



Société Française  
de Microbiologie

**MICROBES**  
15<sup>e</sup> congrès national  
de la SFM ●●●●●

Du  
30 septembre  
au 2 octobre  
**2019**

Cité des sciences et de l'industrie, Paris 19<sup>ème</sup> ●●●

# Selection of effective antifungal cultures in fermented dairy products and identification of antifungal metabolites

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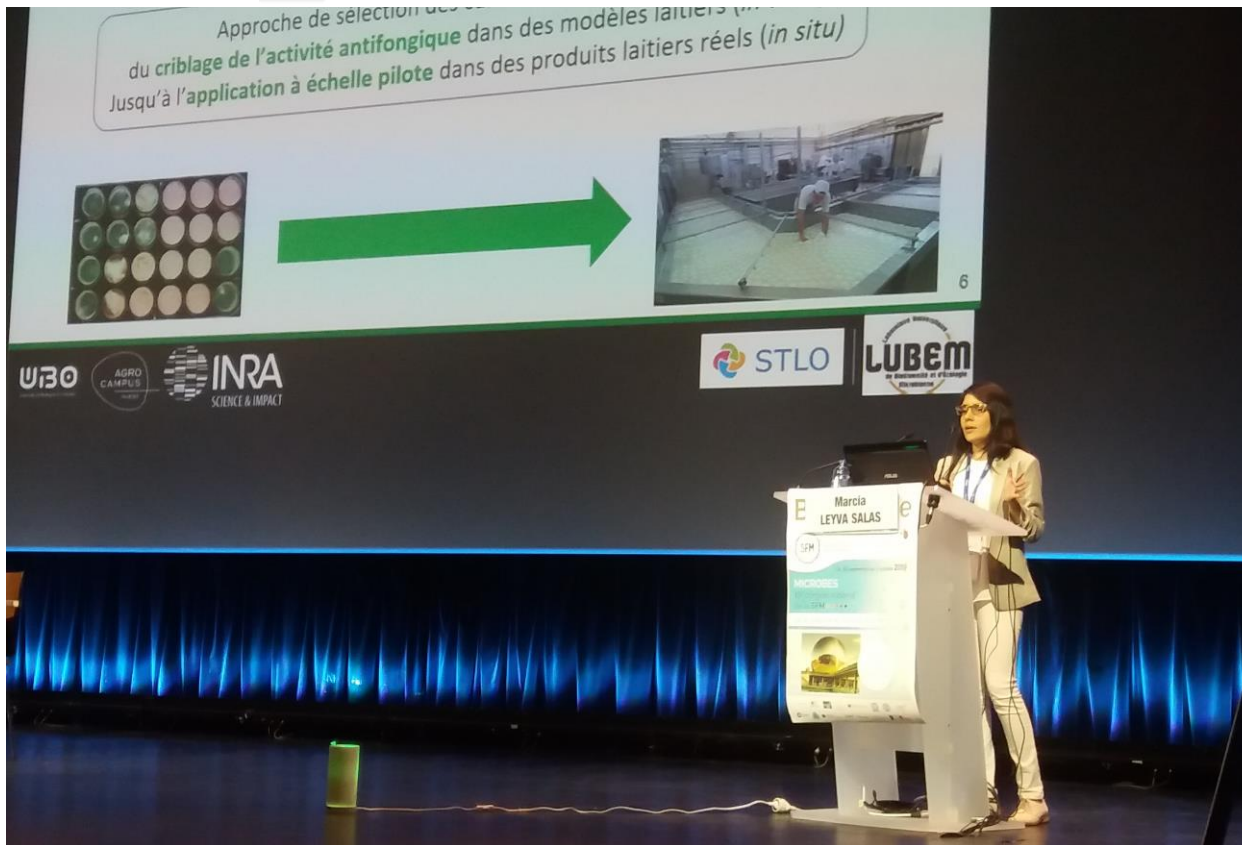
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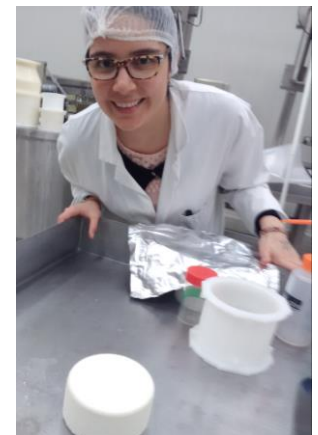
## Déclaration de conflit d'intérêt

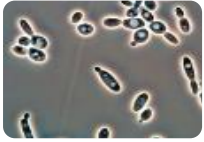
Pour cette présentation, je déclare n'avoir aucun conflit d'intérêt.

# AUDITION Prix de thèse, 30 sept 2019



Marcia Leyva Salas





yeasts



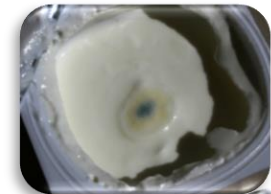
molds

# Fungi of dairy products

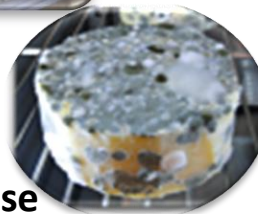
Some essential fungi in dairy products...



... but others responsible for spoilage !



yogurt



cheese

Impact on the organoleptic quality of dairy products  
Food waste and economical losses

**Biopreservation:** « *Extension of food shelf-life and increase in food safety using natural or added microbiota and/or their antimicrobial compounds* » *Stiles, 1996*

- ✓ Can be used to complement preventive and control methods in **replacement of chemical preservatives** (natamycin, sorbates)
- ✓ Responds to the **strong societal demand** for more natural, less severely processed and safer products

LAB and PAB strains as  
**adjunct cultures**

**Fermentates**

**Purified metabolites**

Milk Valley

PROFIL

**PROFIL project**

to develop bioprotective solutions with antifungal activity suitable for use in the dairy industry

# Strategy to identify antifungal strains

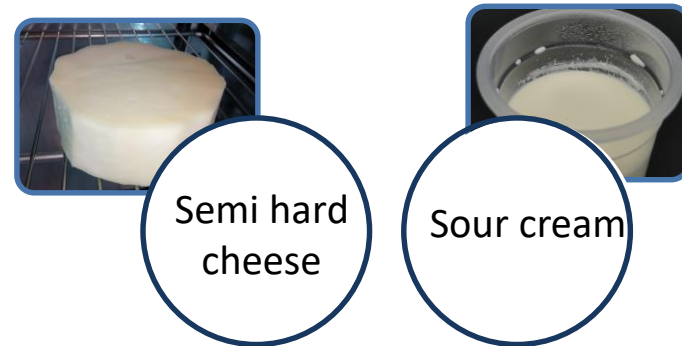
## Scale-up approach

from **high-throughput screening** in dairy models

to **pilot scale validations**

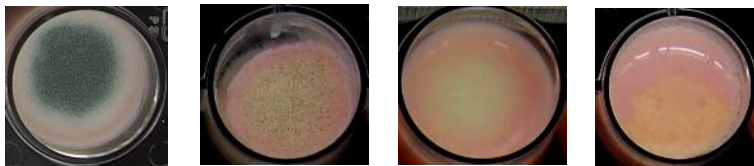


A selection of strains  
Commercial antifungal cultures  
Several inoculation levels  
→ ~7000 tested conditions



*Garnier et al., 2018 J Dairy Science*

**4 fungal targets:**



*P. commune M. racemosus G. geotrichum Y. lipolytica*

- ✓ isolated from spoiled dairy products
- ✓ representative of the most common dairy product spoilers

*Garnier et al., 2017 Int J Food Microbiol*

## Targeted metabolomics

to identify the antifungal metabolites involved in the observed activity in the 4 dairy products

# Screening results

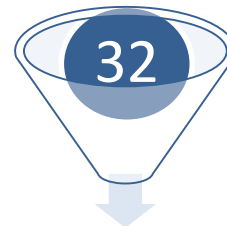
**Previous screening** of the antifungal activity of 500 LAB and PAB strains as fermentates  
*Garnier et al., 2019 Food Microbiol*



→ **17% of the milk fermentates** inhibited at least 1 fungal target

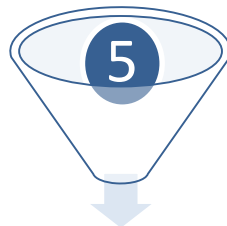
## 32 strains selected

18 *Lactobacillus*  
5 *Leuconostoc*  
9 *Propionibacterium*



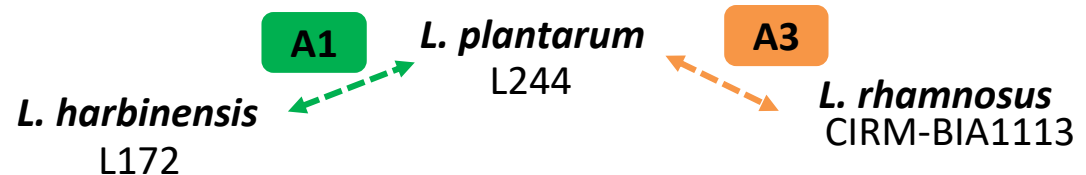
Antifungal activity: both **species- and strain-dependent**  
Many *Lactobacillus* strains active

**5 *Lactobacillus* strains** selected with high activity & broad spectrum of action  
Tested in combination



Safety: biogenic amine production and antibiotic resistance

→ **2 binary combinations** retained to be tested at the pilot scale



# Challenge-test: inoculation of targets

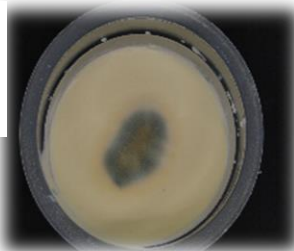
50 spores on the surface

*P. commune*

*M. racemosus*

2 yeast cells/g  
in sour cream  
*R. mucilaginosa*

Sour cream



4 weeks  
incubation  
at 10° C

Cheese



Ripening  
4 weeks at  
12° C

## Shelf life tests: 20 min exposition



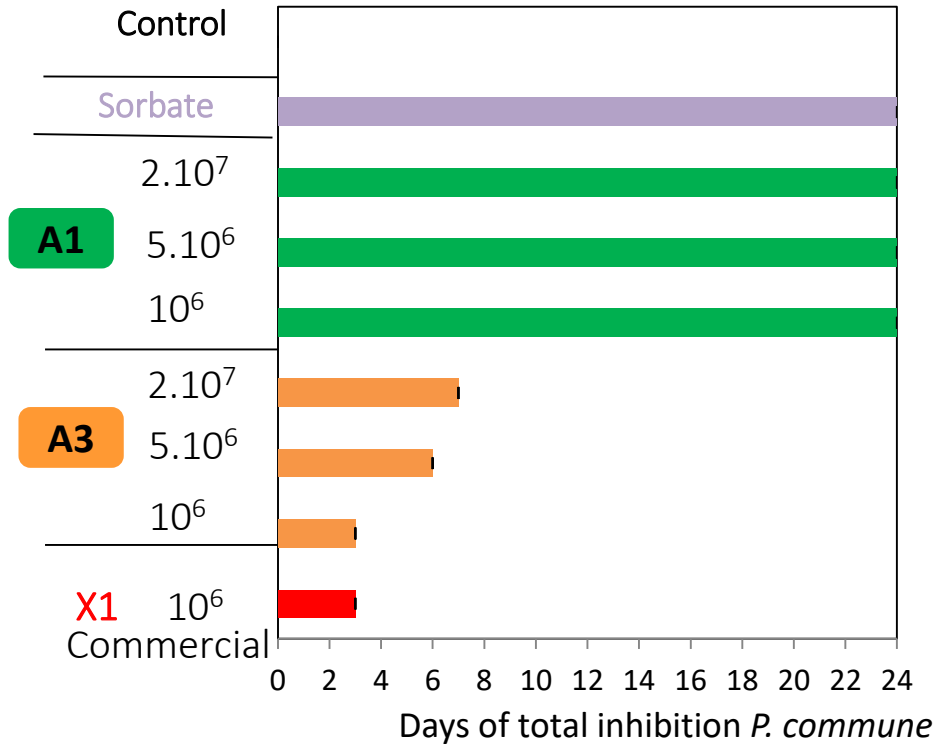
2 weeks incubation at 10° C





## Example of results during scale up (1)

### Sour cream challenge-test against *P. commune*



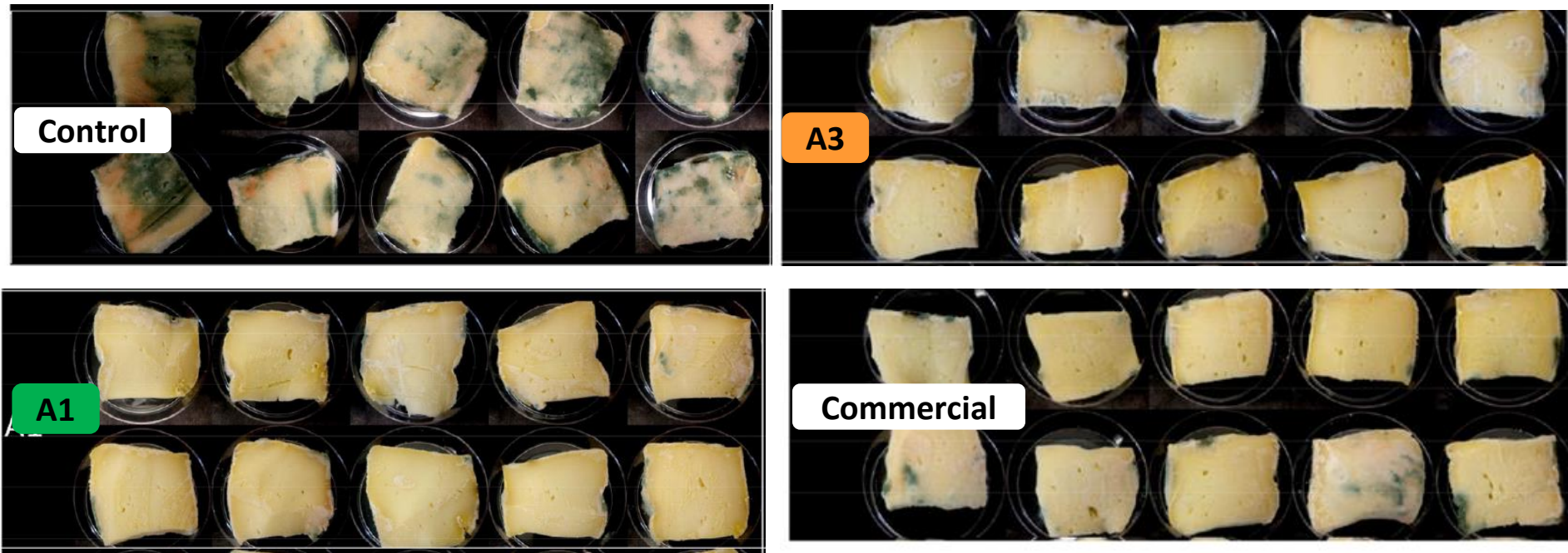
**A1** higher AF activity and spectrum of action compared to **A3** and commercial **X1**

**A1** antifungal activity > *in sour cream* than during screening

Leyva-Salas et al., 2018 *Frontiers in Microbiology*

## Example of results during scale up (2)

### Shelf life test in semi-hard cheese

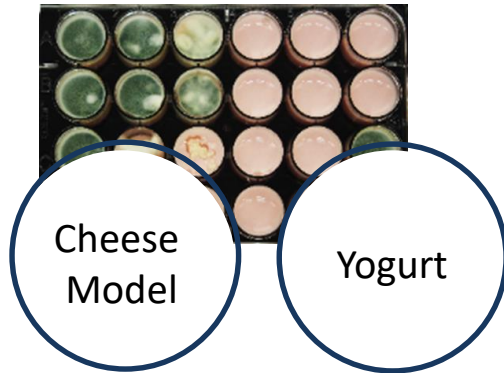


**A1** antifungal activity > **A3** and commercial culture

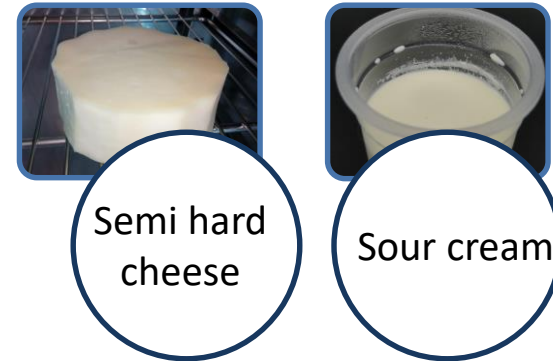
*Leyva-Salas et al., 2018 Frontiers in Microbiology*

# Analyses of antifungal compounds

screening in two dairy models



two dairy products at the pilot scale



**4 dairy products**, 2-3 biological replicates

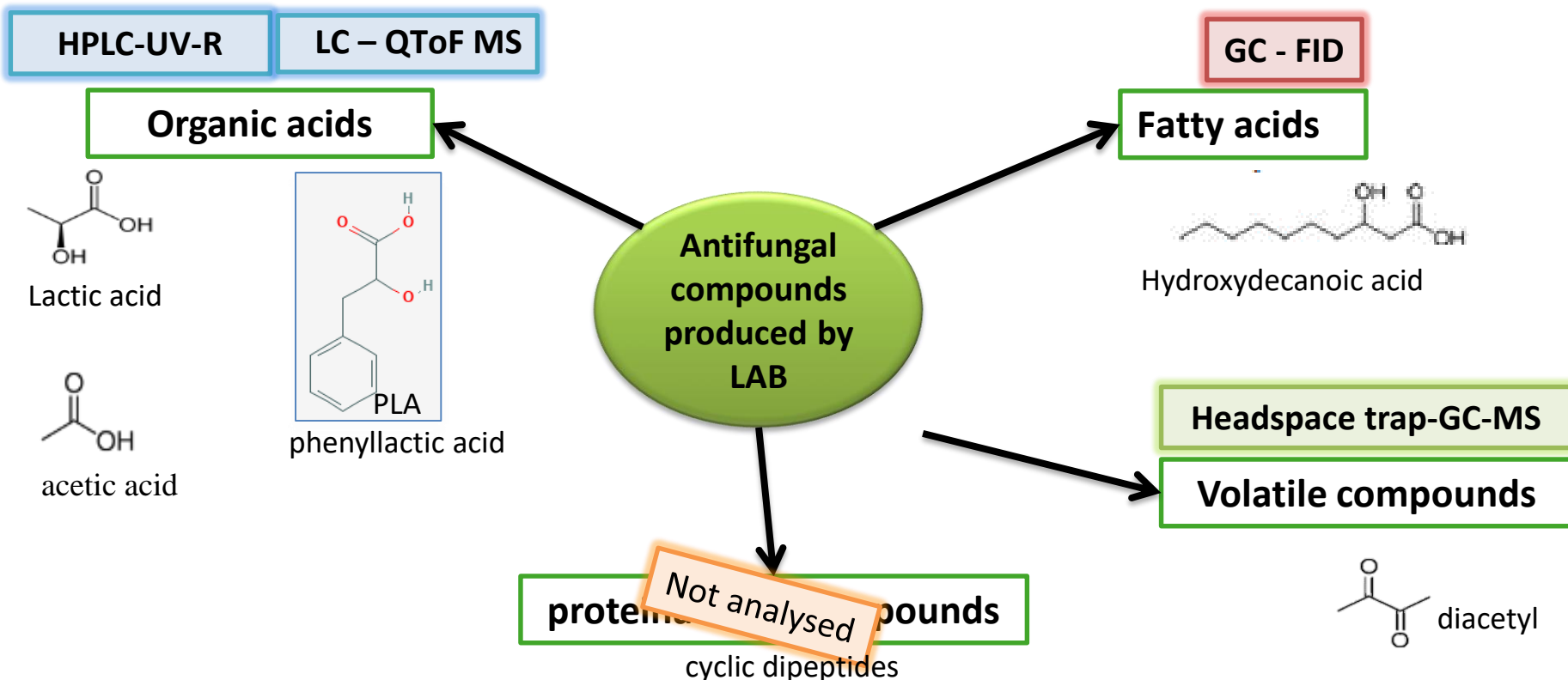
**2 antifungal combinations** tested at 1-3 inoculation levels

→ 100 samples

**Targeted metabolomics**

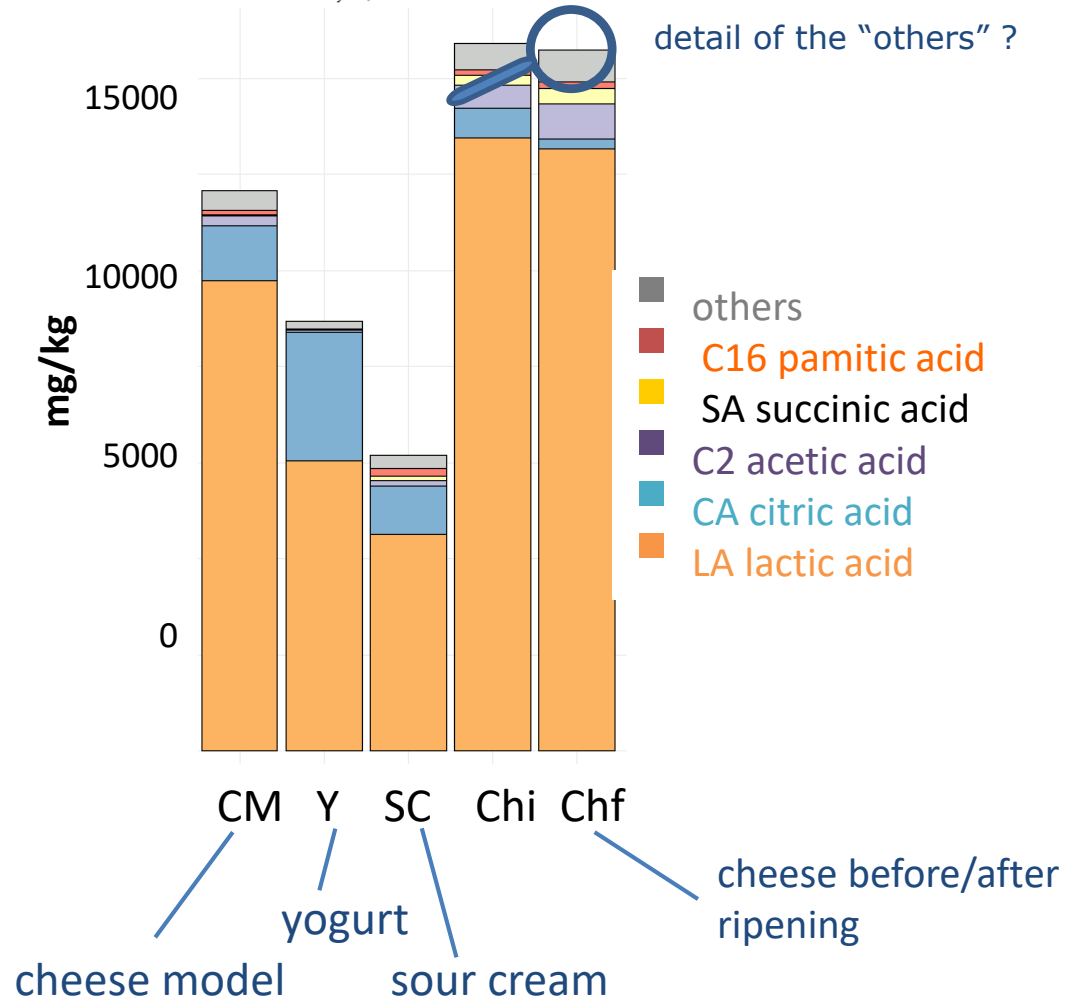
to identify the antifungal metabolites involved in the observed activity

# Four methods combined to quantify antifungal compounds



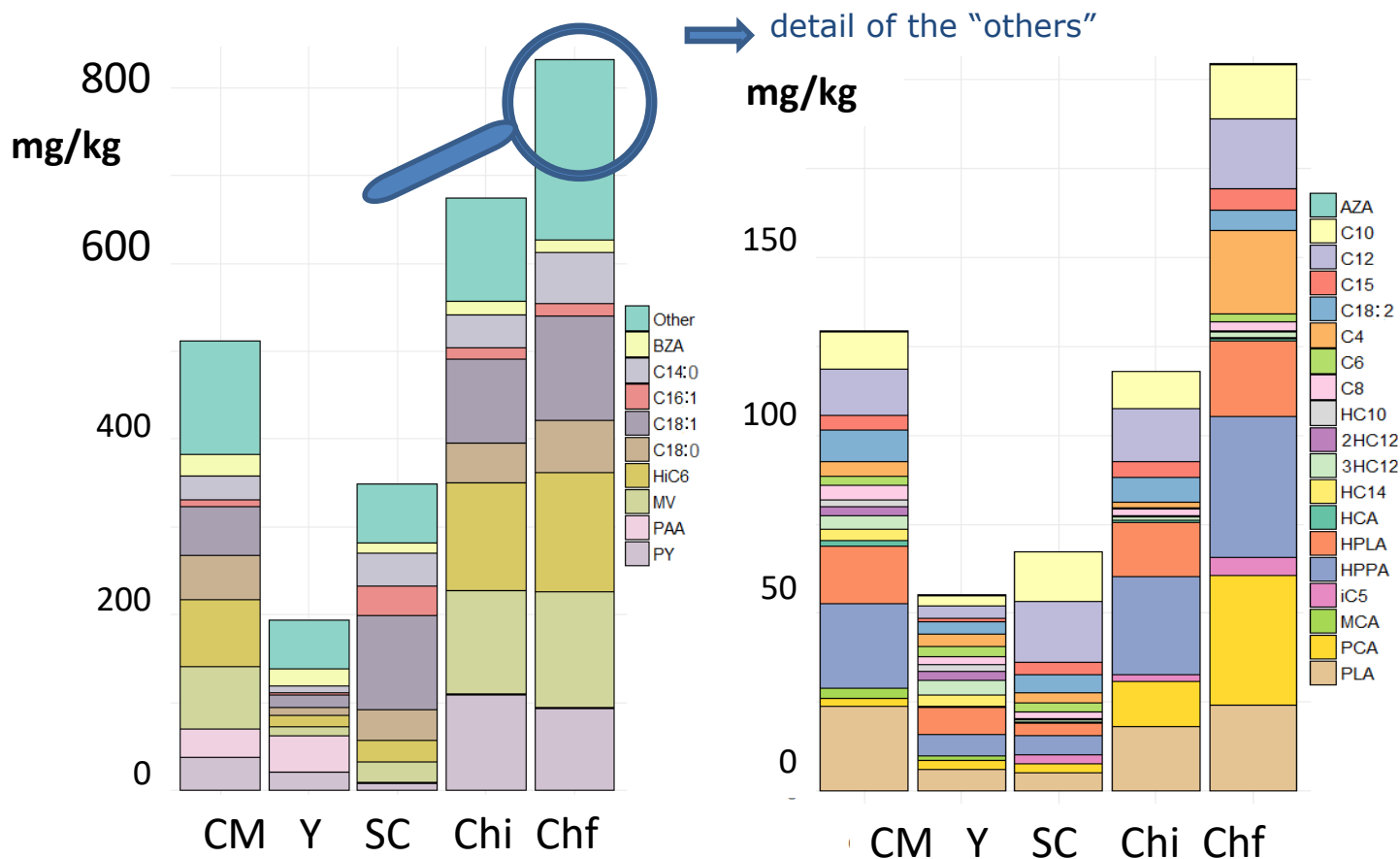
- ✓ 56 antifungal organic acids targeted
- ✓ 34 detected, present at concentrations ranging from > 5 g/kg to < 5 mg/kg

## Main antifungal compounds



Leyva Salas et al; 2019 Food Chem

## Other antifungal compounds



and ~30 volatile  
compounds

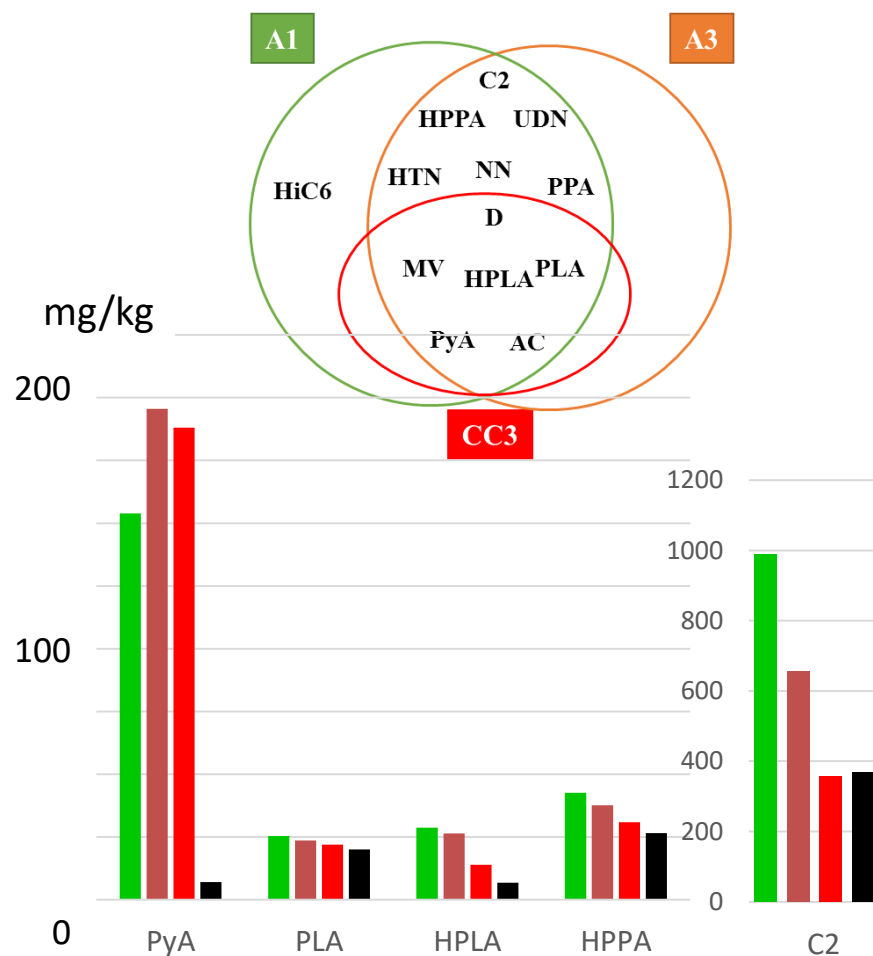
More differences  
between dairy  
products  
than between  
controls and  
products with  
antifungal cultures

→ Compounds  
involved in the  
activity ?

## Compounds produced by antifungal cultures

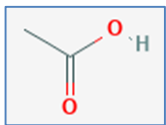
- ✓ ANOVA: 33 compounds present at significantly greater amounts in the presence of an antifungal culture compared to the controls
- ✓ 2 to 16 compounds according to the antifungal culture and the dairy product

### Example of semi-hard cheeses

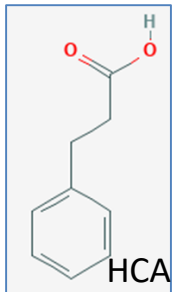


# Some 'key' antifungal compounds

the most correlated with the antifungal activity observed, in 3 or the 4 dairy products

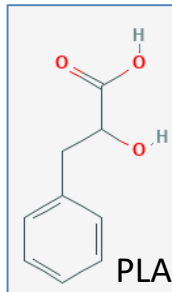


acetic acid



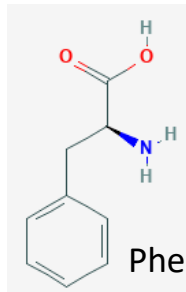
HCA

hydrocinnamic acid



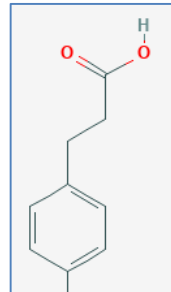
PLA

phenyllactic acid

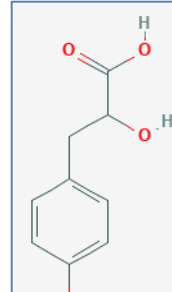


Phe

phenylalanine

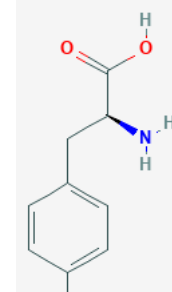


HPPA



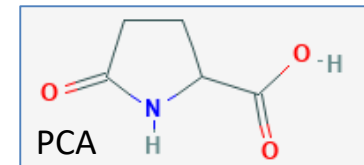
HPLA

hydroxyphenyllactic acid



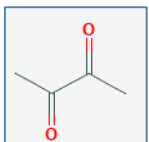
Tyr

tyrosine

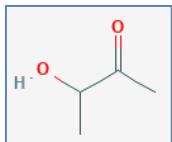


PCA

Pyroglutamic acid



diacetyl



acetoin



# Conclusions

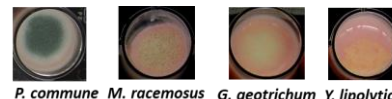


An **efficient approach** to develop flexible antifungal cultures with high antifungal activity and broad spectrum



## Strategy key points for a robust screening approach:

- ✓ Screening in dairy matrices
- ✓ 4 representative and resistant fungal targets
- ✓ Challenge-test performed = worst case contamination scenario  
contamination with 50 spores, incubation at 10-12° C
- ✓ Constraints related to the potential use also considered including safety, sensory and economic constraints (level of inoculation).



→ **Best candidates: 2 combinations of 2 lactobacilli strains:**



# Conclusions

## Antifungal compounds

- ✓ 53 antifungal compounds detected, 33 in significantly higher amounts in at least 1 product inoculated with an antifungal culture compared to the controls
- ✓ **No qualitative differences:** all present in the fermented dairy products without antifungal cultures
- ✓ Present at concentrations below their MIC → act in **synergy**
- ✓ Presence of other (proteinaceous)antifungal compounds not excluded
- ✓ Interactions with matrix: pH, fat content

## Adjunct cultures *versus* fermentates

- ✓ Complementarity between both types of bioprotection solutions

**On going:** exploration of the action mechanisms of some antifungal compounds at the fungal level



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## Funding agencies



## Industrials



## Academic partnership

