



Université
de Bretagne
Occidentale



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

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How to fight spoilers?
06/30/2017

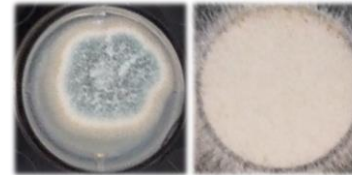


Impact of fungal contaminations in dairy products

Yeast



Filamentous fungi



Growth at low temperature

Growth at low pH

Assimilation of available nutrients

Halotolerant and xerotolerant



Yogurt



Sour cream

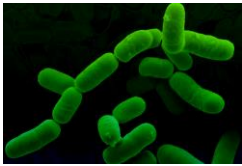


Cheese

Food waste and losses

Consumer dissatisfaction

Economical losses



Preservation of dairy products against fungal spoilage

Preventive methods and hurdle technologies to avoid or delay fungal contaminations in dairy products:

- **Prevention and control methods** (GMP, air filtration,...)
- **Traditionnal hurdle technologies** (heat treatment, fermentation, chemical preservatives)
- **Biopreservation**

« Extend shelf-life and improve food quality through the use of microorganisms and/or their antimicrobial metabolites »
(Stiles, 1996)

Use of **purified
metabolites**

Use of active strain
in situ, as **adjunct
culture**

Fermented substrate



Different steps to develop an antifungal dairy ingredient

Study of the diversity of
spoilage fungi in dairy
products

Screening of
microorganisms

In situ testing and
sensory analysis

Identification of antifungal
molecules and elucidation of
inhibitory mechanism

(adapted from Crowley et al., 2013)



Identification of spoilage fungi isolated from dairy products



Cream



Yoghurt



Cheese

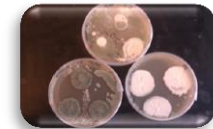
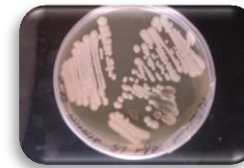


Sour cream

Sample collection (32)



Isolation and purification of 106 isolates



Identification of spoilage fungi by sequencing

- D1-D2 domain of 28S rRNA gene
- ITS region
- β -tubulin
- EF1- α

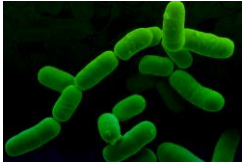


Yeasts



Filamentous
fungi

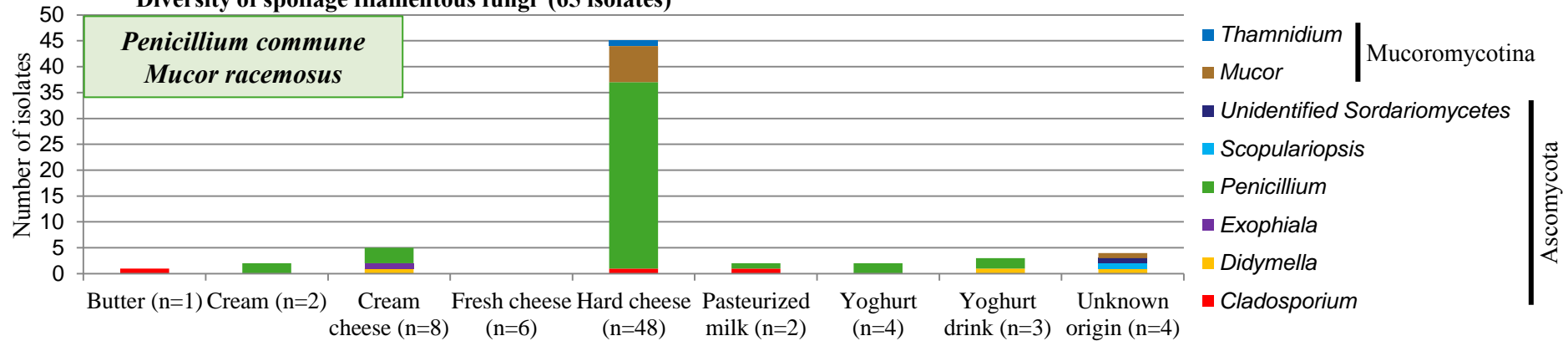
(Garnier et al., 2017) 5



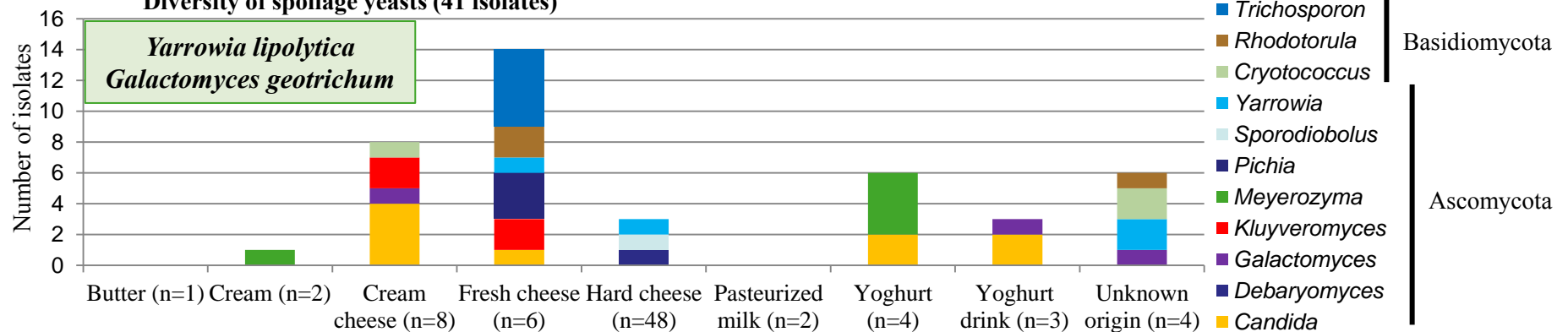
Diversity of spoilage fungi in dairy products

High diversity
19 genera, 41 species

Diversity of spoilage filamentous fungi (65 isolates)



Diversity of spoilage yeasts (41 isolates)



(Garnier et al., 2017) 6



Different steps to develop an antifungal dairy ingredient

Study of the diversity of spoilage fungi in dairy products

Reference collection

Selection of fungal targets

Screening of microorganisms using a standardized, rapid, easy and reproducible method

In situ testing and sensory analysis

Identification of antifungal molecules and elucidation of inhibitory mechanism

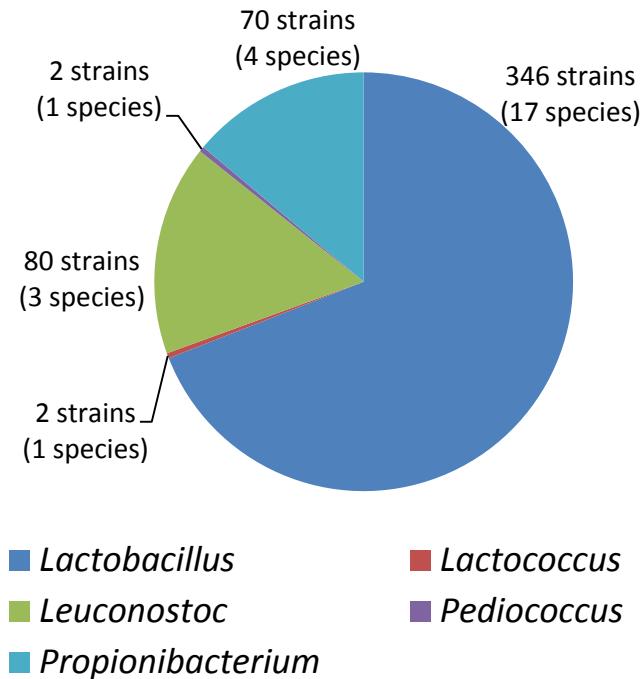
(adapted from Crowley et al., 2013)



Bacteria and fungi selected for the screening of fermentates with antifungal activity

698 selected strains

Lactic acid bacteria and propionibacteria (500 strains)



Fungi (198 strains)

- Non mycotoxin-producing fungi
- **89 species belonging to 28 genera**
- Main genera (>10 strains):
 - Candida*
 - Exophiala*
 - Pichia*
 - Saccharomyces*
 - Mucor*
 - Verticillium*



High-throughput method for antifungal activity screening in a cheese-mimicking model

Dairy matrix

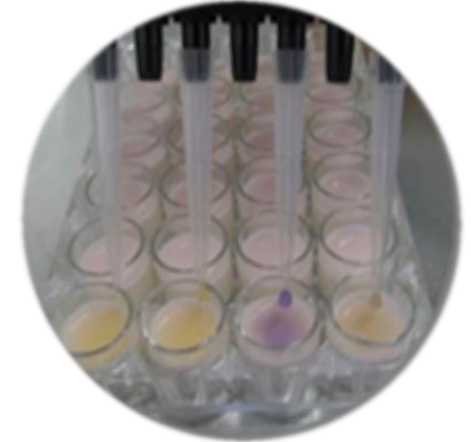
Standardized ultrafiltration cheese with rennet and mesophilic starter culture + pH indicator



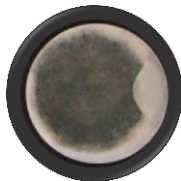
Fermentation substrates

- Low heat milk with anhydrous milk fat + pH indicator
- Milk permeate complemented with yeast extract + pH indicator

Surface application



Surface inoculation with fungal targets (50 spores or cells) and evaluation of antifungal activity after 5-9 days of incubation at 12° C



No effect (0)



Weak effect (1)



High effect (2)



Total inhibition (3)

Target:
P. commune



Results

Impact of fermentation substrates on the expression of antifungal activities

Milk permeate

2.9% with antifungal activity

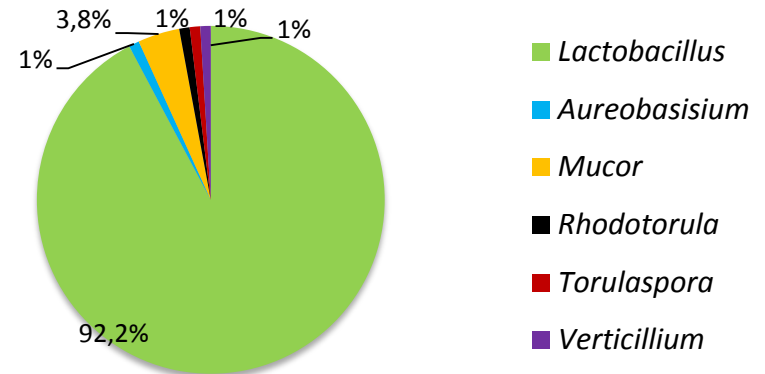
0.3% showed high effect or total inhibition

Low heat milk (LH)

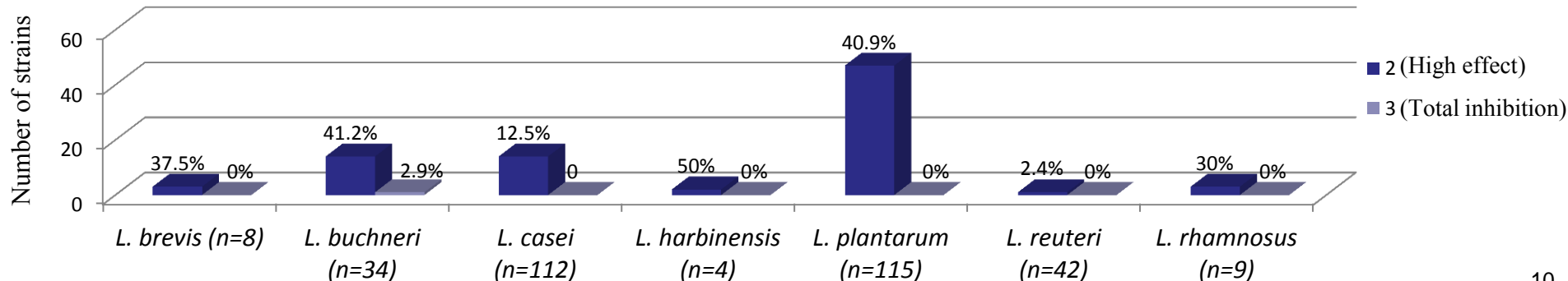
11.9% with antifungal activity

3.7% showed high effect or total inhibition

Distribution of genera presenting high antifungal activities (score > 1) after growth in LH medium

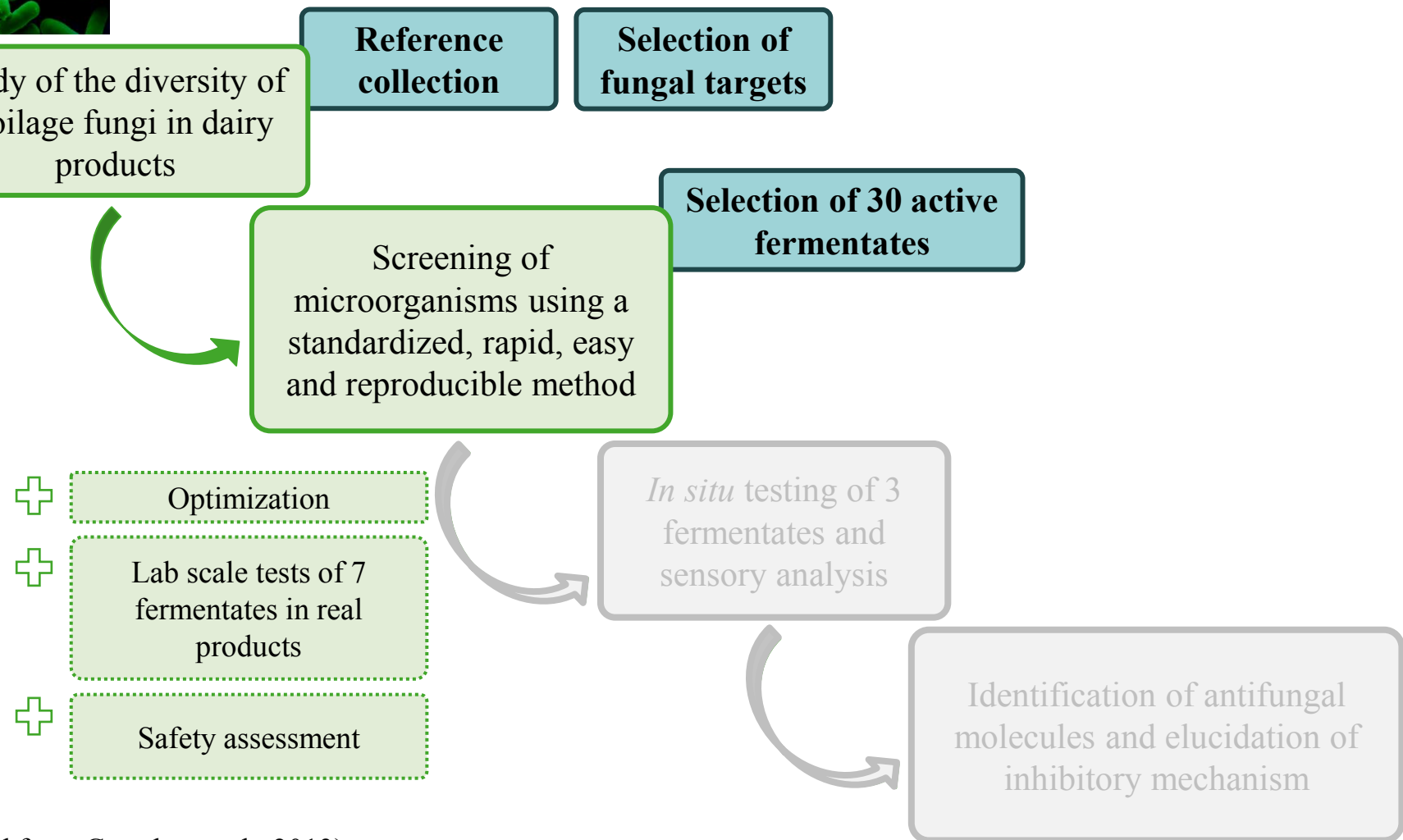


Lactobacillus spp. with high antifungal activities after growth on LH medium





Different steps to develop an antifungal dairy ingredient



(adapted from Crowley et al., 2013)



Pilot scale assay on uncooked pressed smear cheese: surface application of 3 fermentates (*P. jensenii*; *P. jensenii* + *L. rhamnosus*; *L. rhamnosus* + *M. lanceolatus*)



Challenge tests

3 surface-applications at days 1, 4 and 6 of ripening

Surface inoculation
(50 spores)

M. racemosus, *P. commune*

Incubation
(12° C, 95% moisture)

Durability tests

Cheeses contaminated under environmental conditions

Incubation
(12° C and 95% moisture for 6 weeks)

Sensorial tests using FAST

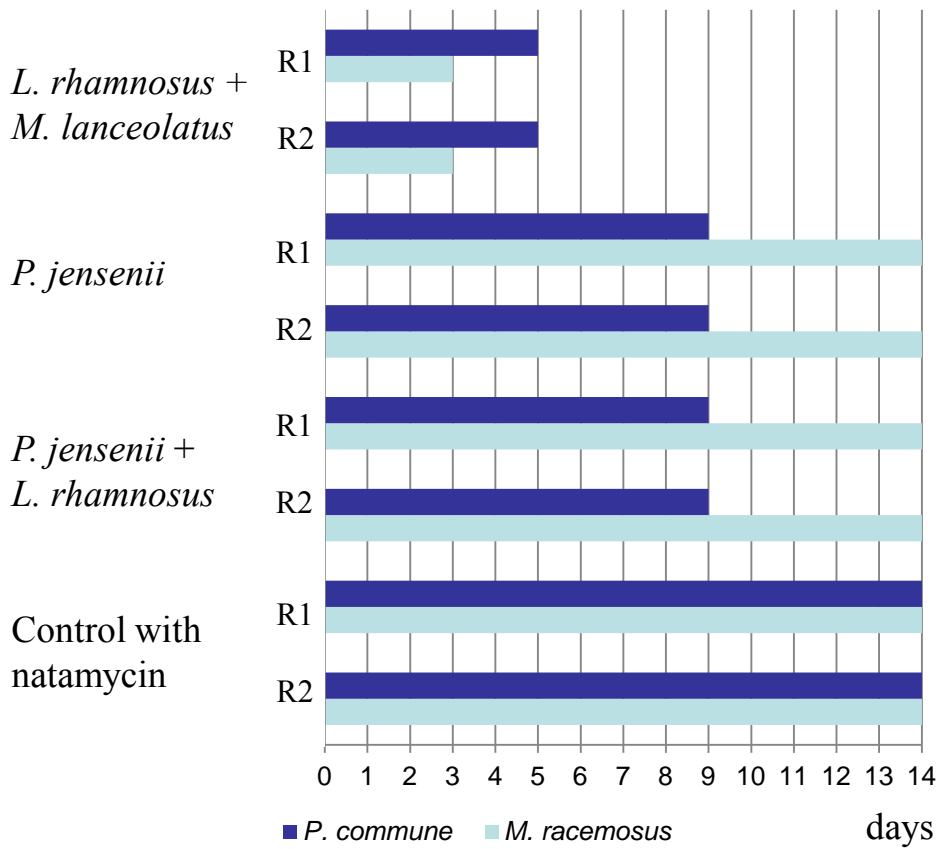
29 panelists

Make groups according to cheese organoleptic properties and attribution descriptors for each group



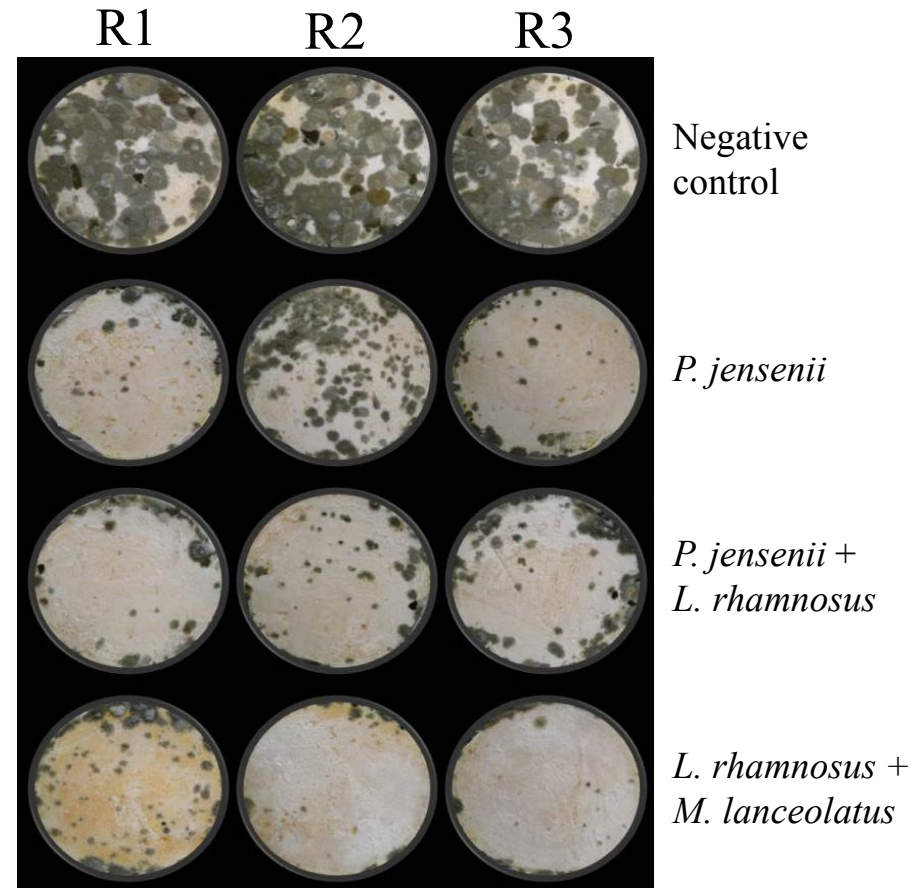
Challenge tests

Results obtained for *P. commune* and *M. racemosus* (growth delay in days)



Durability tests

6 weeks storage at 12° C



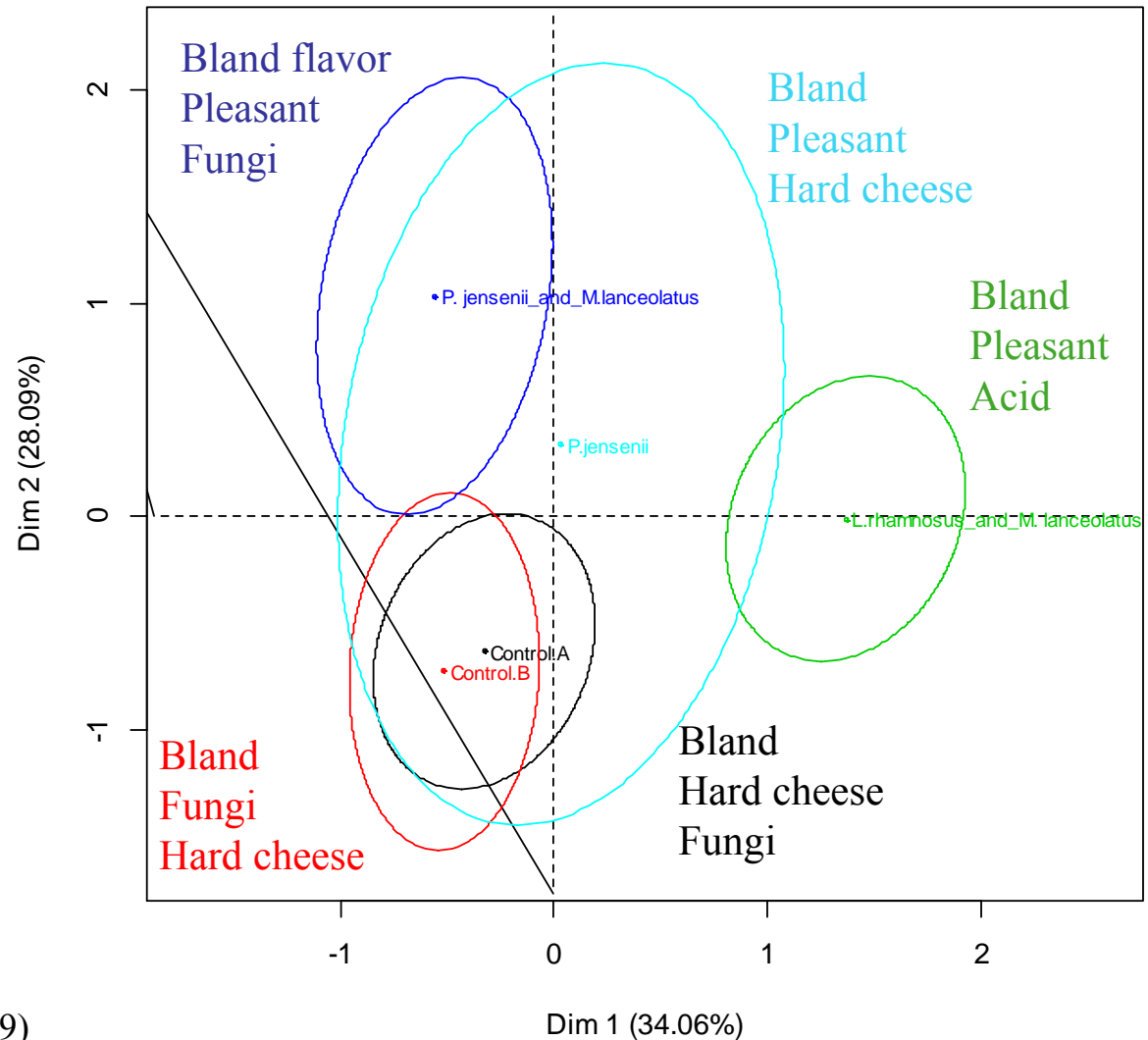


Sensorial tests

- 29 panelists
- 7 panelists made only 1 group

➡ No impact of fermentates on organoleptic properties of uncooked pressed smear cheese

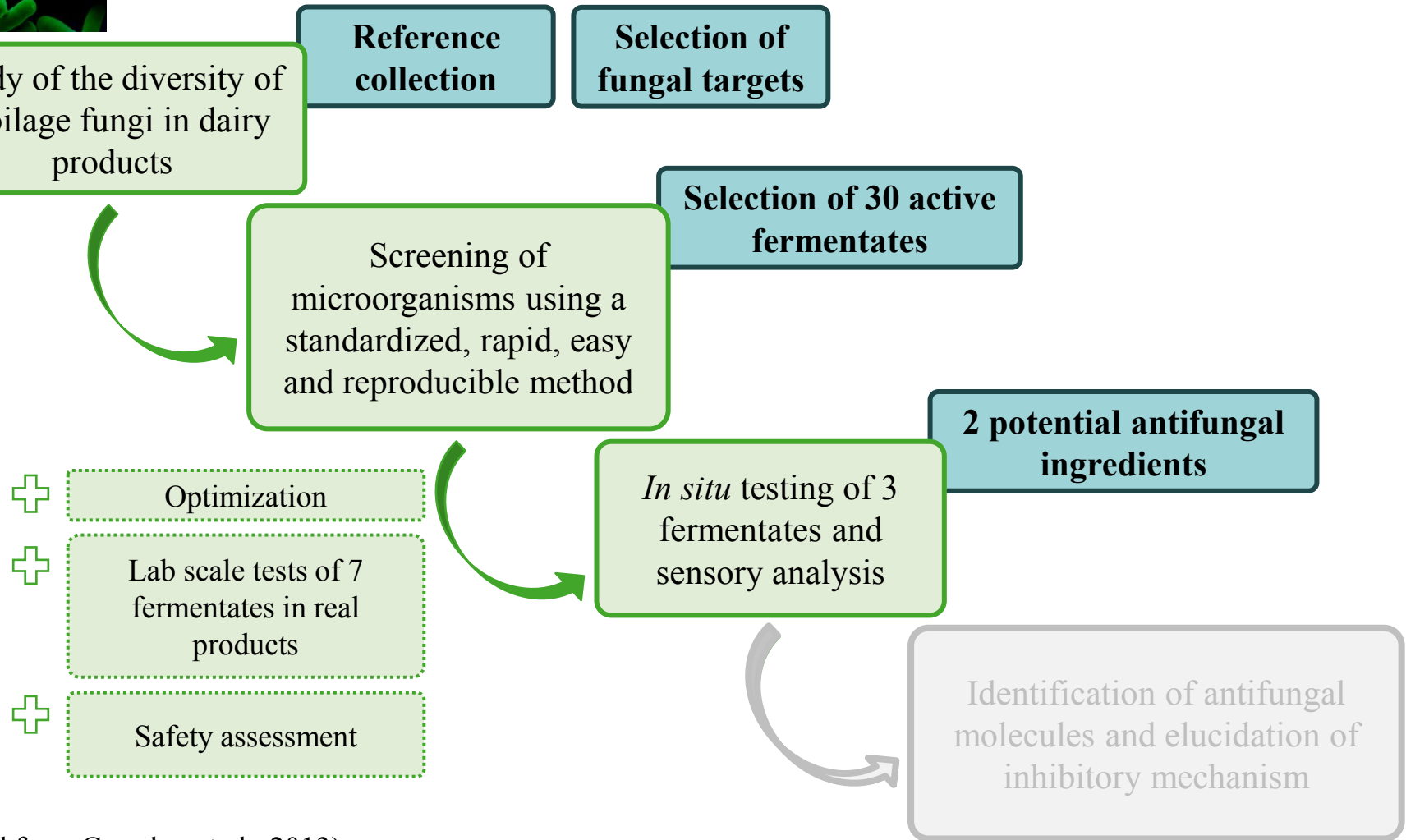
Factorial approach (22 panelists)



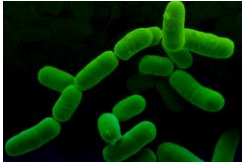
(method adapted from Cadoret et al., 2009)



Different steps to develop an antifungal dairy ingredient



(adapted from Crowley et al., 2013)



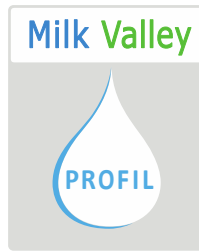
Conclusions

- ➡ High diversity of spoilage fungi in dairy product
- ➡ 698 tested microorganisms
 - < 3% of active fermentates with milk permeate substrate
 - < 12% of active fermentates with low heat milk substrate
- ➡ 3 active fermentates in a real product without any impact on organoleptic properties
- ➡ 2 good candidates for application as antifungal ingredients



On-going research

- ➡ Identification of antifungal compounds
- ➡ Elucidation of action mechanism



Thank you for your attention



Academic partners



Industrial partners



Financial support



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