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### ► To cite this version:

Marcia Leyva Salas, Mathilde Lemaitre, Gilles Garric, Marielle Harel-Oger, Sébastien Lê, et al.. Antifungal lactic acid bacteria combinations as biopreservation tool in dairy products. 5ième Rencontre Nutrition Alimnetation Métabolisme Santé, Oct 2019, Rennes, France. , 2019. hal-02437589

**HAL Id: hal-02437589**

**<https://hal.science/hal-02437589>**

Submitted on 20 Jan 2020

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# Antifungal lactic acid bacteria combinations as biopreservation tool in dairy products

Marcia Leyva Salas <sup>1, 2\*</sup>, Mathilde Lemaître, Gilles Garric <sup>2</sup>, Marielle Harel-Oger <sup>2</sup>, Sébastien Lê <sup>3</sup>, Jérôme Mounier <sup>1</sup>, Florence Valence <sup>2</sup>, Emmanuel Coton <sup>1</sup>, Anne Thierry <sup>2</sup>,

## Context

- Some essential fungi in dairy products... but others responsible for spoilage, causing food waste & economic losses
- Adjunct cultures of bacteria with antifungal activity offer alternatives to the chemical preservatives currently used, in complement to hurdle technologies [1]



## Objective

- To select effective adjunct cultures antifungal with antifungal activity to be used in fermented dairy products
- To identify the antifungal compounds most likely involved in their activity

## 1 High- throughput screening

### Strategy

- Screening in cheese and yogurt models [2]
- 32 strains of lactic and propionic acid bacteria tested as adjunct cultures along with a usual lactic starter
- Antifungal activity evaluated by challenge-test against 4 fungal targets representative of the most common dairy product spoilers [3, 4]
- Safety assessment: biogenic amines production and antibiotic resistance evaluation
- Binary combinations of the 5 most antifungal strains

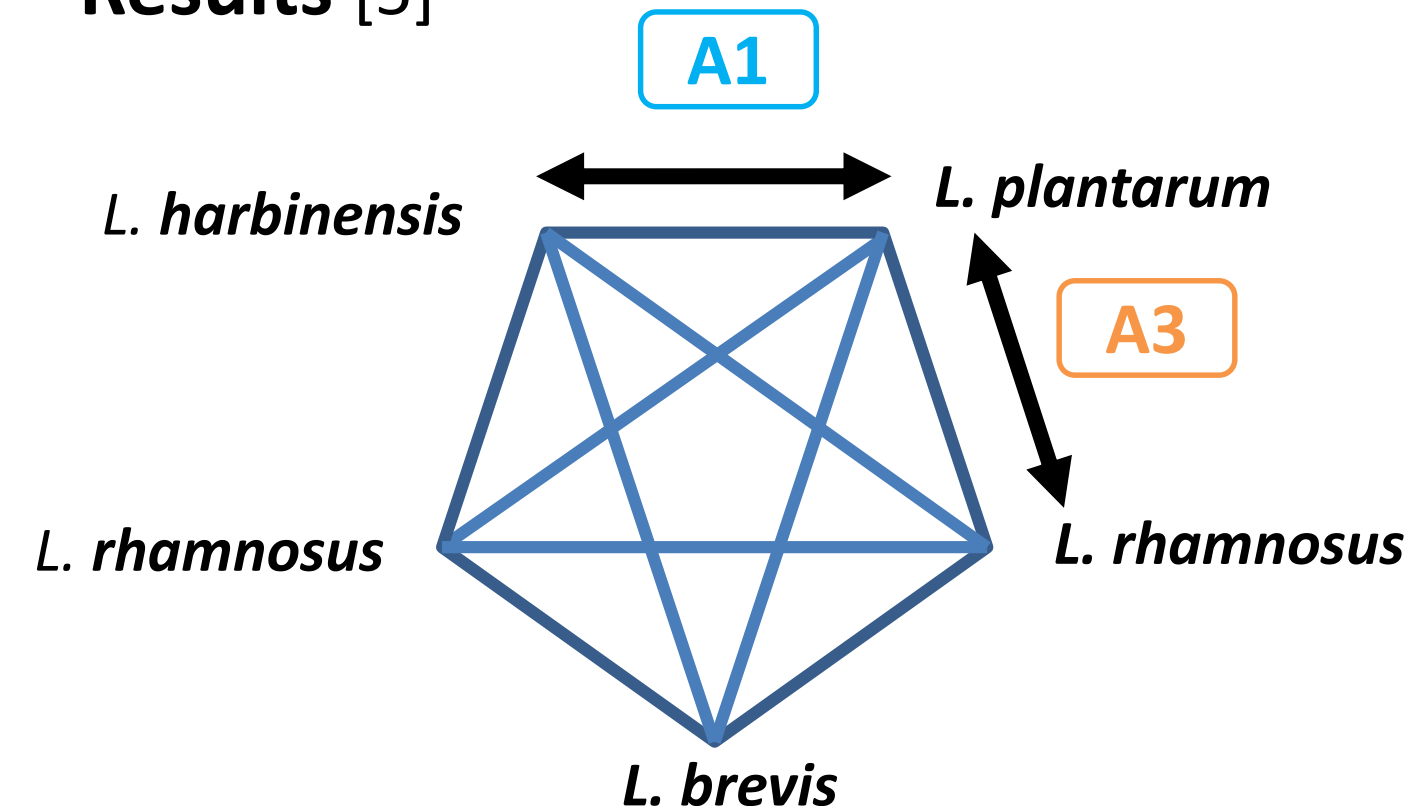


High-throughput screening in microplates

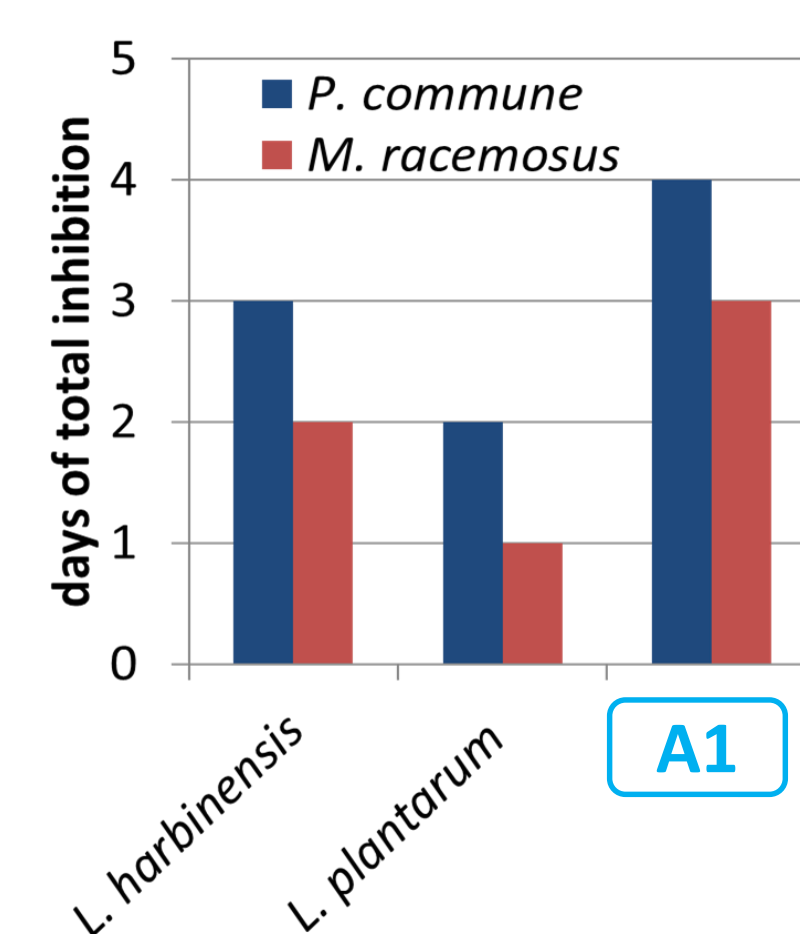


*P. commune*, *M. racemosus*, *G. geotrichum*, *Y. lipolytica*  
4 fungal targets commonly responsible of spoilage in dairy products

### Results [5]



**Figure 1.** 10 binary combinations of the 5 selected *Lactobacillus* strains selected



**Figure 2.** Inhibition of two fungal targets by the combination A1 is higher compared to the corresponding strains in pure culture

- Selection of 2 combinations A1 and A3 of 2 *Lactobacillus* strains with a high antifungal activity and a broad spectrum of inhibition

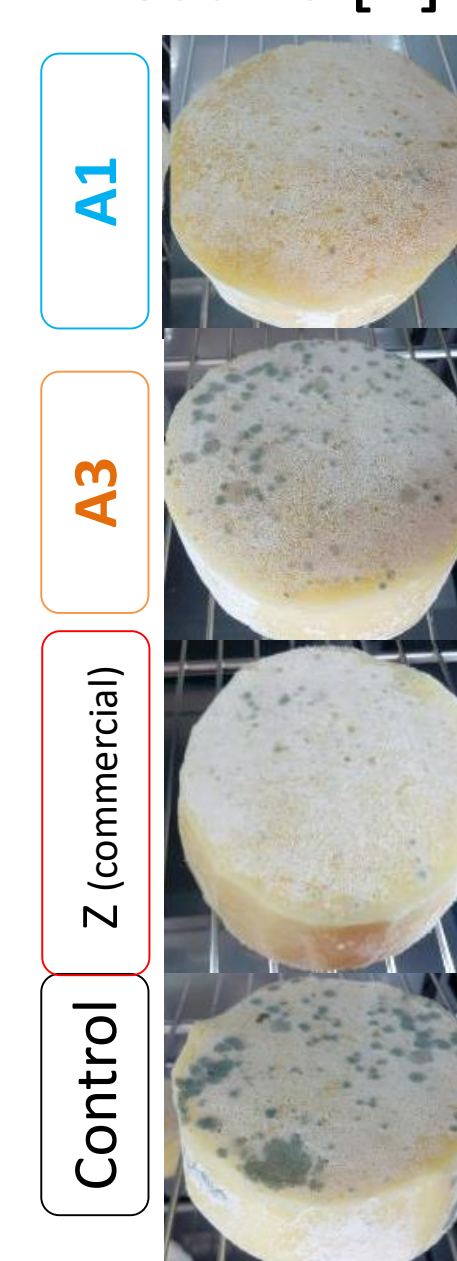
## 2 Validation at the pilot scale

### Strategy

- Combinations A1 or A3 tested in the manufacture of 2 dairy products : cheese and sour cream at the STLO Dairy Platform
- Antifungal activity evaluated using challenge and durability tests

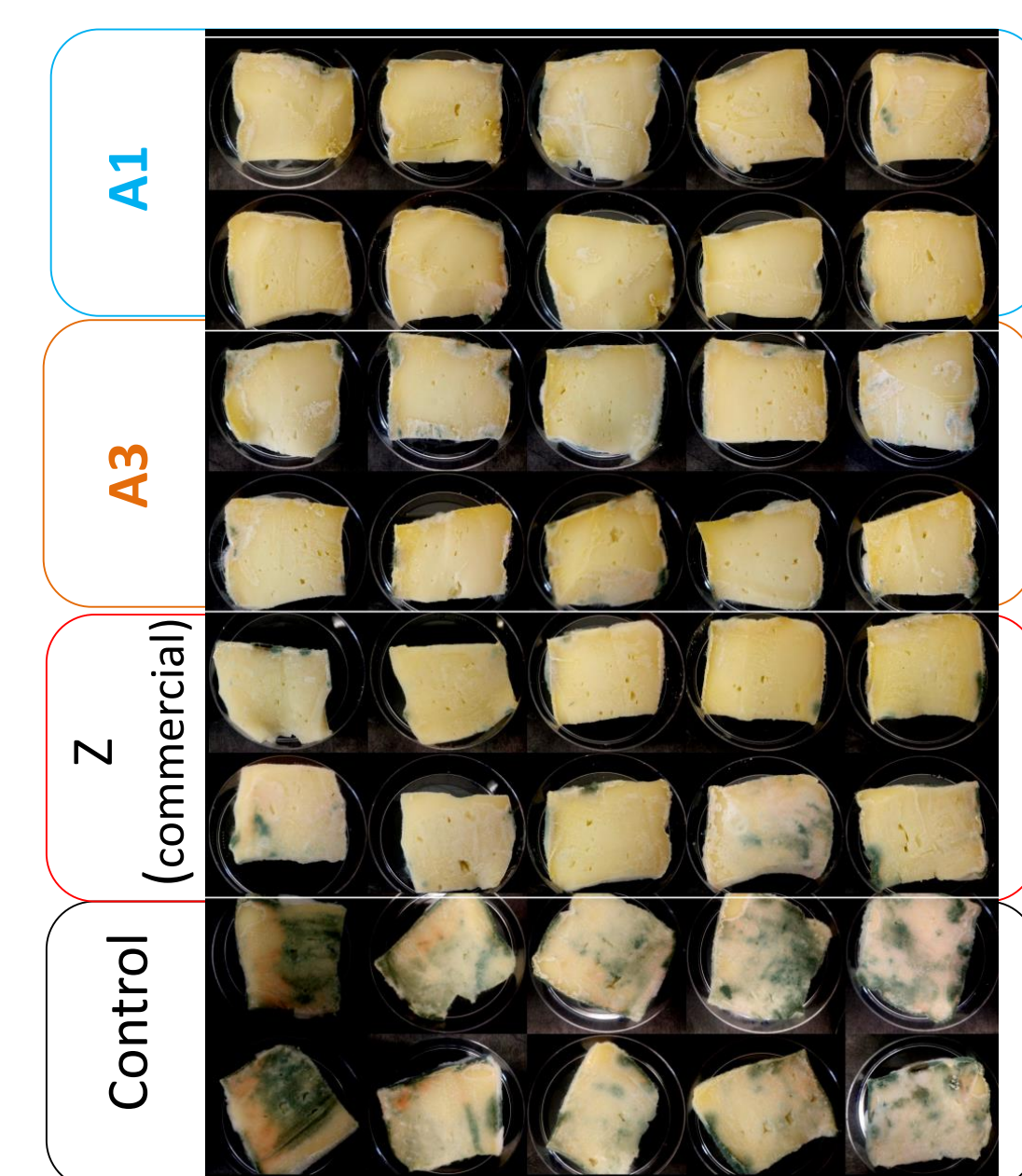


### Results [5]



#### Challenge test

The upper cheese surface is inoculated with a fungal target (here *Penicillium commune*) and growth follow-up in ripening chambers (12°C)



**Durability test**  
antifungal activity after exposition to a naturally contaminated environment and incubation for 13 days at 12°C

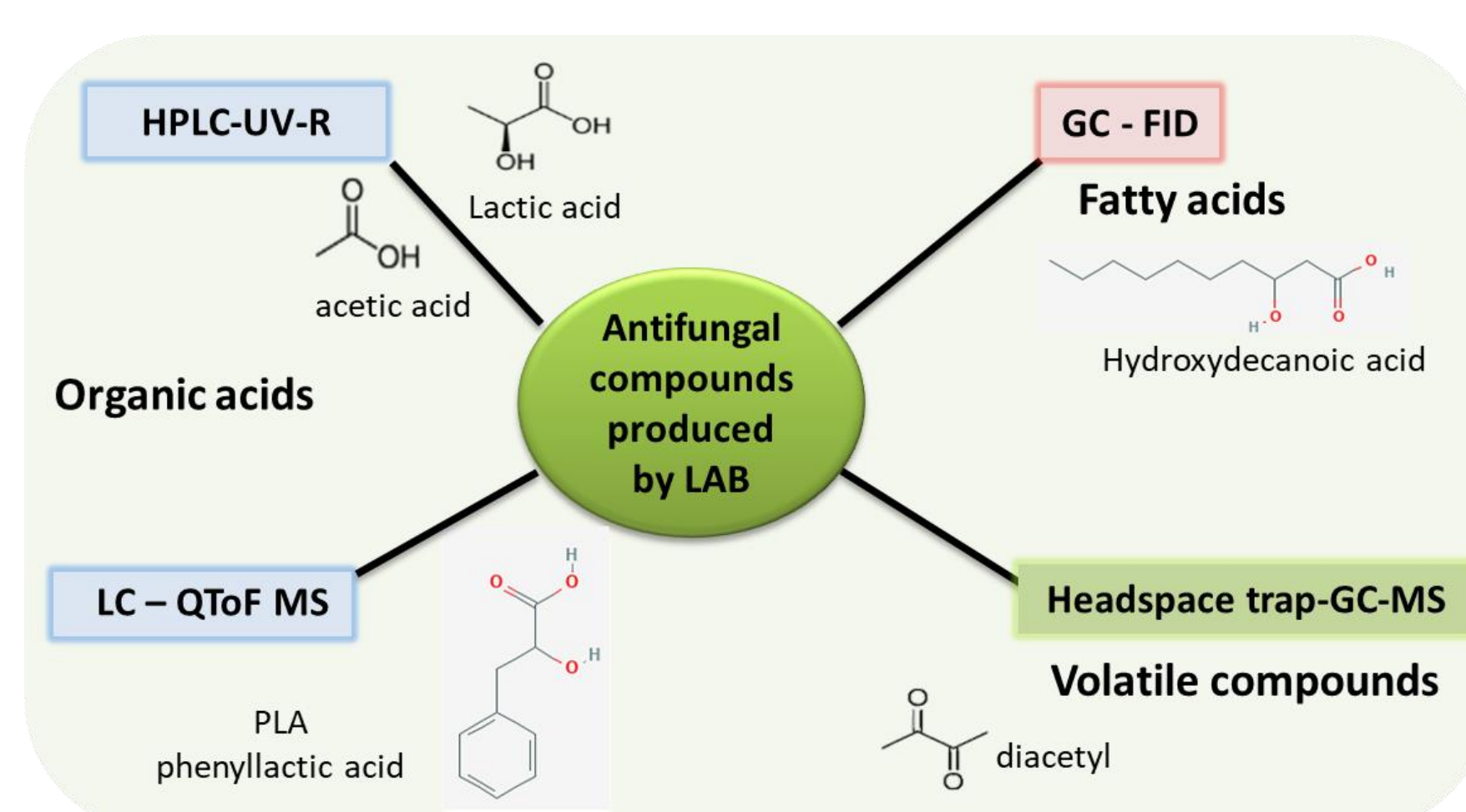
**Figure 3.** Results of challenge-tests and durability test in a semi-hard cheese

- Combinations A1 and A3 exhibited antifungal activity in semi-hard cheese and in sour cream; Their use led to minor sensory changes

## 3 Identification of the compounds potentially responsible for the antifungal activity

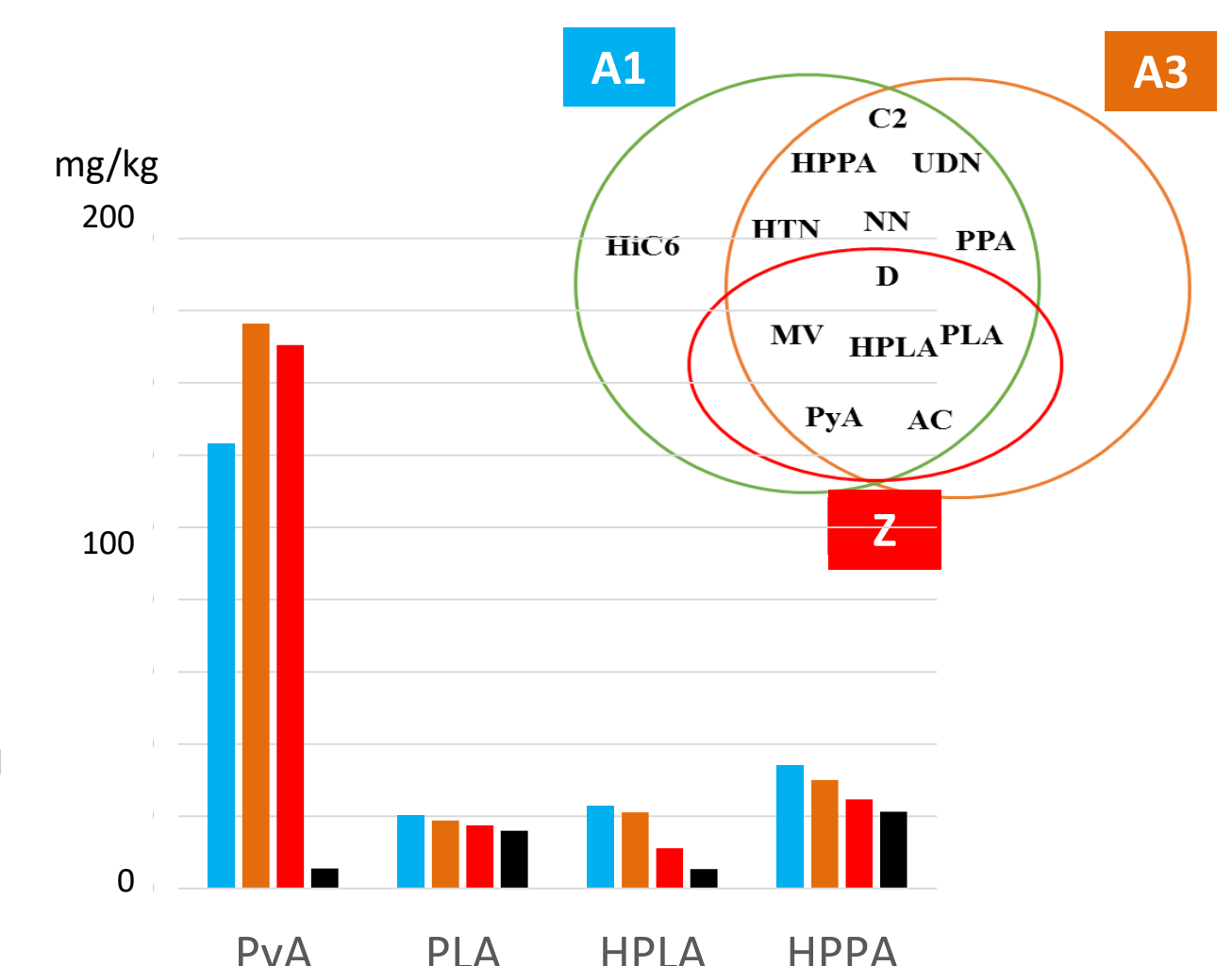
### Strategy

- 4 chromatographic methods combined to target antifungal compounds
- 4 dairy products
- 2 antifungal combinations at 1-3 inoculation levels
- 2-3 biological replicates
- ⇒ 100 samples analyzed



### Results [6]

- 53 antifungal compounds detected in the dairy products
- Antifungal compounds also detected in the controls (fermented dairy products without antifungal cultures)
- Quantitative differences: 33 antifungal compounds in significantly higher amounts in the presence of an antifungal culture
- Cocktails of 2 to 16 compounds according to the antifungal culture and the dairy product



**Figure 4.** Antifungal compounds present in significantly higher amounts in the presence of antifungal cultures A1, A3 or Z (commercial) and concentrations in cheese; ■ Control with only with a usual lactic starter

- 8 compounds highly correlated with the antifungal activity observed and present in 3 of the 4 dairy products analyzed [6]

## Conclusions

- The screening strategy was successful to select cultures of lactic acid bacteria with a high antifungal activity in different dairy products
- Investigation of the action mode of antifungal compounds by studying the physiological response of fungal targets
- Better knowledge about the action mode of antifungal lactic acid bacteria
- Several antifungal strains currently tested by the private partners associated with PROFIL project.
- This work contributes should lead to the development of new bioprotective bacterial cultures.

### Publications associated with these results

- [1] Leyva Salas et al Antifungal Microbial Agents for Food Biopreservation—A Review Microorganisms 2017
- [2] Garnier et al. Technical Note: High-Throughput Method for Antifungal Activity Screening in a Cheese-Mimicking Model. J. Dairy Sci. 2018
- [3] Garnier et al Diversity of spoilage Fungi Associated with Various French Dairy Products. International J. Food Microbiol. 2016.

- [4] Garnier et al Diversity and Control of Spoilage Fungi in Dairy Products: An Update. Microorganisms 2017.
- [5] Leyva Salas et al Antifungal Activity of Lactic Acid Bacteria Combinations in Dairy Mimicking Models and Their Potential as Bioprotective Cultures in Pilot Scale Applications. Frontiers in Microbiology 2018, 9.
- [6] Leyva Salas et al Identification and Quantification of Natural Compounds Produced by Antifungal Bioprotective Cultures in Dairy Products. Food Chem. 2019, 301, 125260. <https://doi.org/10.1016/j.foodchem.2019.125260>.

<sup>1</sup>Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB, Université de Brest, EA 3882, Plouzané, France.

<sup>2</sup>UMR1253 Science et Technologie du Lait et de l'Œuf, INRA, Agrocampus Ouest, Rennes, France

<sup>3</sup>Applied Mathematics Department, Agrocampus Ouest, Rennes, France

