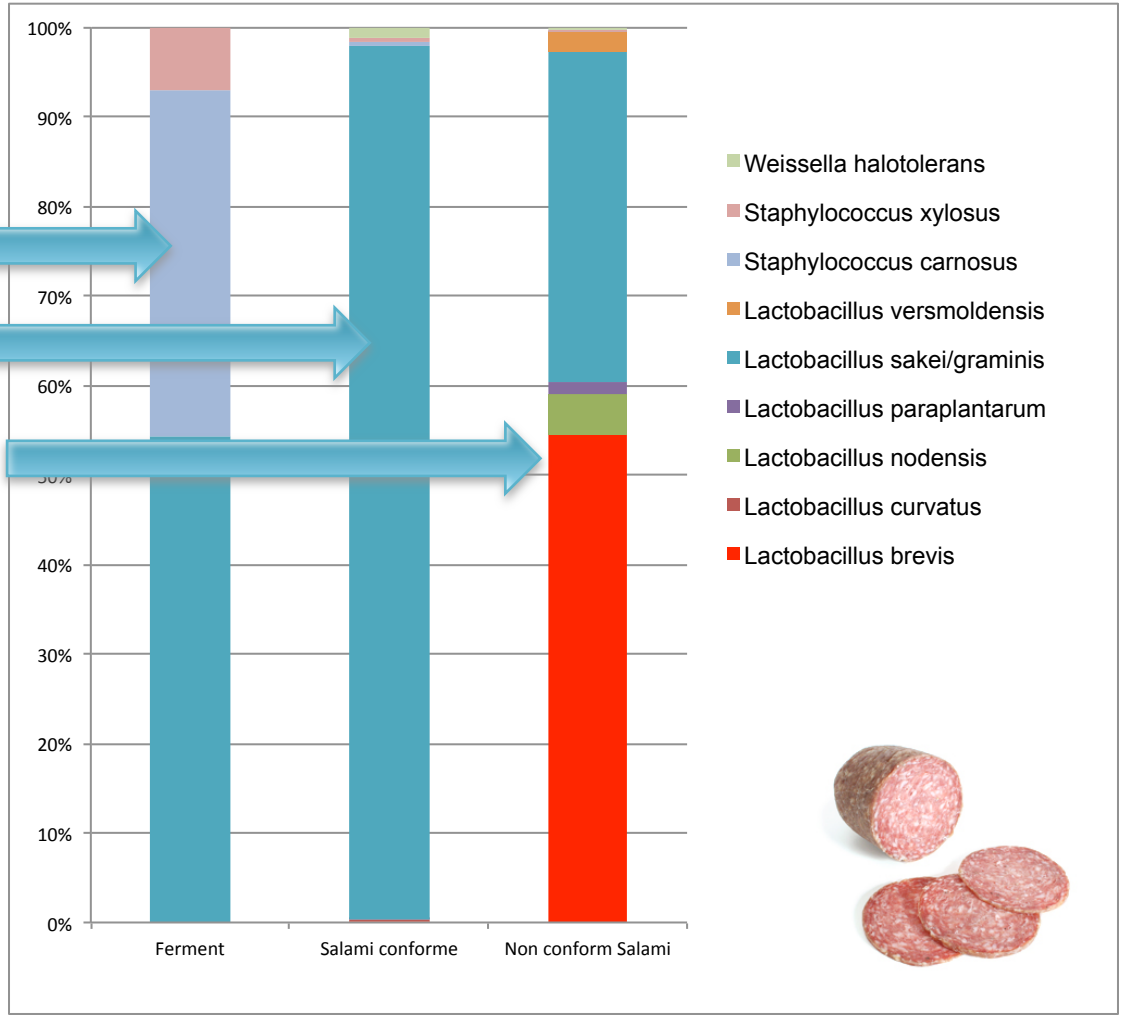
CASE STUDY 1

Incident management

Ferment

Conform product

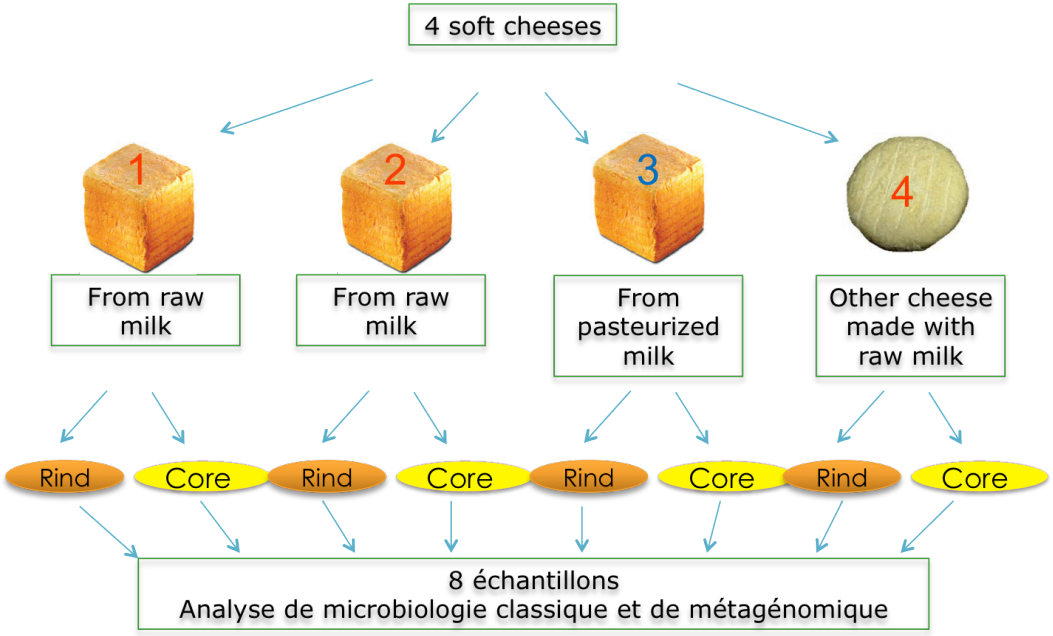
Non conform product



CASE STUDY 2

Process improvement

- Knowledge of the bacterial flora of the cheese
- Comparison between the core and the rind
- Comparison between different manufacturing processes (raw milk / pasteurized)
- Quality control
- Control of the shelf life
- Knowledge of thecompetitors



Results

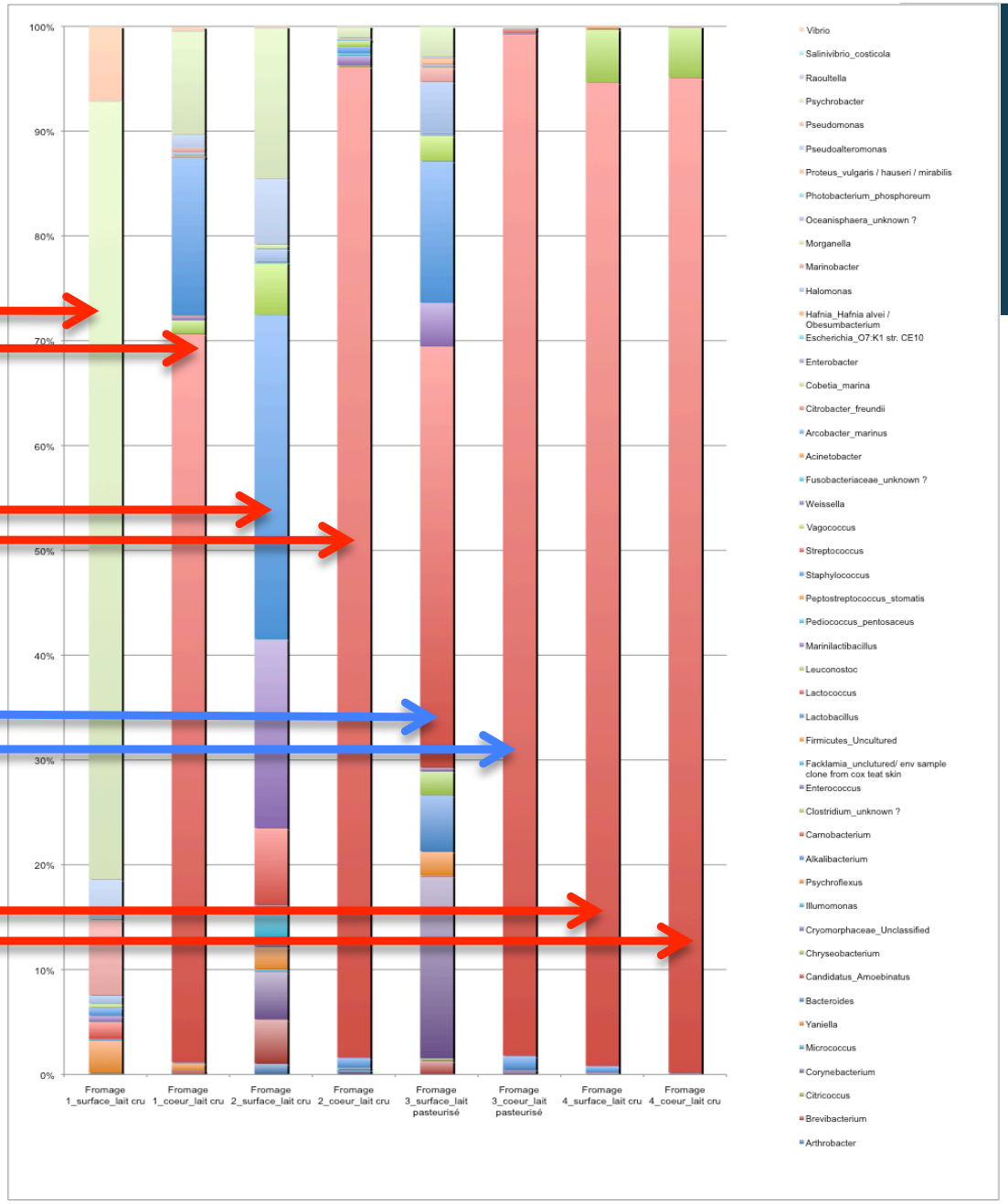
Metagenetic

1 Rind Core

2 Rind Core

3 Rind Core

4 Rind Core

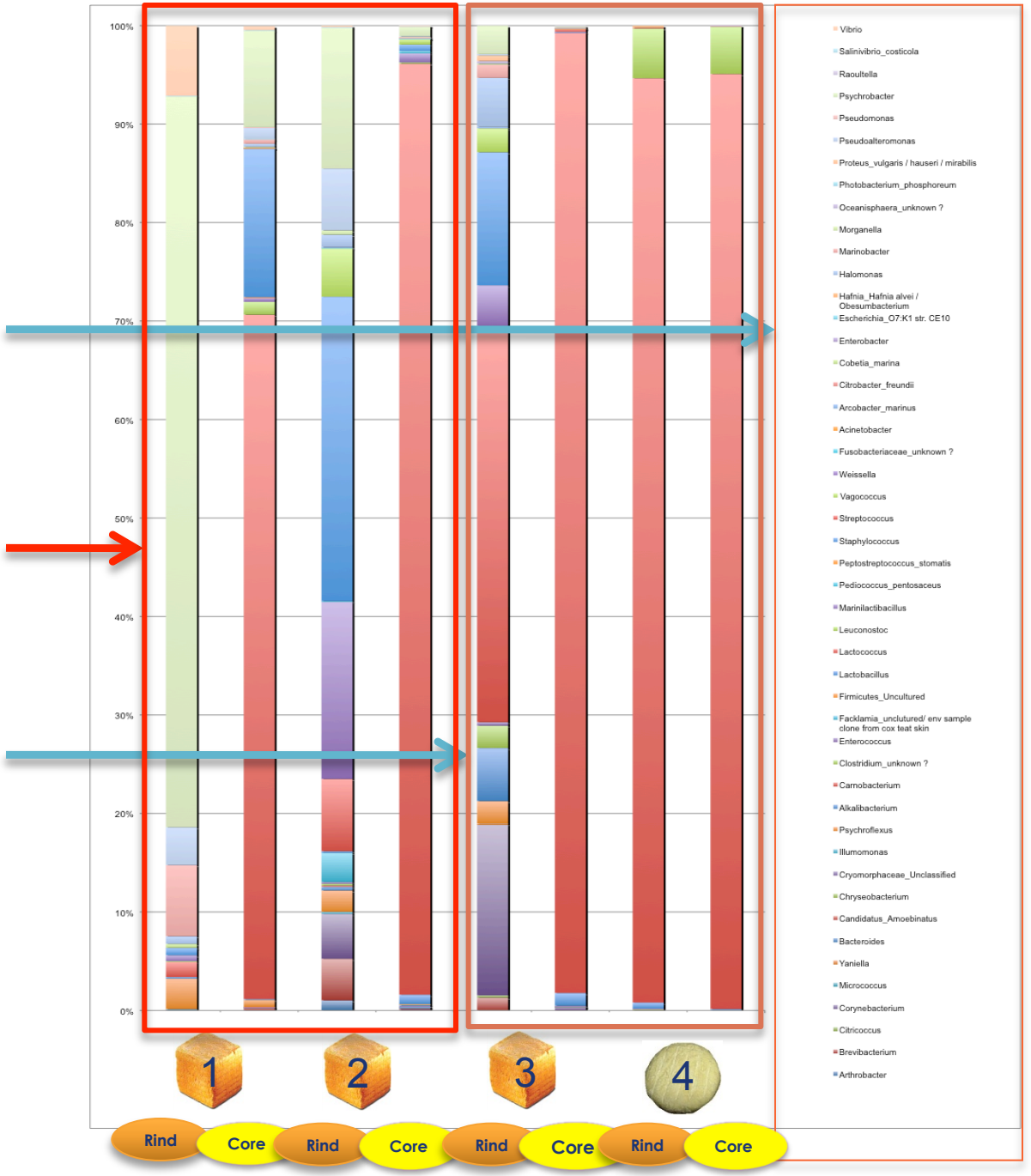


Results

48 genus and 163 species

Many diffrenet bacterial species in the cheeses made with raw milk

Mainly *Lactococcus lactis* (97,6%) in the core

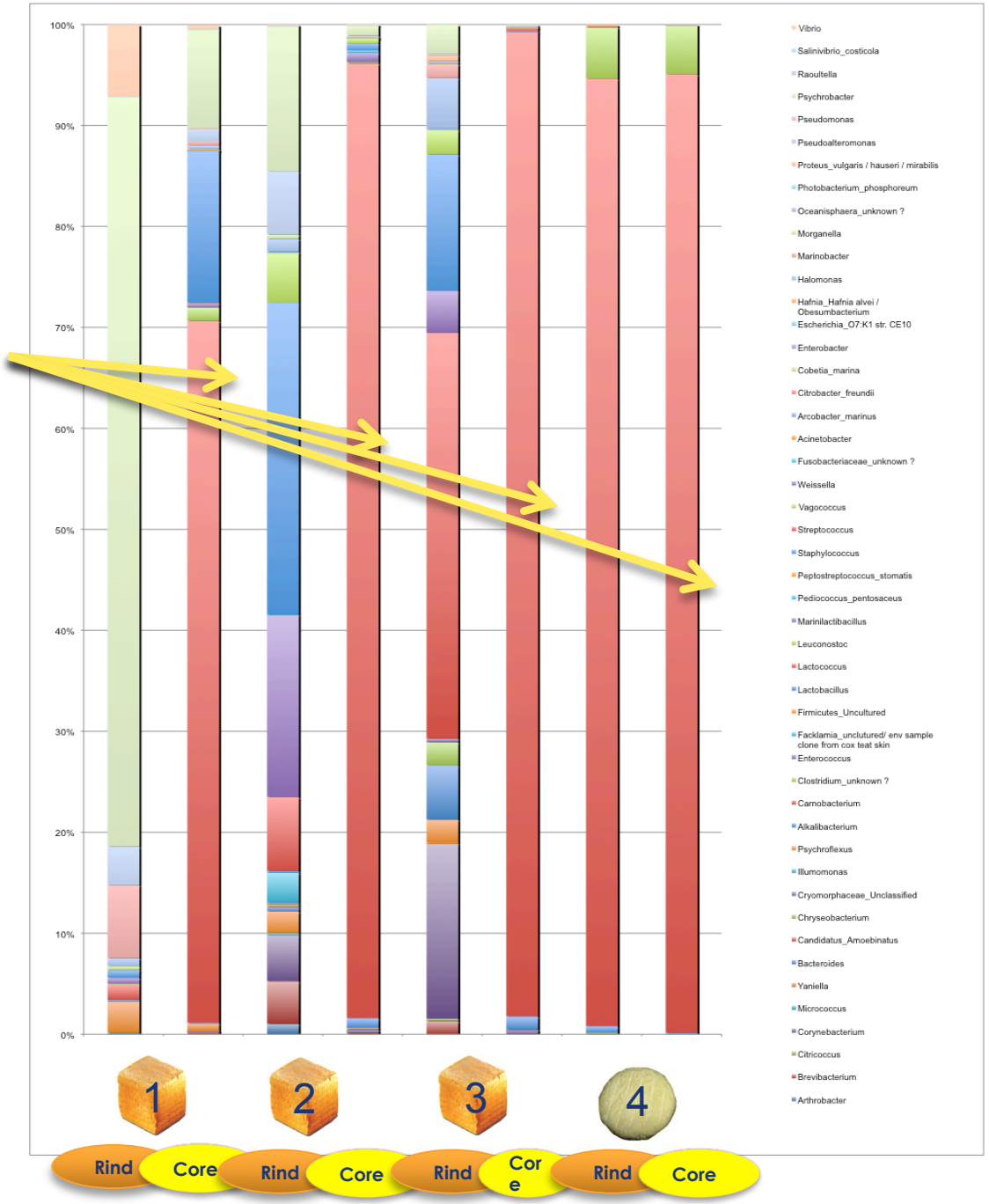


Results

Core: Lactoccocus lactis and/or *cremoris*

Rind :

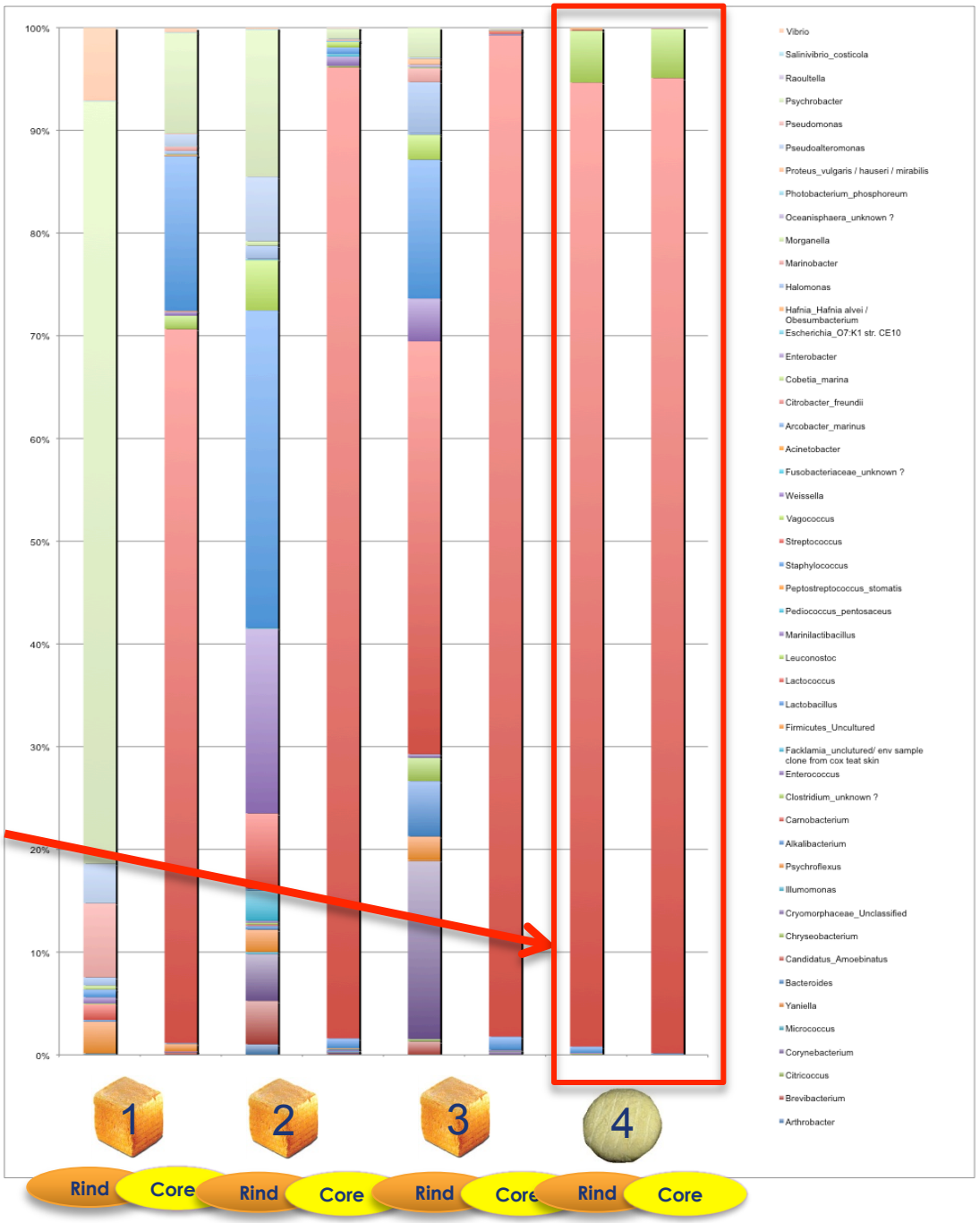
- *Psychrobacter glacinola*
- *Staphylococcus equorum*
- *Corynebacterium casei*
- *Marinilactibacillus psychrotolerans*
- *Brevibacterium* spp
- *Psychroflexus* spp



Results

Only two bacterial species for this cheese

Lactoccocus lactis susp. Cremoris et Leuconostoc citreum



CASE STUDY 3

Microbial quality of fresh meat in Belgium

Steak tartare

n=59

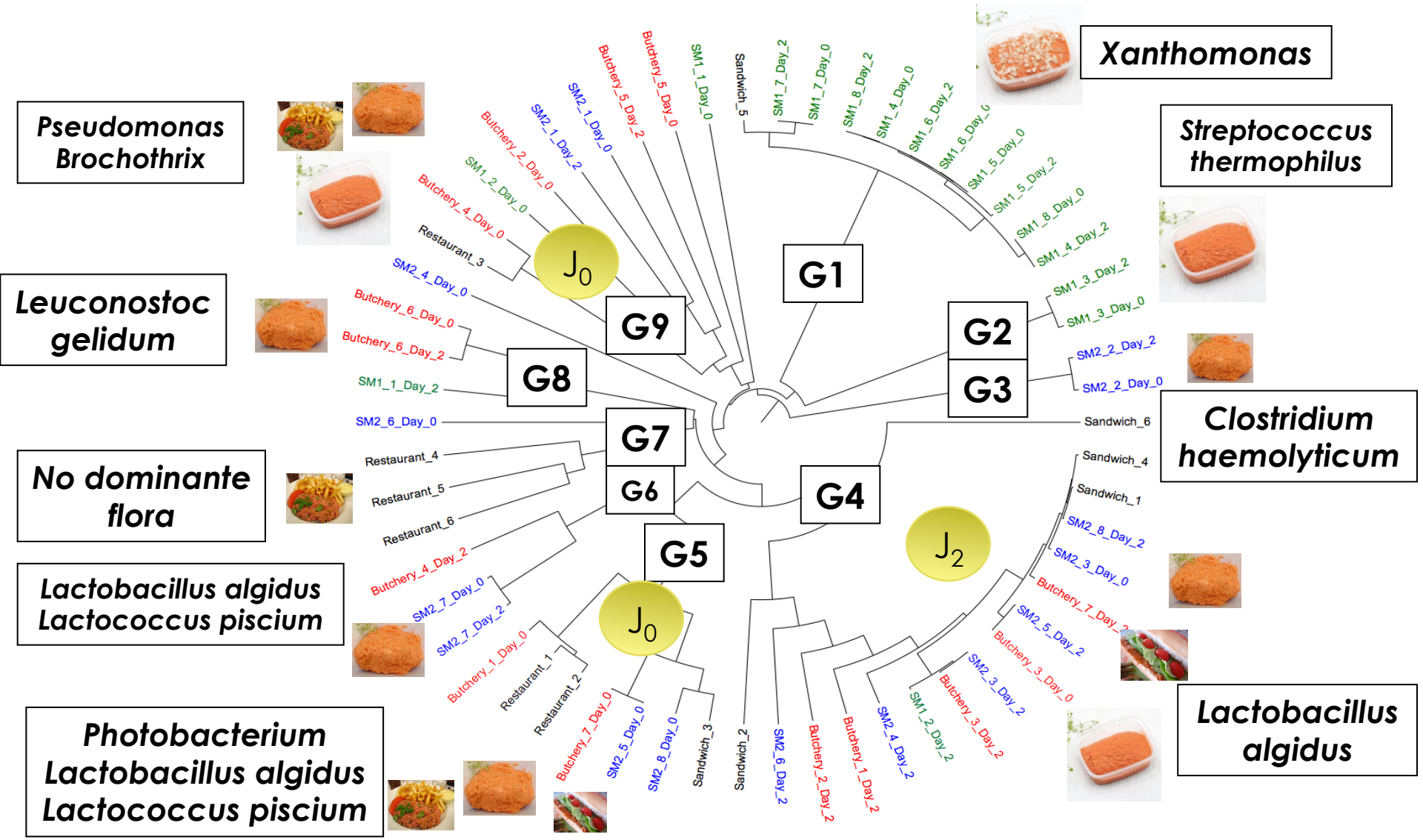
Pre packed in supermarket (SM1;n=8) at day 0 and at day 2

Intern butcheries in supermarket (SM2; n=8) and Butcheries (Butchery; n=7) At day 0 and day 2

Restaurant (n=6) at day 0

Sandwih bars (n=6) at day 0

(Bray-Curtis index)



GROUP

Group II

GROUP III

- *Brochothrix thermosphacta*
- *Clostridium haemolyticum*
- *Lactobacillus algidus*
- *Lactococcus piscium*
- *Leuconostoc gelidum*
- *Phobacterium kishitanii*
- *Pseudomonas antarctica*
- *Streptococcus thermophilus*
- *Xanthomonas oryzae*

GROUP IV

GROUP V

Group VI

Group VII

Group VIII

Group IX

What about the legislation ?

WETENSCHAPPELIJK COMITÉ
VAN HET FEDERAAL AGENTSCHAP VOOR DE VEILIGHEID
VAN DE VOEDSELKETEN

ADVIES 10-2012

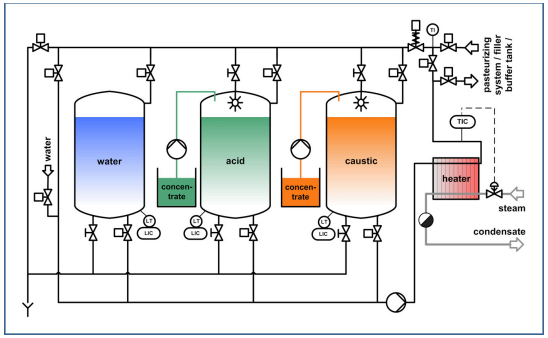
Dit advies vervangt advies 19-2011

Betreft: Evaluatie van het document "Actiegrenzen voor microbiologische contaminanten in levensmiddelen" (dossier Sci Com 2011/21).

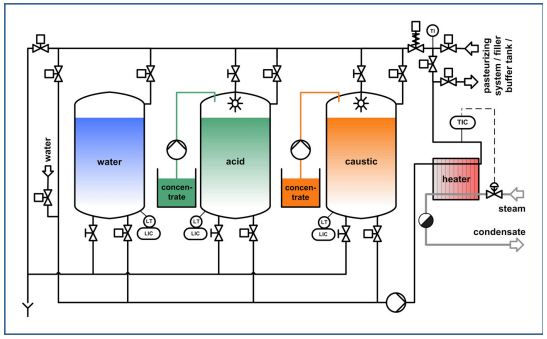
Advies goedgekeurd door het Wetenschappelijk Comité op 16 maart 2012.

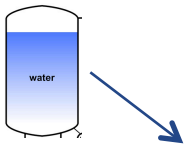
CASE STUDY 4

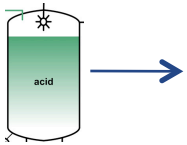
R&D: process improvement

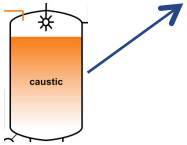


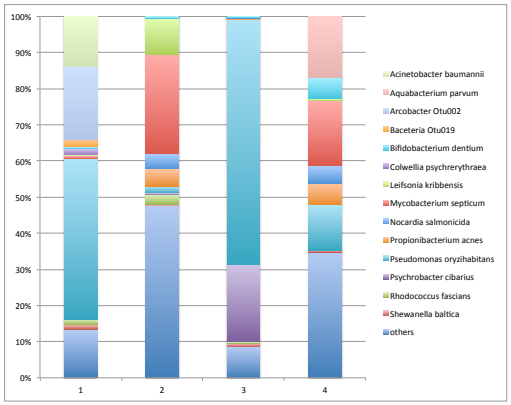
Control quality









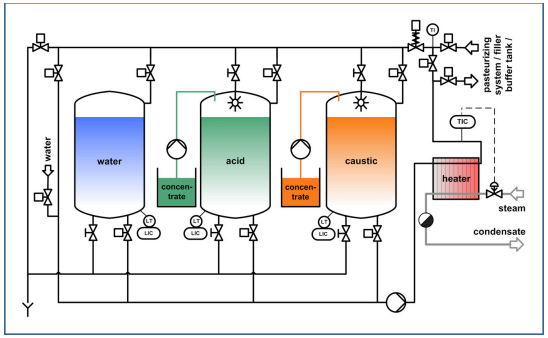


Quality management of the whole process

REALCO ... for Life

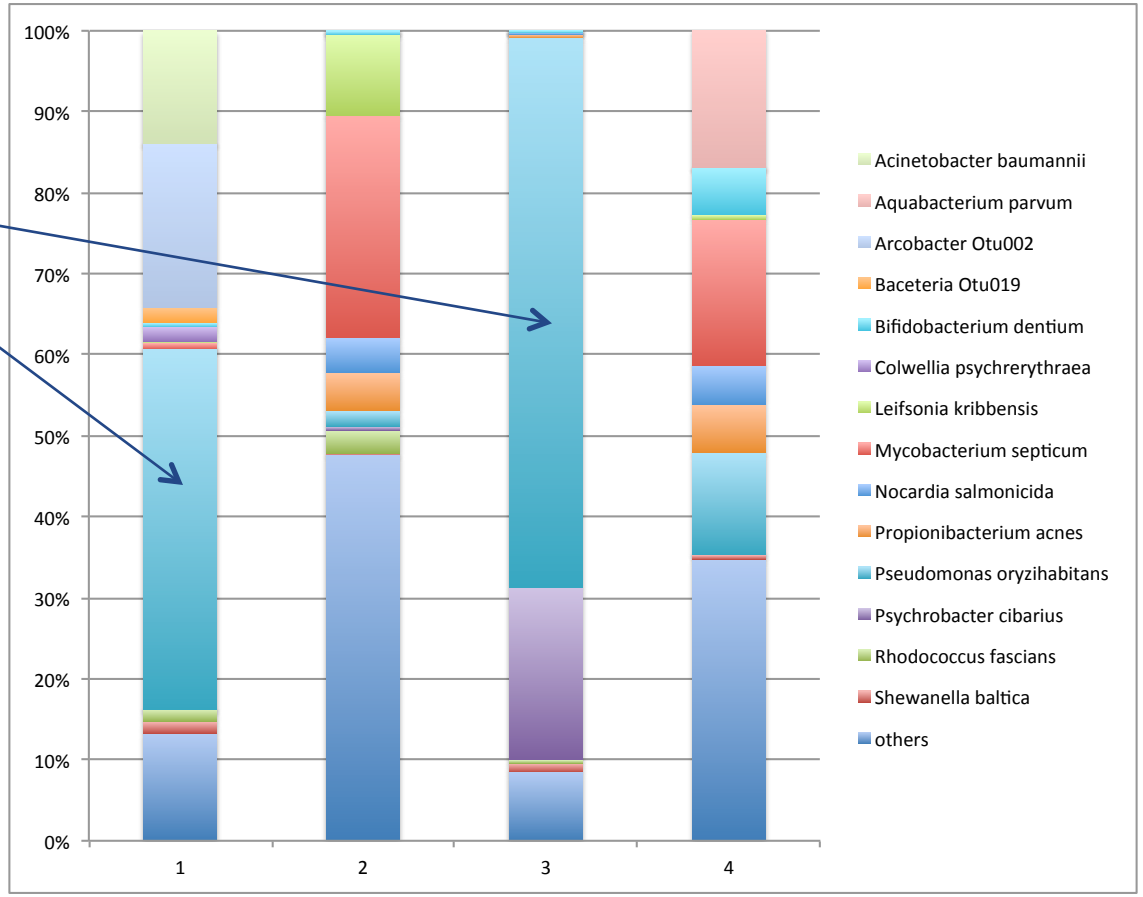
CASE STUDY 4

R&D: process improvement



Cleaning / Disinfection procedures

Pseudomonas from biofilms



REALCO ... for Life

CASE STUDY 6

Patents

Exclusion of dominant taxa/chloroplasts

Exclusion of chloroplasts (Vegetables)
Exclusion of specific taxa

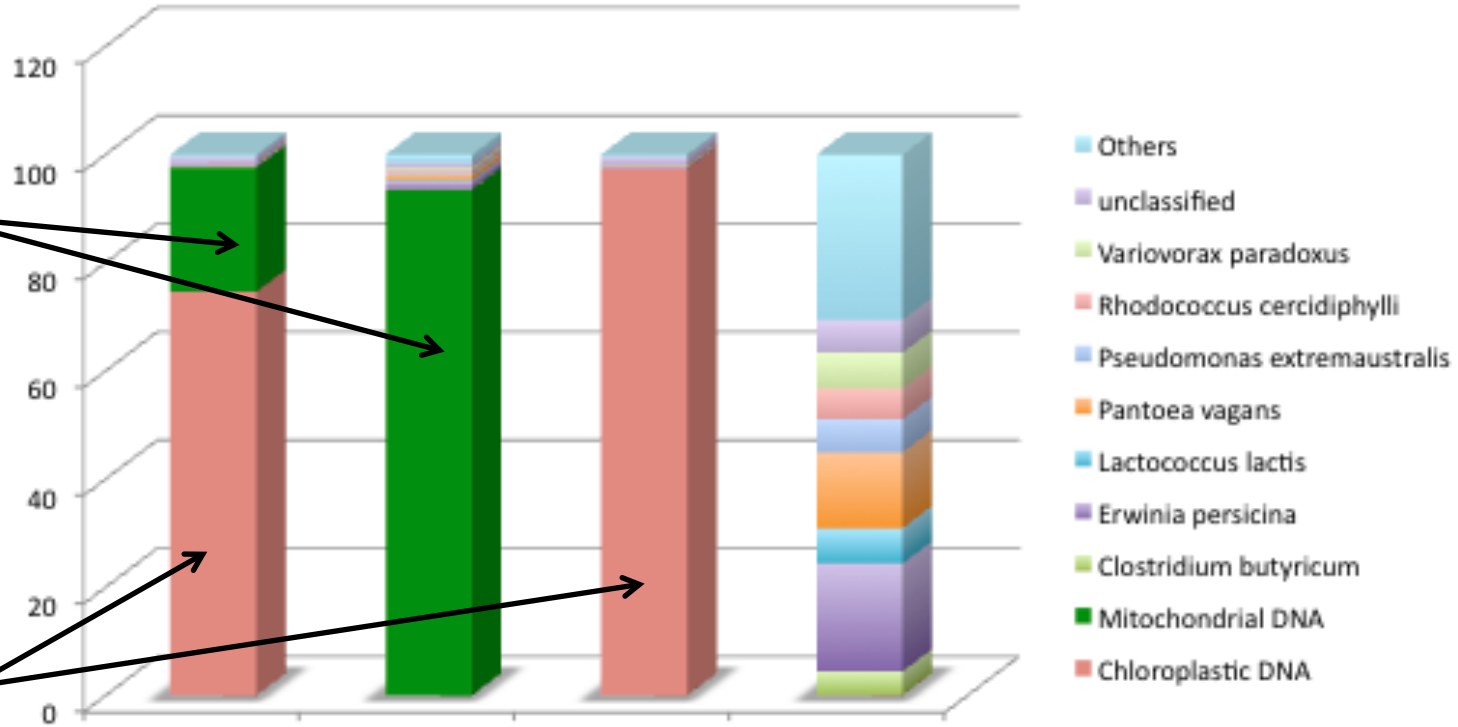
CASE STUDY 6

Patents

Exclusion of dominant chloroplasts

MITOCHONDRIES

CHLOROPLASTES



Without exclusion	With exclusion	With exclusion	With exclusion of chloroplasts and mtochondries

CASE STUDY 6

Yoghourt

PATENTED

Exclusion of dominant taxa in fermented product

Lactobacillus delbruckii subsp. *bulgaricus*

Streptococcus salivarius subsp. *thermophilus*

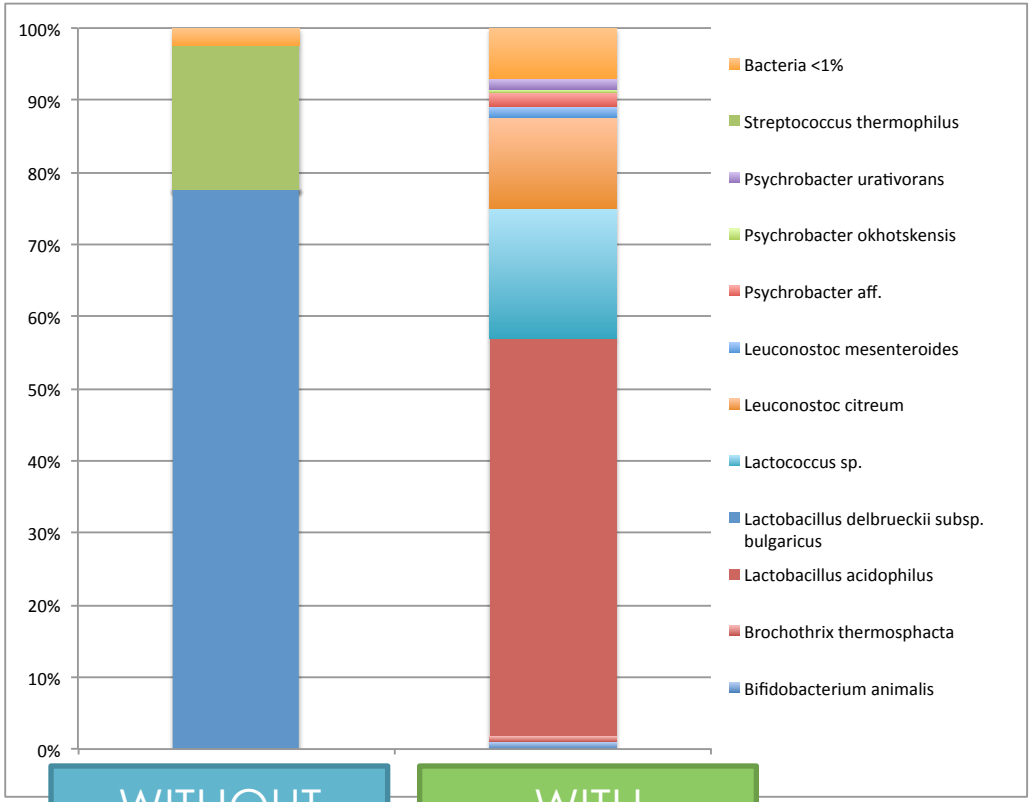
Other bacterial flora ?

CASE STUDY 6

Yoghourt

PATENTED

Exclusion of dominant taxa in fermented product

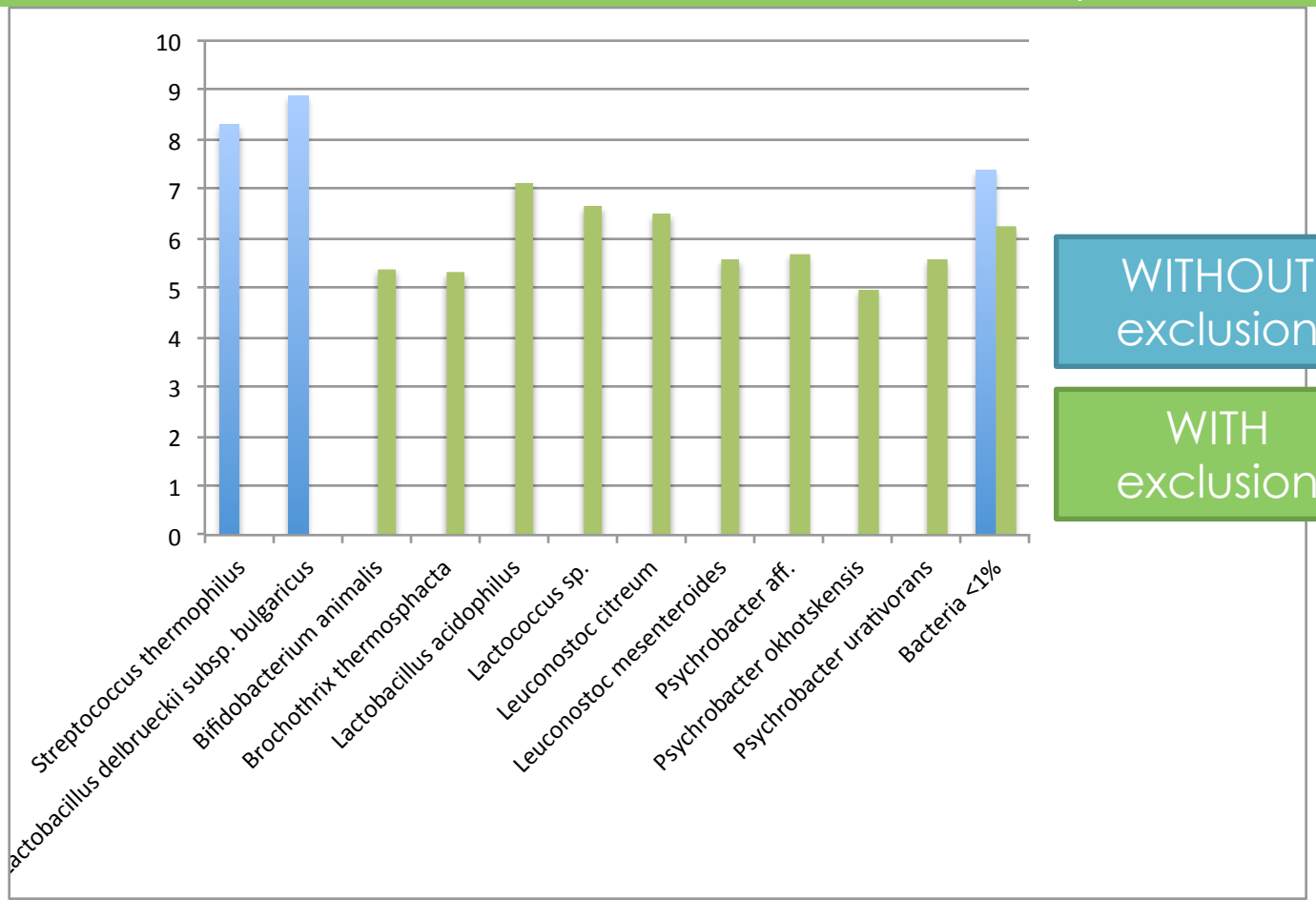


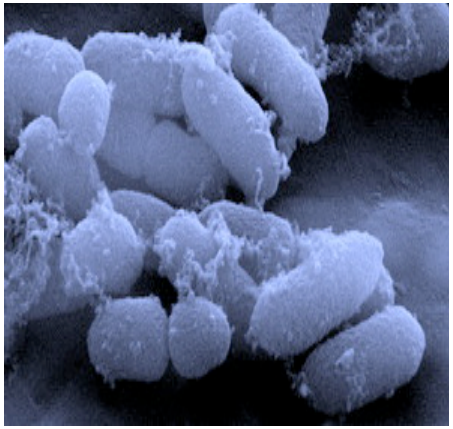
CASE STUDY 6

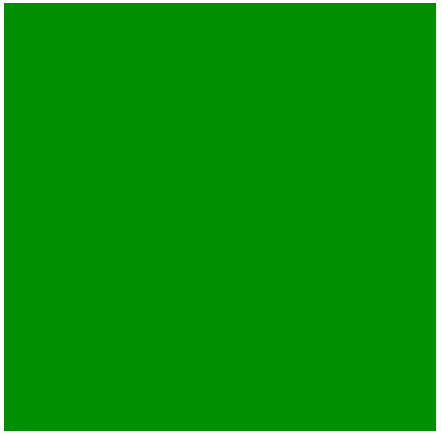
Yoghourt

PATENTED

Exclusion of dominant taxa in fermented product







Conclusions

We are your partner

An integrated approach with exclusive services

- **Sampling preparations**
  - ⇨ Exclusion of specific taxa, dead bacteria or chloroplasts
  - ⇨ Extraction on different types of samples (food , fecal sample, environment, surfaces, etc)
  - ⇨ Complete preparation of samples (from extraction to the bioinformatics)
  - ⇨ Complementary techniques Real-time PCR, flow cytometer

- **High throughput sequencing**
  - ⇨ Most recent and reliable sequencer
  - ⇨ Flexibility
  - ⇨ Fast (10 open days)
  - ⇨ Targeted metagenomics or genome sequencing

- **Bio-Informatics & scientific support**
  - ⇨ Automatic or customized Pipeline
  - ⇨ Flexible
  - ⇨ Statistical analysis
  - ⇨ **Scientific support (microbiologist, helpdesk)**

Take home message

- A new starting era for food microbiology
- A revisited vision of food ecosystems
- Exciting and promising future tools
- Already available for food industries (QC, innovation, trouble shooting)
- Competitive price (comparable with classical microbiology)

The future is :

- Widespread the technology and bioinformatics tools
- Routine technique

QUALITY PARTNER

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