Evaluation of the microbiota of foods with metagenetics
Agenda

• Introduction
  • The food microbial ecosystem
  • -omic technology
  • Metagenetics

• Case study

• Take home message

• Objectives
  • To give an overview of the omics tools available for food industries
  • To present case studies to apply metagenetics 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

Analysis, certification and inspection
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

11 000 audits annuels

Organisme certificateur
ISO 22000

SAC

250 000 tests/an

61 employés

XVI % Croissance

> 4000 clients

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

Ressources

Food matrix: nutrients for microorganisms

Microbiota

Environment

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

Process: temperature, pH, gaz, additives, ...
The food ecosystem

Micro-organism communities

Risk

Benefit

Illness  Spoilage  Preservation  Fermentation
The food ecosystem

- Dehydrated, high sugar or very fatty foods: $10^2/g$
- Raw meat, fish, fruits, vegetables: $10^3$ to $10^9/g$
- Cooked products, chilled ready meals: $10^2$ to $10^9/g$
- Fermented products: $>10^9/g$
- 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)

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

Julius Pétri, 1852-1921
Louis Pasteur, 1822-1895
Robert Koch, 1843-1910

Culture step

Counting
24 h to 5 D

Detection
3-7 D
24 h
Culture-independent tools

**DNA**
- 16S rRNA/Other genes
- Metagenetics*
- Metagenomics

**RNA**
- Random
- Metatranscriptomics

Proteins
- Metabolomics
- Maldi-TOF-F

All components

« Looking large to learn more? »

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

**Sequencing NGS**

- DNA
  - 16S rDNA/Other genes
  - Random
  - Metagenetics*
  - Metagenomics
  - Metatranscriptomics

- RNA
  - Metabolomics
  - Maldi-TOFF

Proteins

All components

« Looking large to learn more? »

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

Sequencing

NGS

16S rDNA/Other genes

DNA

Random

RNA

Metagenomics

Metatranscriptomics

Metagenetics*

Metaproteomics

Diversity

Functionality

Proteins

Metabolomics

All components

Maldi-TOFF

« Looking large to learn more? »

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How does it work?

Next generation sequencing

Non random approach
Metagenetics

A technological breakthrough

Classical approach

Metagenomics

Deposits of 2 European patents: «Metagenomic Analysis of Samples» «Detection Method»
Patenten
Exclusive services

• European patent no 13199610.0 deposited the 24 december 2013. *(2013-54 Metagenetic analysis of food samples)*

• European patent no 13199634.0 deposited the 27 december 2013 *(ref : 2013-55 Detection methods of animal species)*
Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments

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J. Dairy Sci. 98:1–6
http://dx.doi.org/10.3168/jds.2014-9065
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

www.quality-partner.be  www.agrifood-metagenomics.com
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

Incident management

Bifidobacterium ruminantium
Brochothrix thermosphacta
Lactobacillus algidus
Lactococcus piscium
Leuconostoc inhae
Photobacterium kishitanii
Pseudomonas alcaliphila
Psychrobacter aquimaris
Others
Unclassified
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 the competitors
Results
Metagenetic
Results

48 genus and 163 species

Many different bacterial species in the cheeses made with raw milk

Mainly *Lactococcus lactis* (97.6%) in the core
Results

Core: Lactococcus lactis and/or cremoris

Rind:
- Psychrobacter glacinola
- Staphylococcus equorum
- Corynebacterium casei
- Marinilactibacillus psychrotolerans
- Brevibacterium spp
- Psychroflexus spp
Only two bacterial species for this cheese

Lactococcus lactis susp. Cremonis et Leuconostoc citreum
CASE STUDY 3

Microbial quality of fresh meat in Belgium

Steak tartare

- 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
- Sandwich bars (n=6) at day 0

n=59
What about the legislation?

ADVIES 10-2012

Dit advies vervangt advies 19-2011


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

- Acinetobacter baumannii
- Aquabacterium parvum
- Arcobacter Otu002
- Baceteria Otu019
- Bifidobacterium den@um
- Colwellia psychrerythraea
- Leifsonia kribbensis
- Mycobacterium sep@cum
- Nocardia salmonicida
- Propionibacterium acnes
- Pseudomonas oryzihabitans
- Psychrobacter cibarius
- Rhodococcus fascians
- Shewanella bal@ca
- others
CASE STUDY 4
R&D: process improvement

Cleaning / Disinfection procedures

Pseudomonas from biofilms
CASE STUDY 6

Patents

Exclusion of dominant taxa/chloroplasts

Exclusion of chloroplasts (Vegetables)
Exclusion of specific taxa
CASE STUDY 6

Exclusion of dominant chloroplasts

<table>
<thead>
<tr>
<th>Without exclusion</th>
<th>With exclusion</th>
<th>With exclusion</th>
<th>With exclusion of chloroplasts and mitochondries</th>
</tr>
</thead>
</table>

MITOCHONDRIE

CHLOROPLASTES
CASE STUDY 6

Yoghourt

Exclusion of dominant taxa in fermented product

Lactobacillus delbruckii subsp. bulgaricus

Streptococcus salivarius subsp. thermophilus

Other bacterial flora ?
CASE STUDY 6

Yoghourt

Exclusion of dominant taxa in fermented product

WITHOUT exclusion

WITH exclusion

- **Bacteria <1%**
- **Streptococcus thermophilus**
- **Psychrobacter urativorans**
- **Psychrobacter okhotskensis**
- **Psychrobacter aff.**
- **Leuconostoc mesenteroides**
- **Leuconostoc citreum**
- **Lactococcus sp.**
- **Lactobacillus delbrueckii subsp. bulgaricus**
- **Lactobacillus acidophilus**
- **Brochothrix thermosphacta**
- **Bifidobacterium animalis**
Case Study 6

Yoghourt

Exclusion of dominant taxa in fermented product

- *Streptococcus thermophilus*
- *Bifidobacterium animalis*
- *Bifidobacterium thermopilus*
- *Leuconostoc citreum*
- *Psychrobacter aff. okkotskensis*
- *Psychrobacter uraflorans*
- *Leuconostoc mesenteroides*
- *Lactobacillus acidophilus*
- *Lactococcus sp.*
- *Leuconostoc mesenteroides*
- *Psychrobacter orkutskensis*
- *Psychrobacter uraflorans*
- *Bacteria <1%*

Without exclusion

With exclusion
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
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