



HAL
open science

The CIRM -BIA, microbial diversity of food related bacteria in the service of innovation for dairy industry

Florence Valence

► **To cite this version:**

Florence Valence. The CIRM -BIA, microbial diversity of food related bacteria in the service of innovation for dairy industry. STLOpendays, Institut National de Recherche Agronomique (INRA). UMR UMR INRA / AgroCampus Rennes: Science et Technologie du Lait et de l'œuf (1253)., Mar 2019, Rennes, France. hal-02097207

HAL Id: hal-02097207

<https://hal.science/hal-02097207v1>

Submitted on 5 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

AGRO
CAMPUS
OUEST



The CIRM-BIA, microbial diversity of food related bacteria in the service of innovation for dairy industry



STL Open Days
19-21 March 2019



Cirm - Florence Valence



“The role of the infinitely small is infinitely great”

L. Pasteur



Values

Scientific and educational

Social

Economic

Patrimonial

Cultural

- Infinite sources of functionalities (food, health, biotechnology, soil decontamination,...)
- Loss of diversity of microbial resources
- Durability lever
- Intrinsic potential of innovation



Appropriate management of microbial resources



Biological Resources Centers BRCs

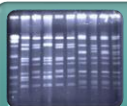


2005, creation of the CIRMI BRC, a thematic network dedicated to the management of its microbial resources

BRC, Biological Resources Center functions



Acquisition



Identification



Conservation



Valorisation



Distribution

- Preserve the biodiversity
- Give access to biological resources for scientific, industrial, agricultural environmental and medical R&D applications
- Be repositories of biological reference material
- Be repositories of BR for protection of intellectual property
- Facilitate and favorize their use → performance of R&D
- Be resources for public information and policy formulation



High criteria of quality and expertise (Normative and standard procedure)

Balance between R & D activities and service activities

Traceability Quality and consistency of all the data associated with the MB

CIRM = A thematic and geographical collections network of 5 specific BRCs

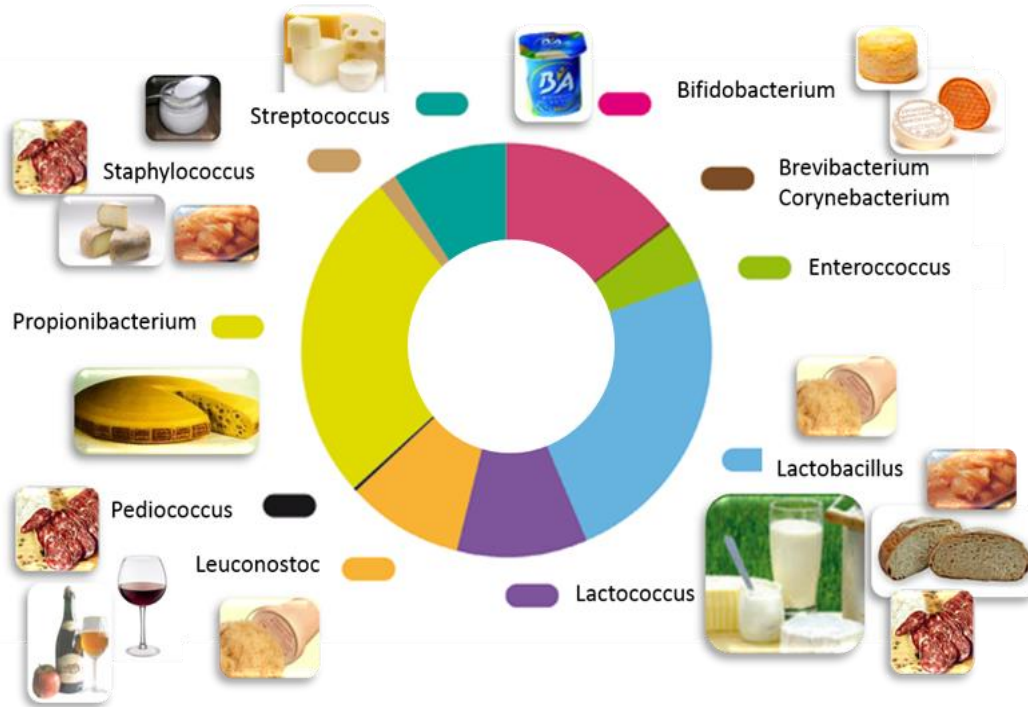
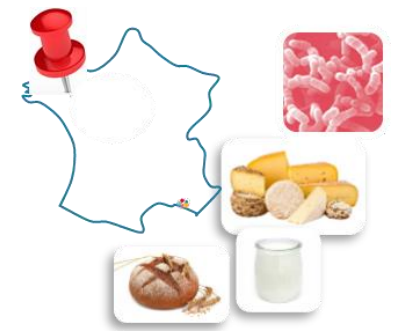
18 full time dedicated people

- Five Specialized and sector-oriented collections
- High intra-species diversity
- Backed by expertise and equipments of research laboratories where BRCs are located
- All ISO9001 certified
- Involved in European and international projects
- Sharing common research fields



- More than 20,000 strains of food, plant and pathogenic bacteria, yeasts and filamentous fungi
- Partner of MIRRI, the pan-European Microbial Resource Research Infrastructure

CIRM-BIA dedicated to Food Bacteria STLO-Rennes

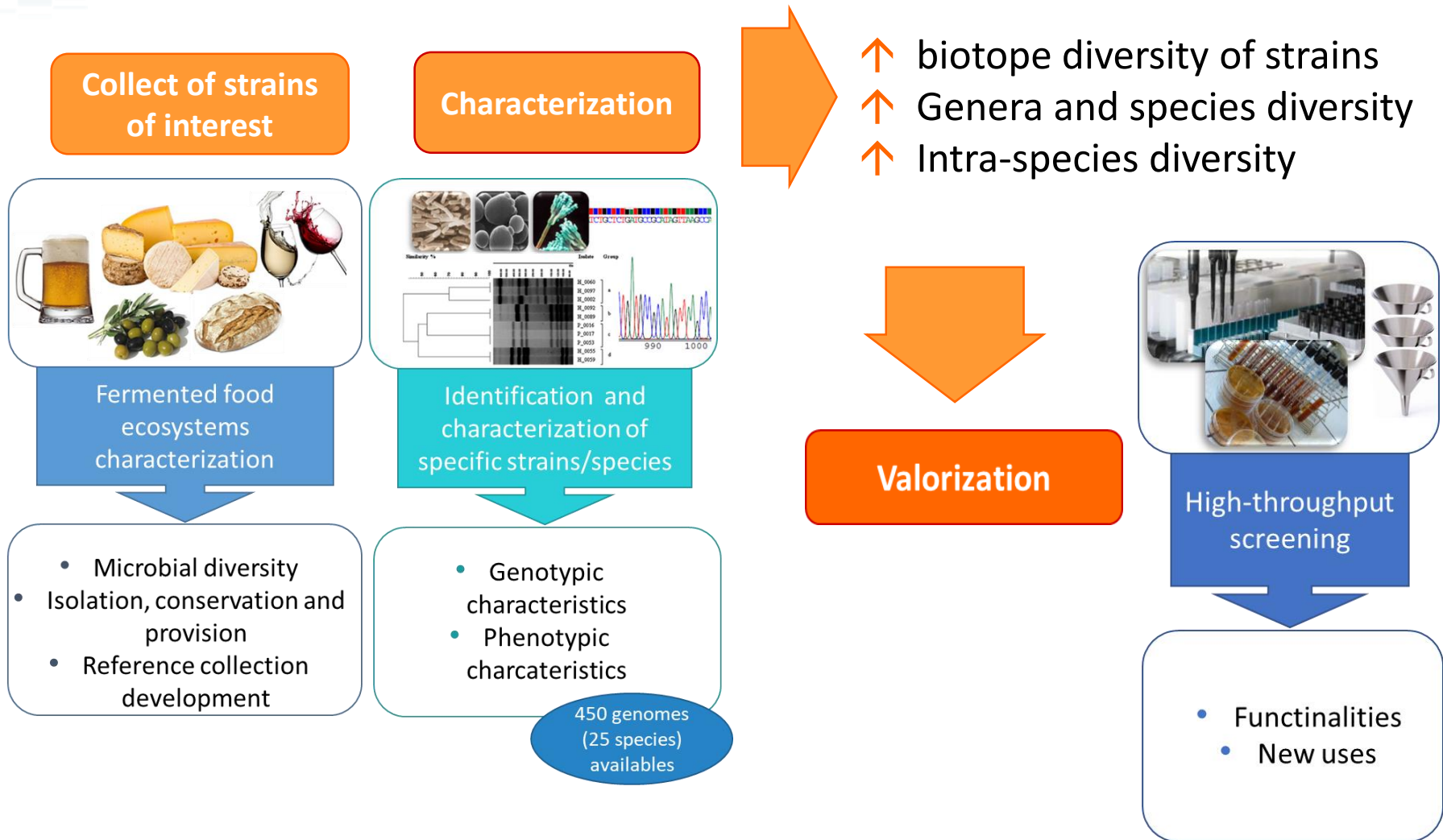


- 4,000 strains of bacteria of food interest (> 150 species) incl. 2,000 lactic acid bacteria
- A collection continuously enriched by exploration of bacterial diversity of various food ecosystems
- Securing and storing data system with associated to a strain catalog online



<http://collection-cirmbia.fr>

Bacterial diversity: contribution to innovation



Bacterial diversity: contribution to innovation

Collection of strains of interest

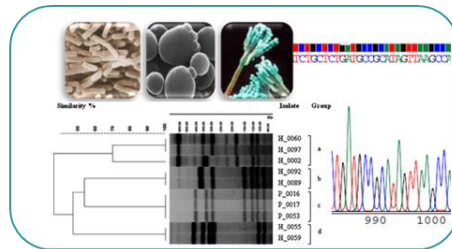
Characterization

- ↑ biotope diversity of strains
- ↑ Genera and species diversity
- ↑ Intra-species diversity



Fermented food ecosystems characterization

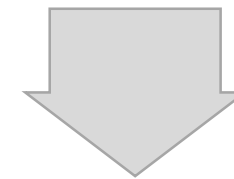
- Microbial diversity
- Isolation, conservation and provision
- Reference collection development



Identification and characterization of specific strains/species

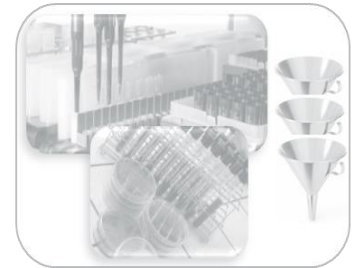
- Genotypic characteristics
- Phenotypic characteristics

450 genomes (25 species) availables



Valorization

> Exples of bacterial diversity collection and characterization in dairy and non dairy fermented products




High-throughput screening


- Functionalities
- New uses

Collection and characterization




- **BAKERY ANR Project** 
Microbial diversity (Bacteria and Yeast) of sourdough used for traditional bread

50 original LAB strains from integrated into our collection. One new yeast species: *Kazachstania saulgeensis*


 Lhomme et al. , 2016

- **Lemzeit Project**
Bacterial diversity of traditional fermented wheat (Constantine University, Algeria, PhD R. Merabti)

69 LAB isolates (6 genera, 16 species), among them 10 LAB strains belonging to 7 species = functional starters for wheat fermentation

 Merabti et al. , 2018 - Submitted

 Merabti et al. , 2015

- **PEAKYEAST ANR Project** 
Microbial diversity (Bacteria and Yeast) of grape must used for wine making

420 vegetal LAB and ACB strains with high level of species diversity (26 genera, 82 species) integrated into our collection



Strains biotope diversification ↑↑↑

→ New starters for fermentation of non dairy matrix or milk-legumes mixes

Collection and characterization



- European project “Gwell”



Development of a traditional fermented milk product from the milk of local Cows “bretonne Pie Noire: the Gwell” (Preparatory action EU plant and animal genetic resources in agriculture)

No starters, only backslopping technic for production of Gwell

Sometimes accidental lost of the “Gwell” → **Gwell production securing**

- 1 Diversity characterized by
 - > Culture dependant methods
 - > Metabarcoding 16S

~ 100 LAB isolates,
18 strains of interest (*Lactococci*)

- 2 Test of strains in associations as “functionnal gwell starters”



How to define the “functional associations” ?
How to predict their functionalities ?

Bacterial diversity: contribution to innovation

Collect of strains of interest

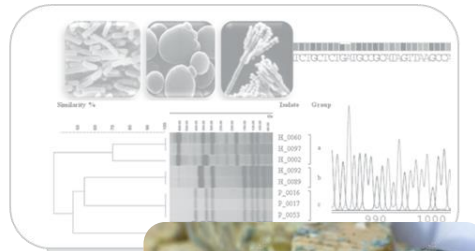
Characterisation

- ↑ biotope diversity of strains
- ↑ Genera and species diversity
- ↑ Intra-species diversity



Fermented food ecosystems characterization

- Microbial diversity
- Isolation, conservation and provision
- Reference collection development



Identification of specific



- Genotypic characteristics
- Phenotypic characteristics

450 genomes (25 species) availables



Valorization

> Exple :
Development of antifungal ingredients for dairy products

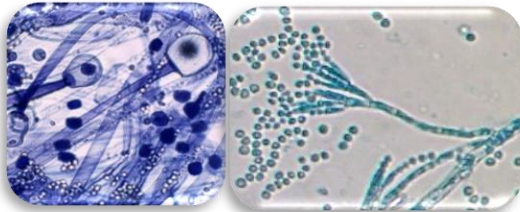


High-throughput screening

- Functionalities
- New uses

Development of antifungal ingredients for dairy products: *from in vitro screening to pilot-scale applications*

Moulds



- Growth at low temperature and low pH
- Assimilation of available nutrients
- Halotolerant and xerotolerant



Yeasts

Spoilage



Visible alterations, off-flavours, ethanol, CO₂

Cheese



Cream



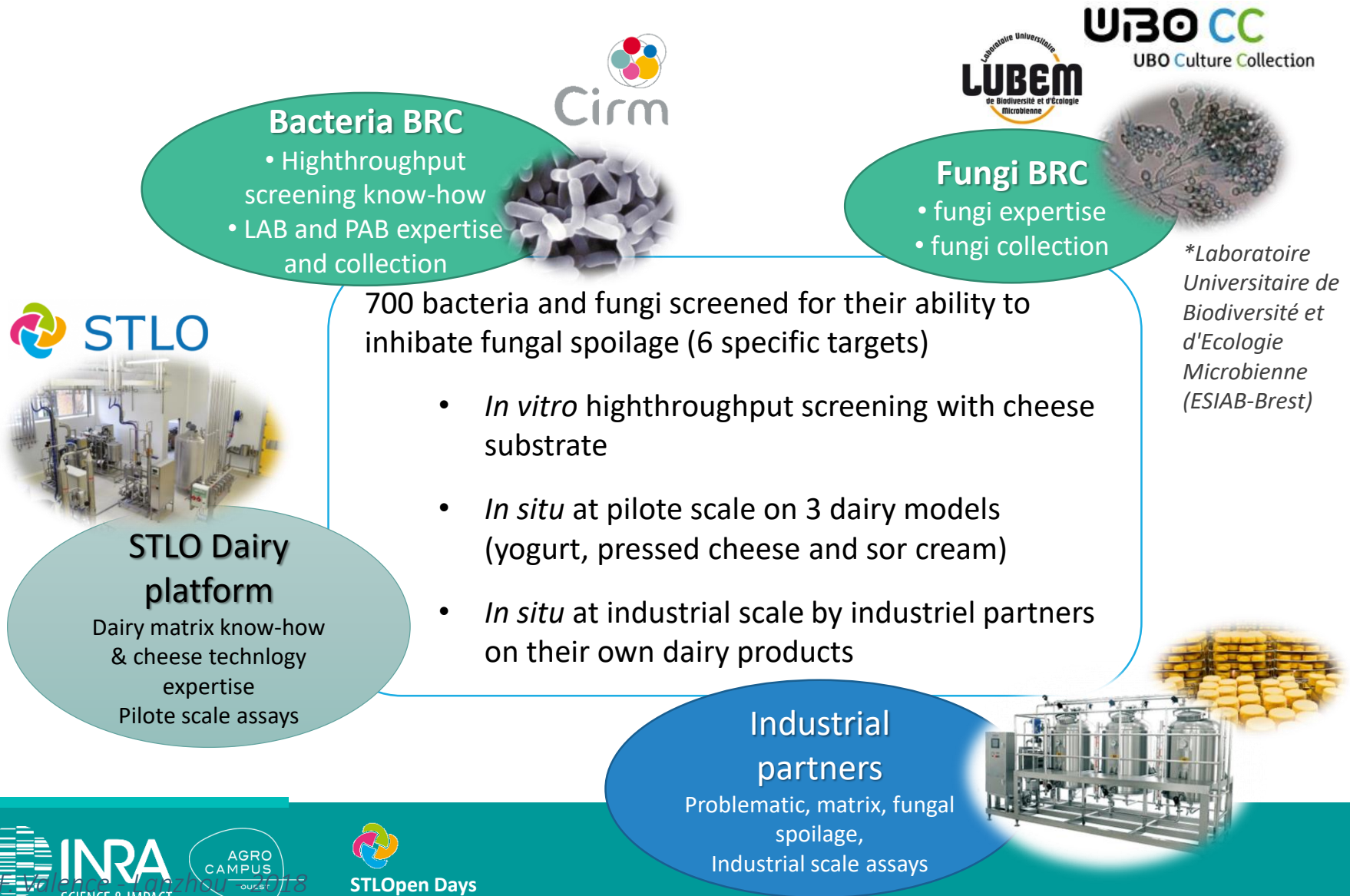
Yogurt

Food waste and economical losses

Consumer dissatisfaction

Limitation of export possibilities

Development of antifungal ingredients & adjunct for dairy products: a close research partnership



Integrative approach for the development of antifungal adjuncts and dairy fermentates

- Choice of fungal targets and screening matrix
- Development of an easy (high throughput) and reproducible method

Screening
and Selection

- Scale up: *In situ* activity (challenge & shelf-life tests)
- Sensorial analysis
- Safety assessment (acquired antibiotic resistance, biogenic amines production)

Application

- Which Antifungal compounds ?
- Mode of action
- Fungal target response

Identification and
action mode

Adapted and modified from Crowley et al. 2013

Choice of fungal targets

- Choice of fungal targets and screening matrix
- Development of an easy (high throughput) and reproducible method

Screening and Selection

- Scale up: *In situ* activity (challenge & shelf-life tests)
- Sensorial analysis
- Safety (acquired antibiotic resistance, biogenic amines production)

Application

- Which Antifungal compounds ?
- Mode of action
- Fungal target response


Identification and action mode

« If you know the enemy and know yourself, you need not fear the result of a hundred battles... » Sun Tzu, *The Art of War*.

> Collect and identification of fungal diversity of industrial spoiled dairy products and isolates

- ✓ A reference collection of 105 dairy fungal contaminants (18 genera, 40 species)
- ✓ 4 fungal targets selected, representative of the diversity of fungal contaminants in dairy industry:

- *G. geotrichum* UBOCC-A-216001
- *M. racemosus* UBOCC-A-116002
- *P. commune* UBOCC-A-116003
- *Y. lipolytica* UBOCC-A-216006

 Garnier et al. 2017, IJFM

Development of a relevant screening tool

- Choice of fungal targets and screening matrix
- Development of an easy (high throughput) and reproducible method

Screening and Selection

- Scale up: *In situ* activity (challenge & shelf-life tests)
- Sensorial analysis
- Safety (acquired antibiotic resistance, biogenic amines production)

Application

- Which Antifungal compounds ?
- Mode of action
- Fungal target response

Identification and action mode

☀ Traditionally performed using lab media favoring the growth of screened microorganisms

Easily usable and high throughput compatible

- **Lab media can enhance artificially** antifungal activities (e.g. MRS medium)
- Lab media composition **differs from that of real food products**
- **Interactions** with starters not taken into account

➔ Development of a new method for antifungal activity screening

- Dairy matrix as substrate
- In miniaturized system compatible with High throughput application

Mini-Cheese model

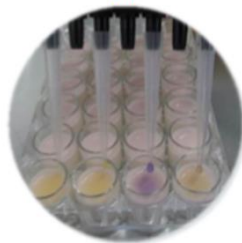
Standardized UF milk retentate with rennet and mesophilic starter cultures + pH indicator

Mini-Yogurt model

Semi-skimmed milk supplemented with skimmed milk powder at 4% + starter cultures + pH indicator

24 well plates

Fermented dairy ingredients, fermentates: surface application



Adjunct cultures: added cocomitantly with starter cultures



Screening rate of 1,600 assays per week

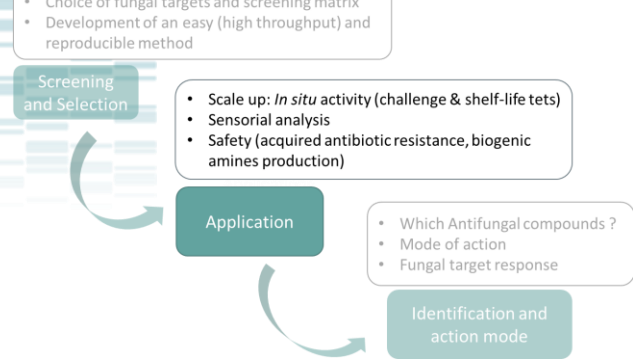
500 LAB and PAB strains screened

32 AF strains



Technical note: High-throughput method for antifungal activity screening in a cheese-mimicking model. Garnier et al. 2018.

Development of antifungal adjuncts and dairy fermentates: main results




Challenge-tests

M. racemosus, P. commune
Surface-inoculation (50 spores)

R. mucilaginoso
(50 CFU/g)

Incubation at 10°C




Shelf life tests

Storage for 2 weeks at 4°C

Natural contamination by ambient air

Incubation at 10°C for 10 days




Sensorial analysis: Sorting task approach

Storage for 2 weeks at 4°C

27 panelists

Group samples according to their organoleptic properties and give descriptors for each group



Safety (acquired antibiotic resistance, biogenic amines production)

32 AF strains

Impact of adjunct cultures on sensorial properties ?

📖 Development of antifungal ingredients for dairy products: from in vitro screening to pilot scale application. L. Garnier et al., 2018.

📖 Antifungal Activity of Lactic Acid Bacteria Combinations in Dairy Mimicking Models and Their Potential as Bioprotective Cultures in Pilot Scale Applications. Leyva-Salas et al., 2018

📖 Biogenic amine and antibiotic resistance profiles determined for lactic acid bacteria and a propionibacterium prior to use as antifungal bioprotective cultures. Coton et al., 2018

> **7 "antifungal" strains** (2 single strains and 3 associations) available for industrial partners, safety validated

> **Usable as "adjunct cultures" or "fermentate"**, depending of the strains and associations, currently under test by industrial partners

Development of antifungal ingredients for dairy products, conclusion and perspectives

- Interest of bioprotection to reduce fungal spoilage of dairy products → 2 complementary tools, fermentates and adjunct cultures (7 strains/3 associations currently in test in real products with our industrial partners)
- Evaluation of *in situ* activity and sensorial impact : a crucial step
- This research was able to succeed thanks to the availability of strains in both complementary **collections with a high level of intra-species diversity**

Functionalities: highly strain dependant !!!

Acknowledgements



Pr. Jérôme Mounier (LUBEM)



Dr. Anne Thierry (STLO-INRA Rennes)



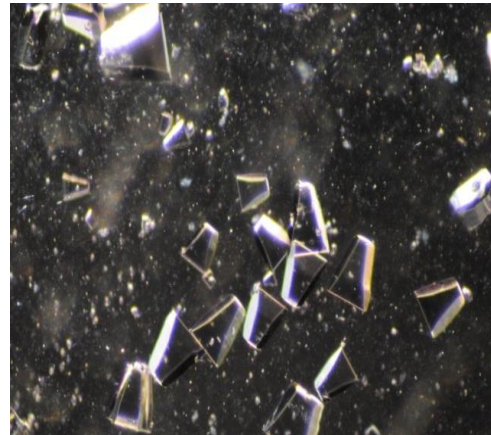
Dr. Lucille Garnier (PhD. at LUBEM/INRA STLO)



Marcia Leyva-Salas (PhD. at INRA STLO/LUBEM)



MERCI



***THANK YOU FOR
YOUR ATTENTION***