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# Antifungal lactic acid bacteria combinations as biopreservation tool in cheese production

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# Introduction

#### Context

Dairy products are susceptible to acidtolerant fungal contaminants, causing food waste & economic losses





Antifungal cultures are of growing interest as an alternative to chemical preservatives or a complement tool to hurdle technologies

**Opportunity** 



### **Objective**

To develop lactic acid and/or propionic acid bacteria antifungal cultures for the biopreservation of dairy products

# In-vitro: screening of antifungal activity

## Strategy

### Screening approach

- 24 well plates in cheese-mimicking & yogurt models
- 32 tested strains:  $\bullet$ 
  - 19 Lactobacillus spp.
  - 4 *Leuconostoc* spp.
  - 9 Propionibacterium spp.
  - Added as adjunct cultures along with the commercial starter
- 4 fungal targets:

Mucor racemosus, Penicillium commune, Galactomyces geotrichum and Yarrowia lipolytica

Binary combinations of the most antifungal strains

### Safety assessment

Biogenic amines production and antibiotic resistance evaluation

### **Results**

Antifungal activity higher in cheese-mimicking than in yogurt model

> 5 antifungal *Lactobacillus* strains selected for test in combinations

Three binary combinations showed an improvement of the antifungal activity compared to the corresponding pure cultures



Figure 1. 10 binary combinations of the 5 selected Lactobacillus tested

\*A2 discarded because *L. brevis* harbors biogenic amines associated genes



Figure 2. Illustrating the antifungal activity improvement of combination A1 against 2 fungi, compared to that of the corresponding strains in pure culture. A1 antifungal activity was  $\geq$  to that of the X and Y commercial antifungal cultures

> 2 combinations (A1& A3) selected for evaluation in semi-hard cheese

# *In-situ:* evaluation in cheese at pilot scale

# Strategy

#### Semi-hard cheese manufacture at pilot scale using:

- combination A1 or A3
- or a commercial antifungal culture Z
- or without antifungal culture (control)



### **Evaluation of antifungal activity:**

- Challenge-test: upper cheese surface inoculated with fungal targets and growth follow-up in ripening chambers (12°C)
- Durability test: antifungal activity in a naturally contaminated environment



Figure 3. Inhibition of *Penicillium commune* (challenge-test) and natural contamination (durability test) after 13 days at 12°C



Correspondence analysis with confidence ellipses made from the contingency table of sensory descriptors in the different cheeses with A1, A3, Z and no antifungal culture (C1 and C2)

Dim 1 (32.19%)

#### **Evaluation of organoleptic impact** by a sorting task approach

> A1 & A3 combinations exhibited antifungal activity in semi-hard cheese. Cheese A1 and A3 showed a more marked, but acceptable, flavor compared to the control cheeses and were close to cheese with the commercial antifungal culture

# Conclusions



- The screening strategy was successful to select antifungal cultures active in semi-hard cheese.
- The two selected combinations are natural antifungal alternatives of interest for the dairy industry.

The production of antifungal molecules and pH decrease are the main factors contributing to lactic acid bacteria antifungal activity in foods. The identification of the antifungal molecules involved in the observed antifungal activity is thus in process.

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