

Potential of antifungal lactic acid bacteria combinations as bioprotective agents in pilot scale dairy products

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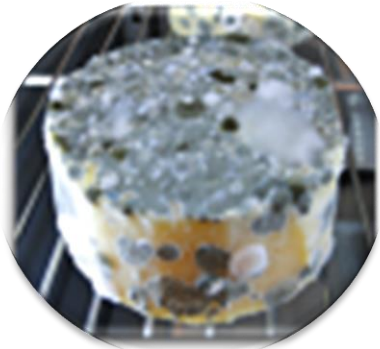
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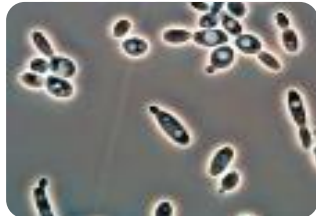
Fungal spoilage of dairy products



yogurt



cheese



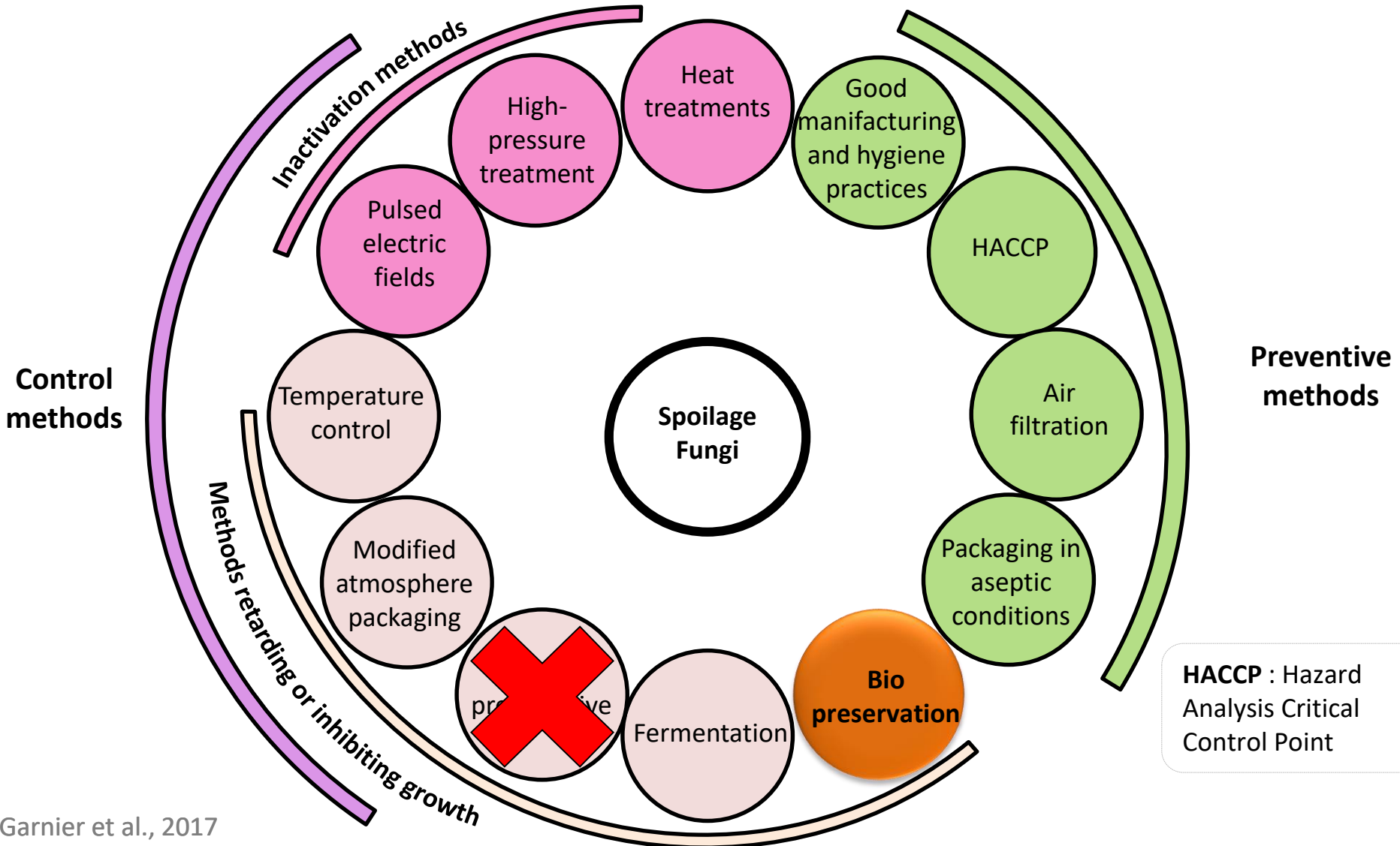
yeast



molds

Impact on the product
organoleptic qualities

Food waste and
economical losses



Garnier et al., 2017

Biopreservation

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

Stiles, 1996

Strains as **adjunct cultures**

Fermentates

Purified metabolites

Biopreservation responds to the strong societal demand for more natural, less severely processed and safer products

Which microbial candidates ?

Lactic acid bacteria known
to have a **long history of safe use**
in the fermentation
of dairy products

Species **adapted**
to dairy products
e.g *Lactococcus lactis*,
Lactobacillus spp.,
Leuconostoc mesenteroides

Safety
Qualified Presumption of Safety

(antibiotic resistance and
production of biogenic amines)

**LAB are good candidates for the development
of bioprotective antifungal cultures**

Objective

to develop bioprotective cultures
with antifungal activity suitable for use
in the **dairy industry** as **adjunct cultures**



Scale-up approach going
from **antifungal activity screening** in dairy models (*in vitro*)
to **pilot scale applications** in actual dairy products (*in situ*)



Screening of the antifungal activity

In vitro

Selection of antifungal strains added as adjunct cultures

Which screening medium?

Cheese Model

Yogurt

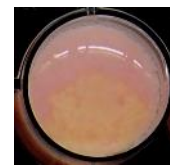
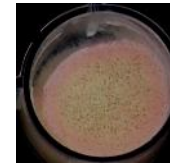
Cheese Model:

Heat-treated salted ultrafiltered milk retentate with rennet and starter

Which microorganisms?

- a selection of strains
- 2 commercial antifungal cultures
- 2 levels of inoculum

Fungal targets?



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

Garnier et al., 2017 International Journal of Food Microbiology 7

High-throughput screening method



Garnier et al., 2018 Journal of Dairy Science

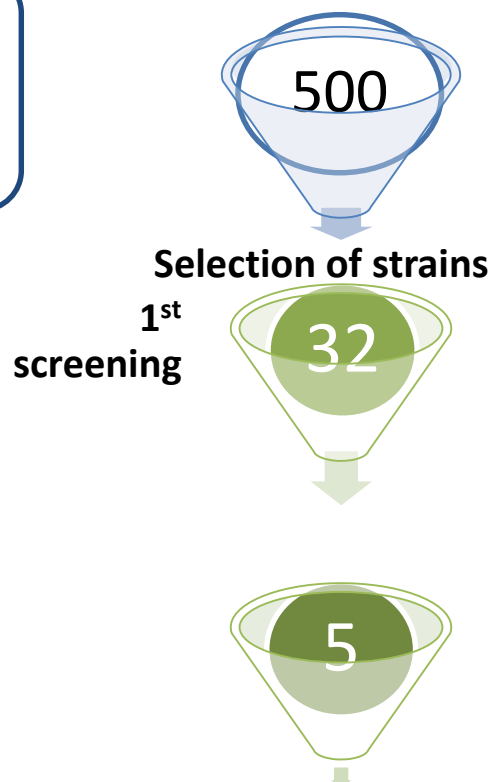
Screening results



Previous study: Screening of
the AF activity of fermentates
*Garnier et al., 2018 Food
Microbiology*

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

High activity, broad
spectrum of action,
reduced number of
strains



31/32 strains inhibited at least 1 of the
4 fungal targets

Matrices impact the AF activity: **CM > Y**

AF activity is **species- and strain-**
dependent

**Selection of 5 lactobacilli strains with
antifungal activity \geq to commercial antifungal cultures**

*AF : antifungal

Antifungal activity improvement

Effect of combining antifungal strains (increase of activity or spectrum of action)?

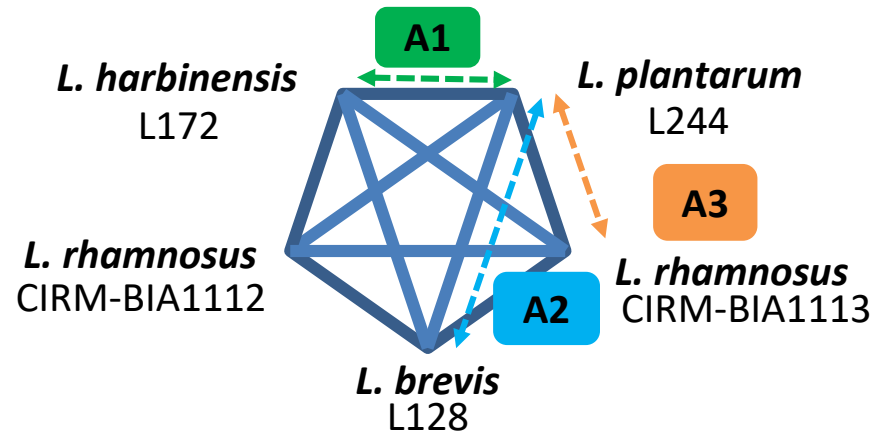
- combinations of the 5 selected strains
- additional fungal targets (n=6)

Combination selection

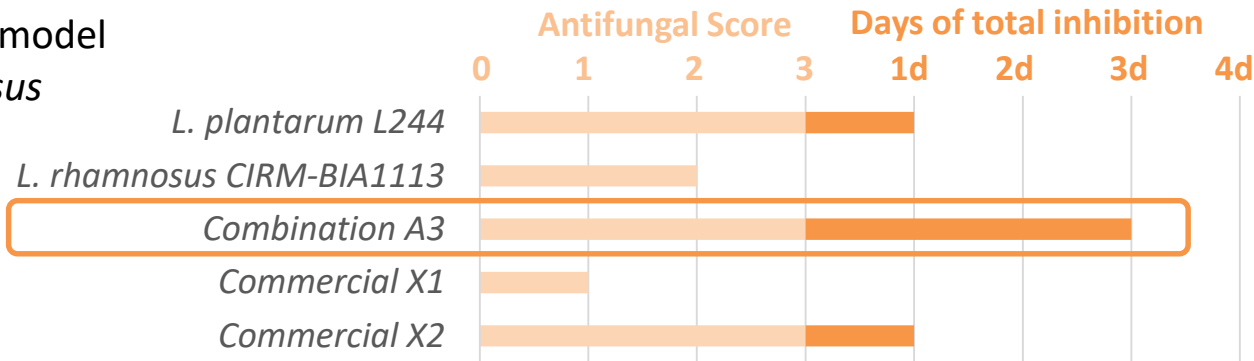
Safety?

Biogenic amine production and antibiotic resistance

Results: Antifungal activity of strain combinations



Example in cheese model
against *M. racemosus*



Improvement of AF activity in combination
A1, A2 and A3 AF activity \geq to commercial antifungal cultures
Broad spectrum (additional fungal targets more sensitive)

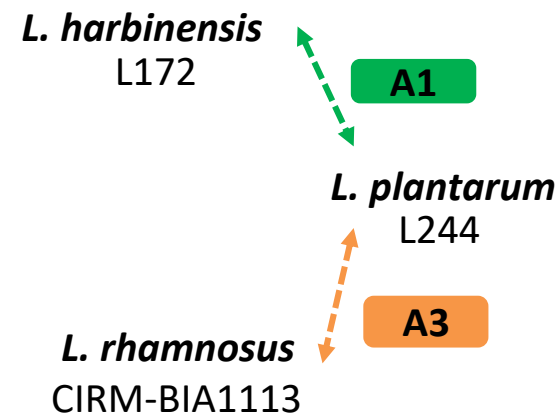
In vitro antifungal activity

~7000 tested conditions

5 lactobacilli with high AF activity

Safety: 2 combinations selected
(A2 discarded due to **BA production** by
L. brevis L128)

AF activity > commercial cultures
Broad spectrum of action





Pilot-scale production

Sour cream

Sterile
standardised
cream



Packaging
and
fermentation



~80kg of cream

Semi hard cheese

Semi hard cheese



tank 150 kg
pasteurised
milk

~120 cheeses

Challenge-tests

Inoculation of targets
on the product surface
50 spores

P. commune

M. racemosus

Inoculation of the yeast
in sour cream
2 cells/g

R. mucilaginosa

Sour
cream



Storage
4 weeks at
12° C

Cheese

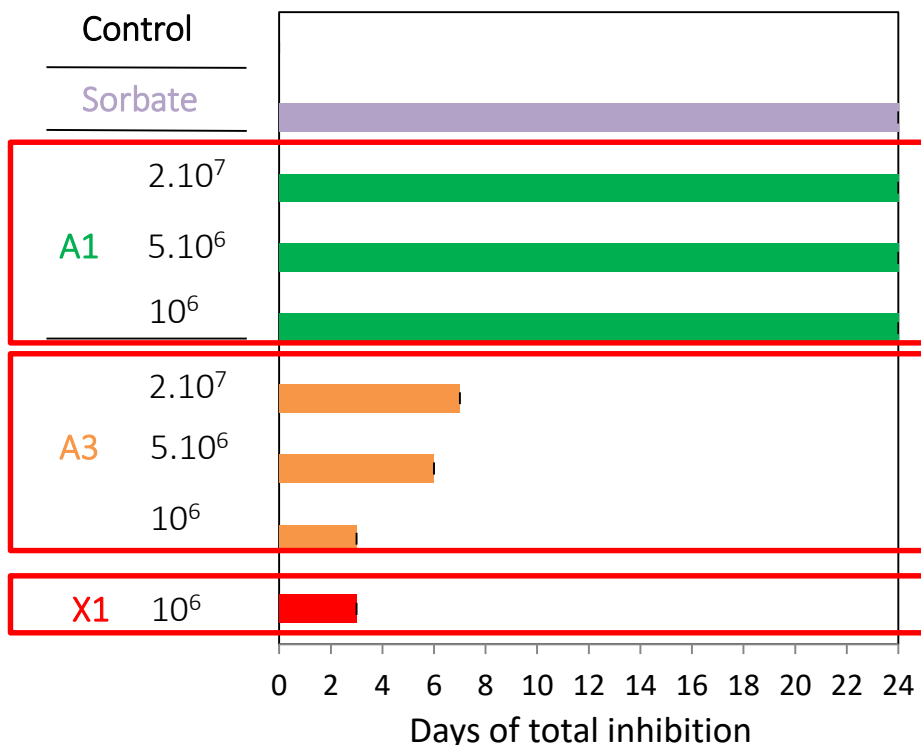


Ripening
4 weeks at
12° C

Both the concentration of fungal targets and storage
temperature are a « *Worst case scenario* »

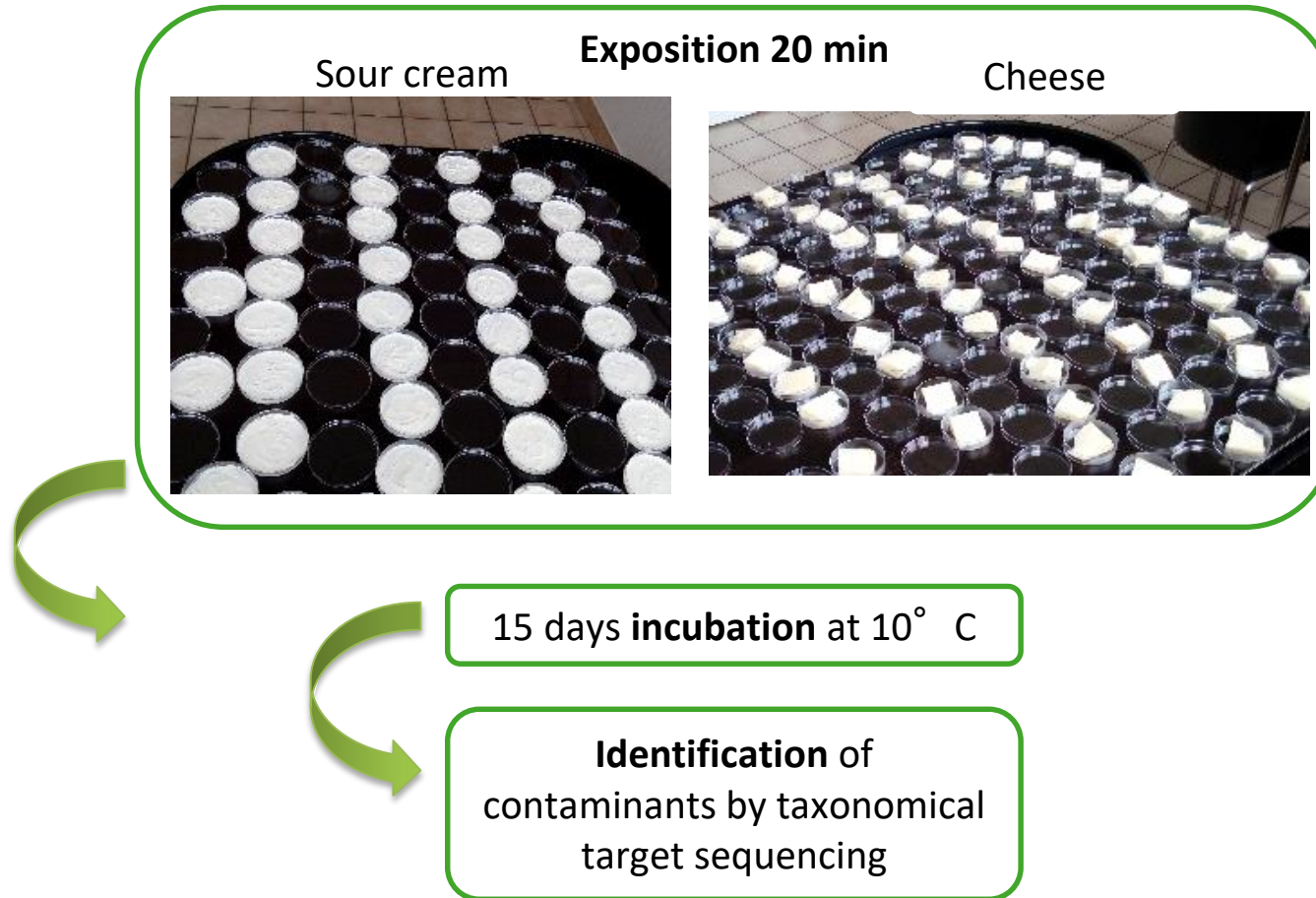
Sour cream challenge-test results

P. commune



A1 antifungal activity > *in situ* than *in vitro*
A1 higher AF activity (= sorbate) and spectrum of action than **A3** and commercial **X1**
A3 antifungal activity similar to **X1**

Shelf life tests



Results of shelf life test in sour cream

Control



8 species identified

Penicillium crustosum
Penicillium glabrum
Cladosporium allicinum
Cladosporium
cladosporoides
Debaryomyces hansenii
Sporidiobolus metaroseus
Exophiala xenobiotica
Bulleromyces albus

A1
 2.10^7

A1
 5.10^6

A1
 1.10^6

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



A3
 2.10^7

A3
 5.10^6

A3
 1.10^6



2 species identified

Cladosporium allicinum
Candida zeylanoides

A1 and A3 : broad spectrum of action (A1 > A3)

Fungal targets inhibited *in vitro* + inhibited during shelf life test: at least 17 fungi inhibited
 at < 5.10^6 CFU/mL

Organoleptic impact ?

Sorting Task

30 panelists

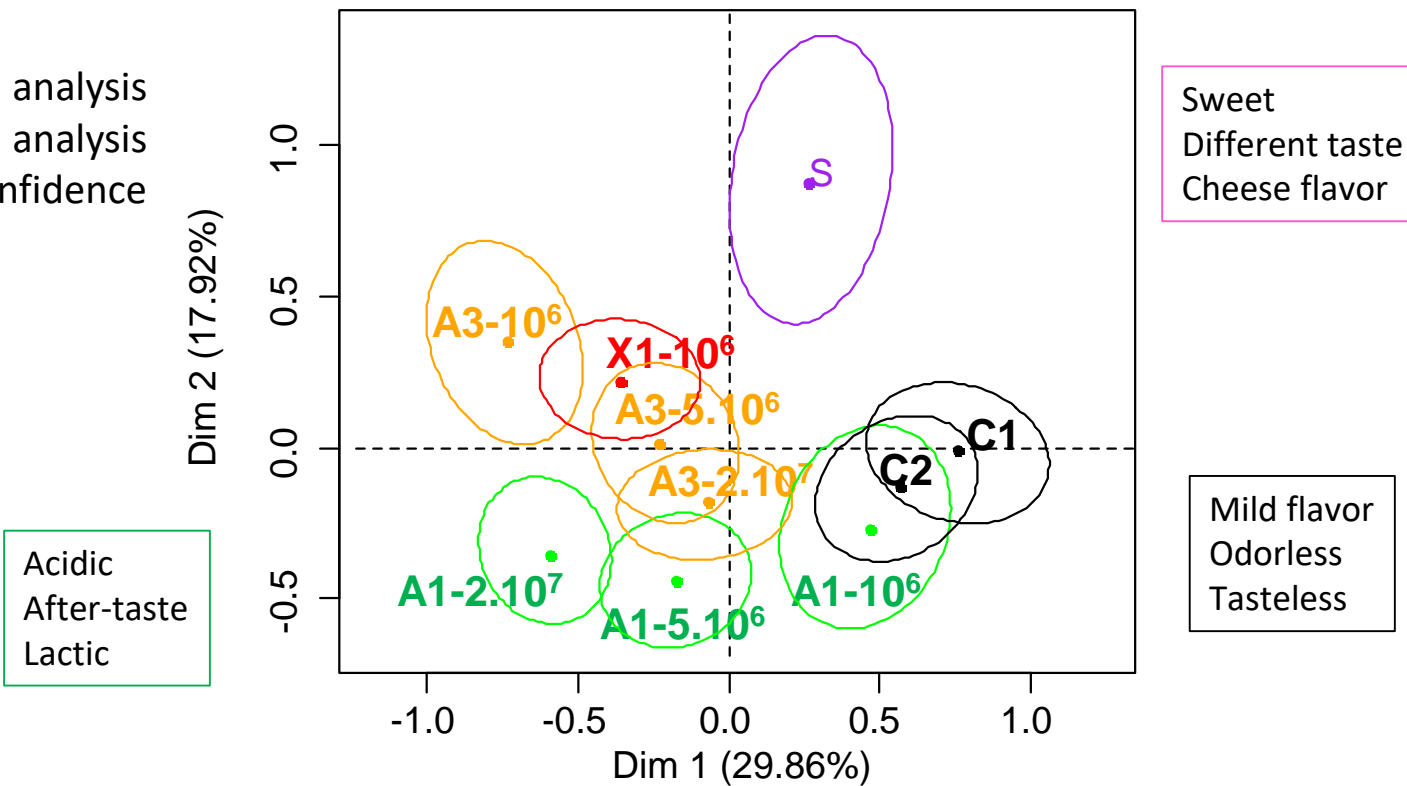


Panelists grouped together the samples perceived as the most similar
After grouping, they proposed specific descriptors for each group

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

Organoleptic impact: Sour cream

Correspondence analysis map of sensory analysis data with confidence ellipses



Impact of **A1** et **A3** in terms of acidity were equal to the commercial antifungal cultures
A1 inoculated at 10⁶ CFU/mL similar to control samples
 Samples were all acceptable for the judges.

Conclusions

Efficient approach to develop antifungal combinations with high antifungal activity and broad spectrum

A1 and **A3** combinations are good candidates for the antifungal biopreservation of dairy products

Broad spectrum of action, flexibility and no impact on the lactic starters

Perspectives

Deciphering molecules supporting the observed AF activity (see poster)

Understanding these molecule targets and action mechanisms at the fungal level



Academic partnership



Prof. Emmanuel Coton



Dr. Anne Thierry



Funding agencies



Industrials



UBO

Université
de Bretagne
Occidentale



INRA
SCIENCE & IMPACT

Thank you for your attention

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