Schweizerische Eidgenossenschaft
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Federal Department of Economic Affairs,
Education and Research EAER

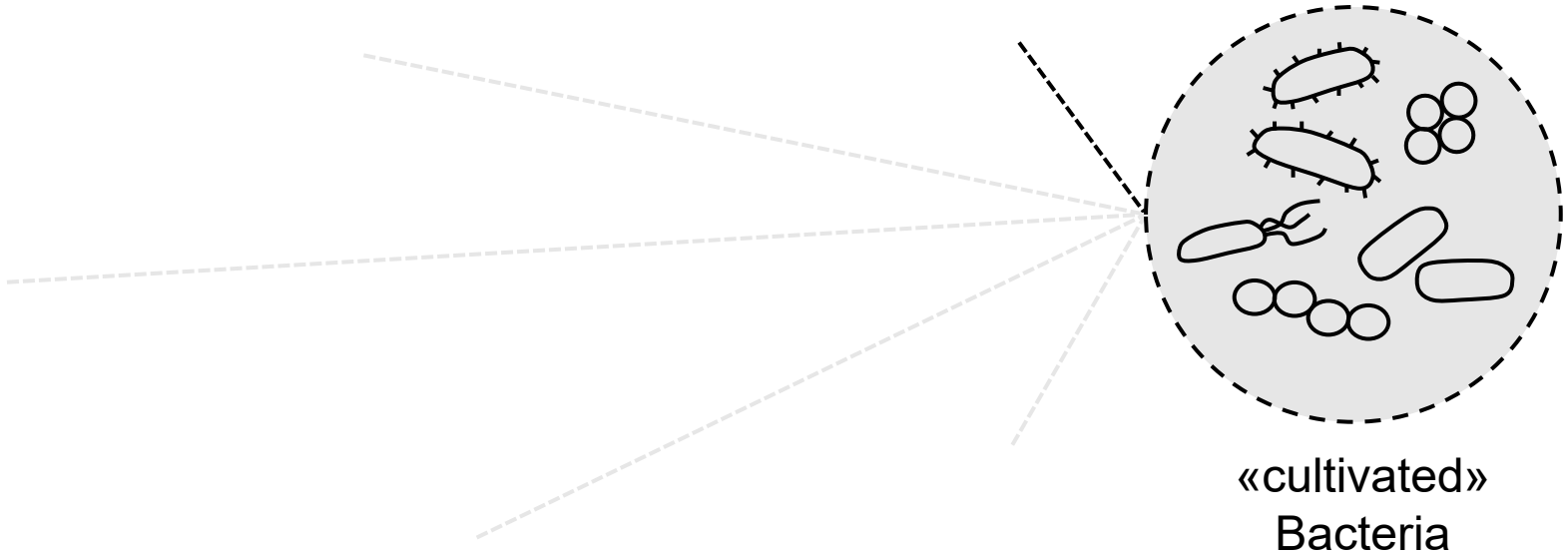
**Agroscope**

# Small consortia of microorganisms enhancing the taste and safety of our food

**Gabriela Purtschert Montenegro**

25.01.2024

# Fermented foods: when humans involuntarly started cultivating microbes



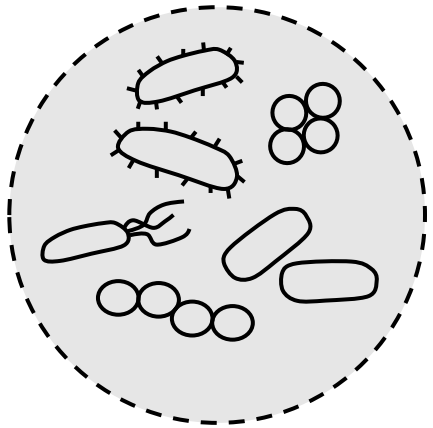
«cultivated»
Bacteria

**Cheese**
**Lactic acid bacteria**
~8,000 years ago

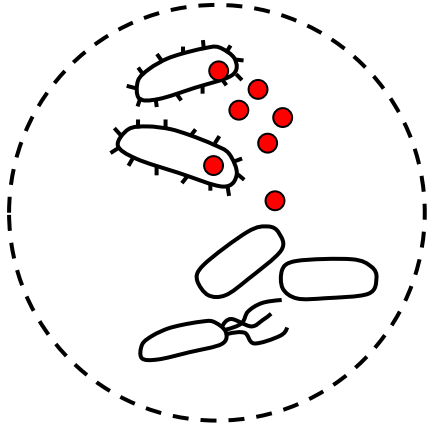
Toussaint-Samat et al, 2008

# Other interesting characteristics of lactic acid bacteria (LAB)

**Lactic acid bacteria (LAB)** are essential for cheese making
→ fermentation & flavour development



→ **Interactions strategies** between bacteria



**Bacteriocins**

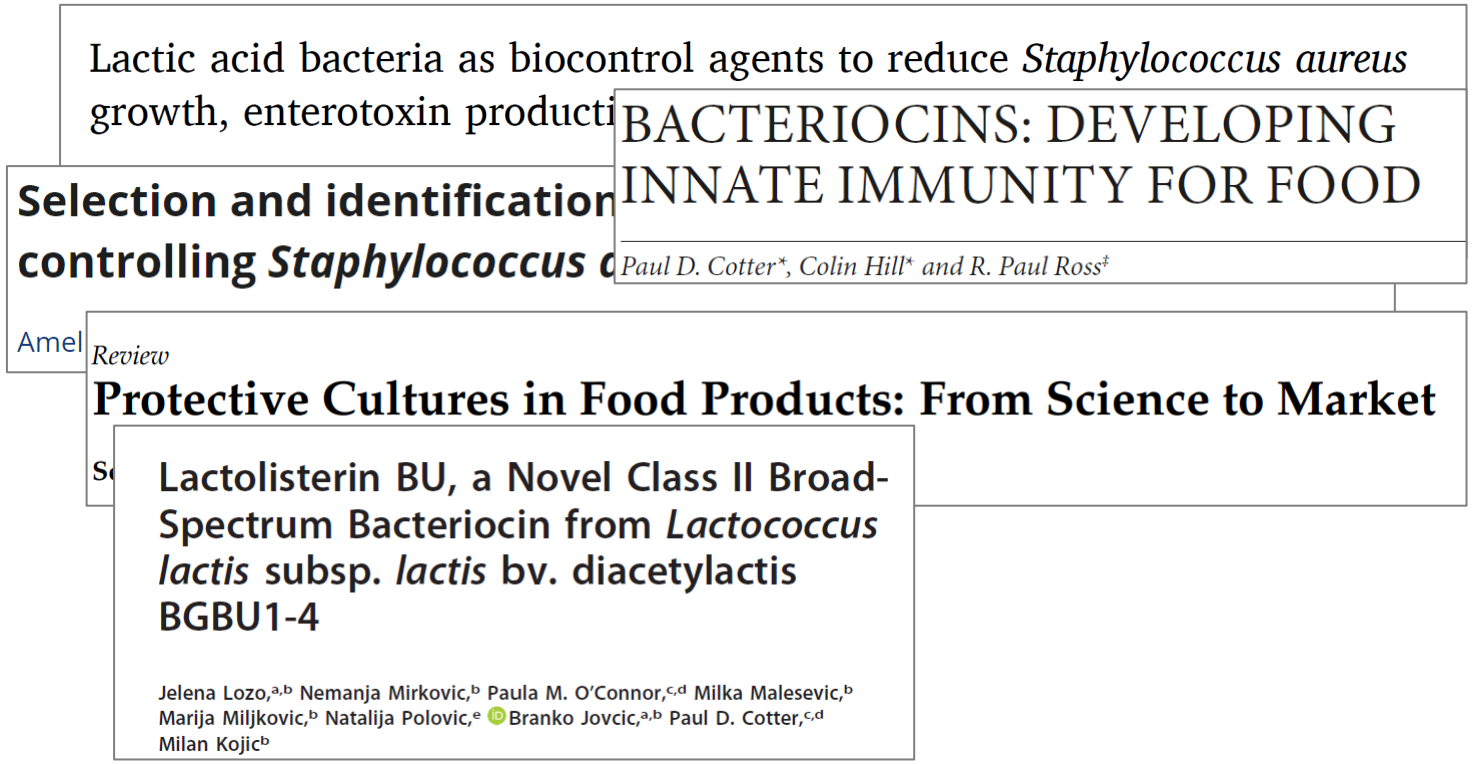
Small diffusible molecules (toxins) produced by bacteria that are **active against other bacteria**, either in the same species or across genera

Bacteriocins are a **heterogeneous group** of peptides and proteins

Vijayakumar et al, 2015, Cotter et al, 2005

# Pathogens that can cause spoilage or food borne disease in cheese

- *Staphylococcus aureus*
- *Salmonella* spp.
- *Listeria monocytogenes*
- EHEC *(E. coli)*
- *Campylobacter* spp.
- *Bacillus* spp.
- *Clostridia* spp.
- …




Choi et al, 2016 – Nileshkumar et al, 2015 – Bastan et al, 2021

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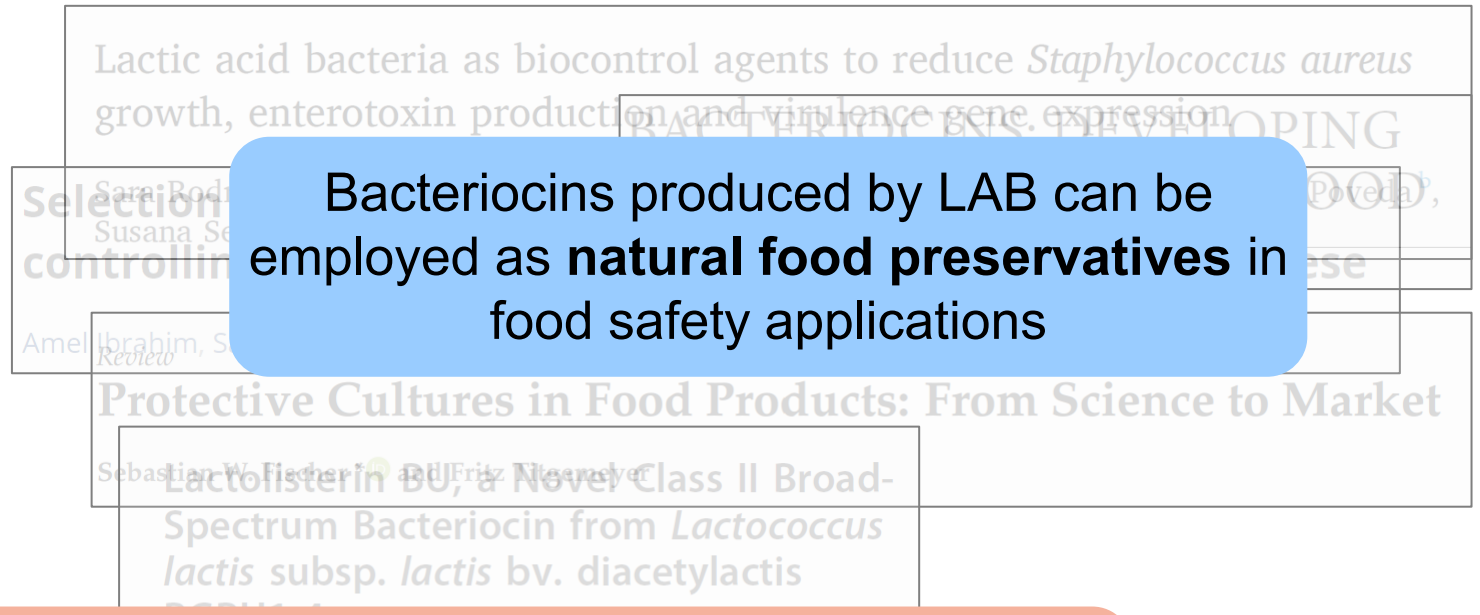



Bacteriocins produced by LAB can be employed as **natural food preservatives** in food safety applications

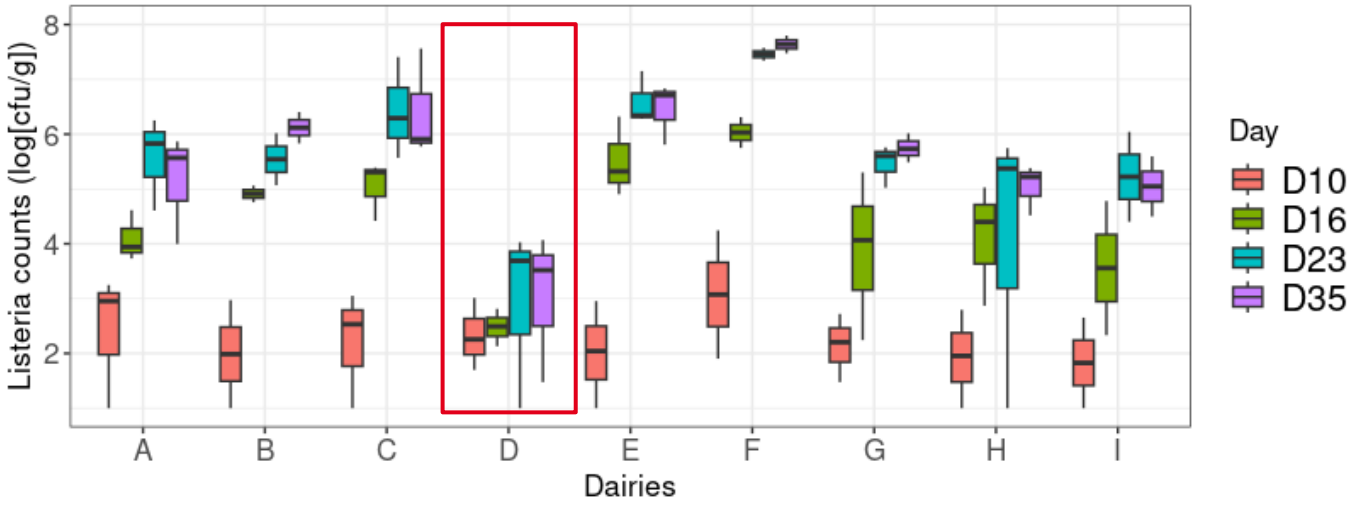
**Lactic acid bacteria as biocontrol agents against food borne pathogens & spoilers**

Choi et al, 2016 – Nileshkumar et al, 2015 – Bastan et al, 2021

# LAB as biocontrol agents: *Leuconostoc* spp. in the rind of soft smear cheese

**Vacherin Mont-d'Or cheese**

- 24h-old-cheeses were collected from 9 dairies (A-I)
- Maturation ocurred at 13°C / 23 days + storage at 4°C / 12 days
- Surface was inoculated with *Listeria innocua* on days 7-10




Antagonistic properties were observed in cheese from Dairy D

→ **Facility-dependent antagonism against *Listeria***

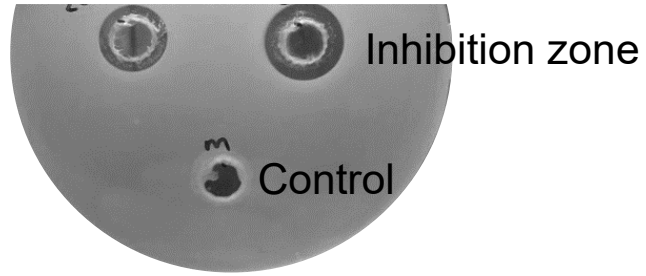
**Emmanuelle Arias-Roth**
Alexandra Roetschi
Florian Gschwend

Roetschi *et al*, Manuscript in preparation

# LAB as biocontrol agents: *Leuconostoc* spp. in the rind of soft smear cheese

Isolation and characterization of antagonists → *Leuconostoc mesenteroides* strains → known producers of **Mesentericin**, an active bacteriocin against *Listeria*

| Strain FAM- | Dairy | Inhibition *L. innocua*[1] | **mesY** [%] AY286003 | Other bacteriocin[2] | **citrate** operon |
|---|---|---|---|---|---|
| **24179** | Raw milk | +++ | **100** | B1 | yes |
| **25299** | A | (-) | 0 | B2 | no |
| **25300** | A | + | **100** | B2 | no |
| **24636** | D | + | **100** | B2 | yes |
| **25292** | D | + | **100** | B2 | yes |
| **25293** | D | ++ | **100** | B2 | yes |
| **25301** | G | (-) | 0 | B2 | no |
| **25302** | G | (-) | 0 | B2 | no |
| **25281** | H | (-) | 0 | B2 | no |
| **25285** | I | (+) | 0 | B2 | no |





Fremaux *et al*, 1995 - Roetschi *et al*, Manuscript in preparation

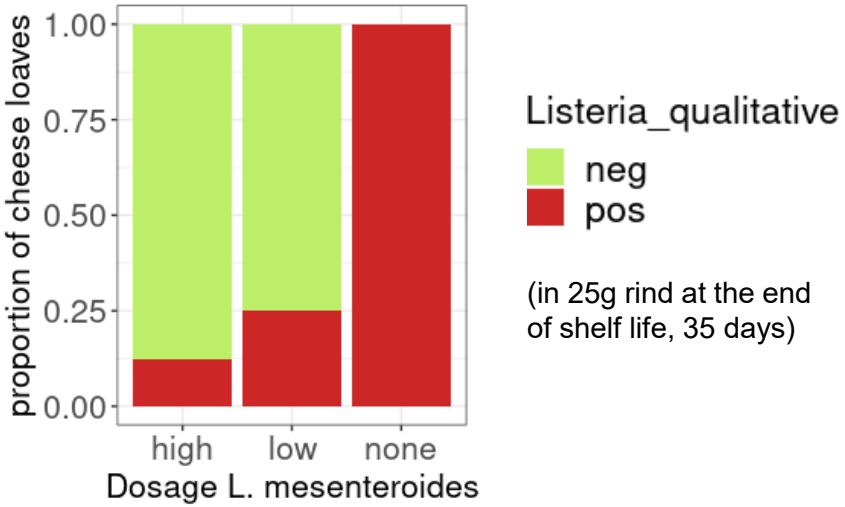
# *L. mesenteroides* provided protection against *Listeria* in a challenge test at pilot scale

*L. mesenteroides* inoculum:

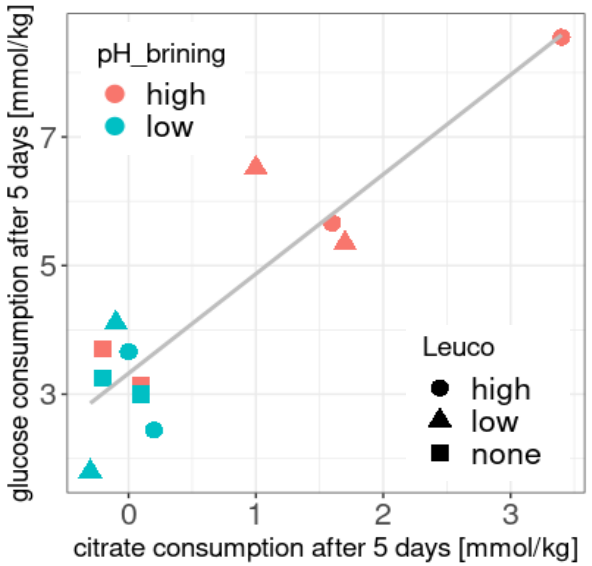
High: $10^4$ cfu/ml
Low: $10^3$ cfu/ml
None

*Listeria innocua* inoculum:

$3*10^4$ cfu/ml




(in 25g rind at the end of shelf life, 35 days)




*L. mesenteroides* **provided protection to 25%** of *Listeria* positive loaves vs 100% of the control cheeses

Positive correlations were observed between **citrate and glucose consumption** by *L. mesenteroides*

Roetschi *et al*, Manuscript in preparation



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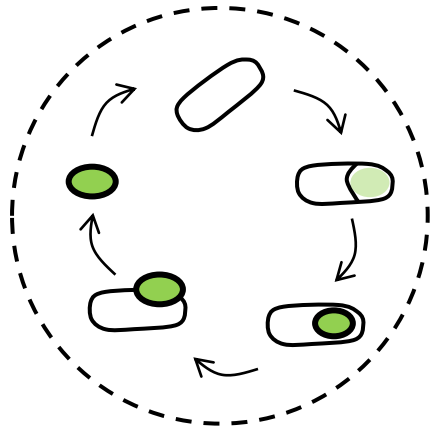
# Pathogens that can cause spoilage or food borne disease in the diary industry

- *Staphylococcus aureus*
- *Salmonella* spp.
- *Listeria monocytogenes*
- EHEC *(E. coli)*
- *Campylobacter* spp.
- *Bacillus* spp.
- *Clostridia* spp.
- …



**Spores** can survive harsh environmental conditions for years

→ Attributed to the thickness of the spore coat

**Spore forming bacteria!**

Spores are **resistant** to: heat, pressure, radiation, chemicals, and desiccation!

**Pasteurization** can damage the spore coat which causes germination of the bacterial cell.

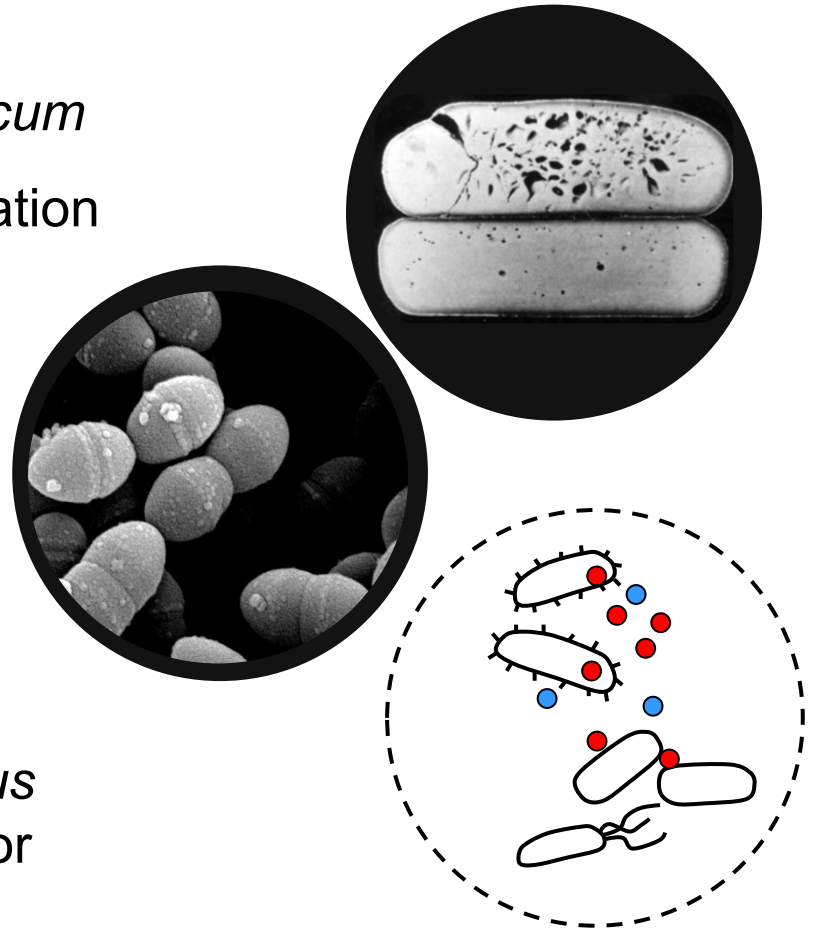
Choi *et al*, 2016 – Nileshkumar *et al*, 2015 – Bastan et al, 2021 – Tao et al, 2014

# Anti-clostridial activity of *Lactoccous lactis* strain FAM17919

The **spore-forming** bacterium *Clostridium tyrobutyricum*

→ Causes gas formation during butyric acid fermentation

→ late blowing in cheese

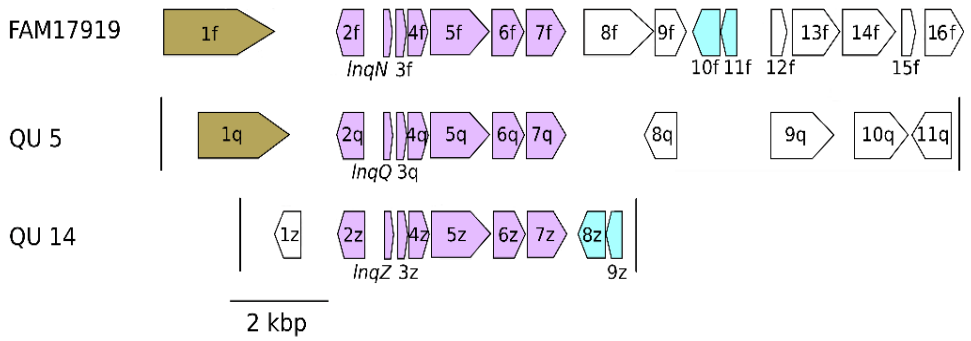


*Lactococcus lactis* strains are know producers of bacteriocins *e.g.* Nisin

*In silico* analysis of the WGS showed that *Lactoccous lactis* strain **FAM17919 carries 2 genes** that code for bacteriocins

Ryser et al, Manuscript in preparation – Pahlow et al, 2023 – Colins et al, 2011

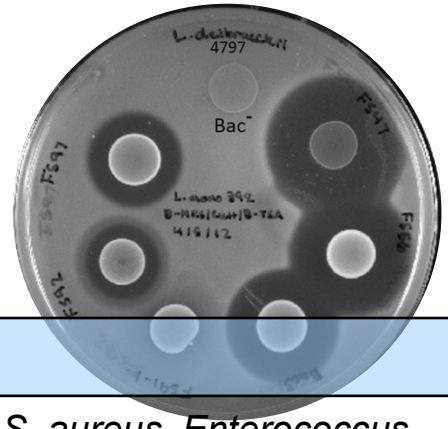
# Anti-clostridial activity of *Lactoccous lactis* strain FAM17919

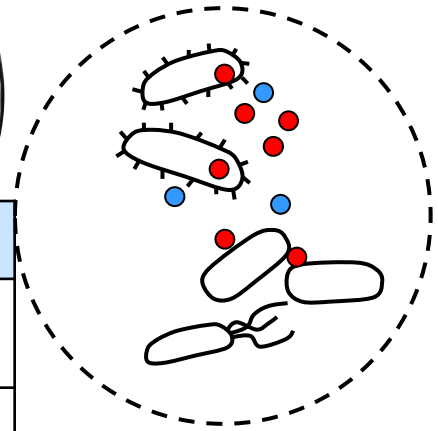



100% identity to Lactolisterin BU

94.3% identity to Lacticin Q → Lacticin N

Anti-clostridial activity of Lactolisterin BU & Lacticin N

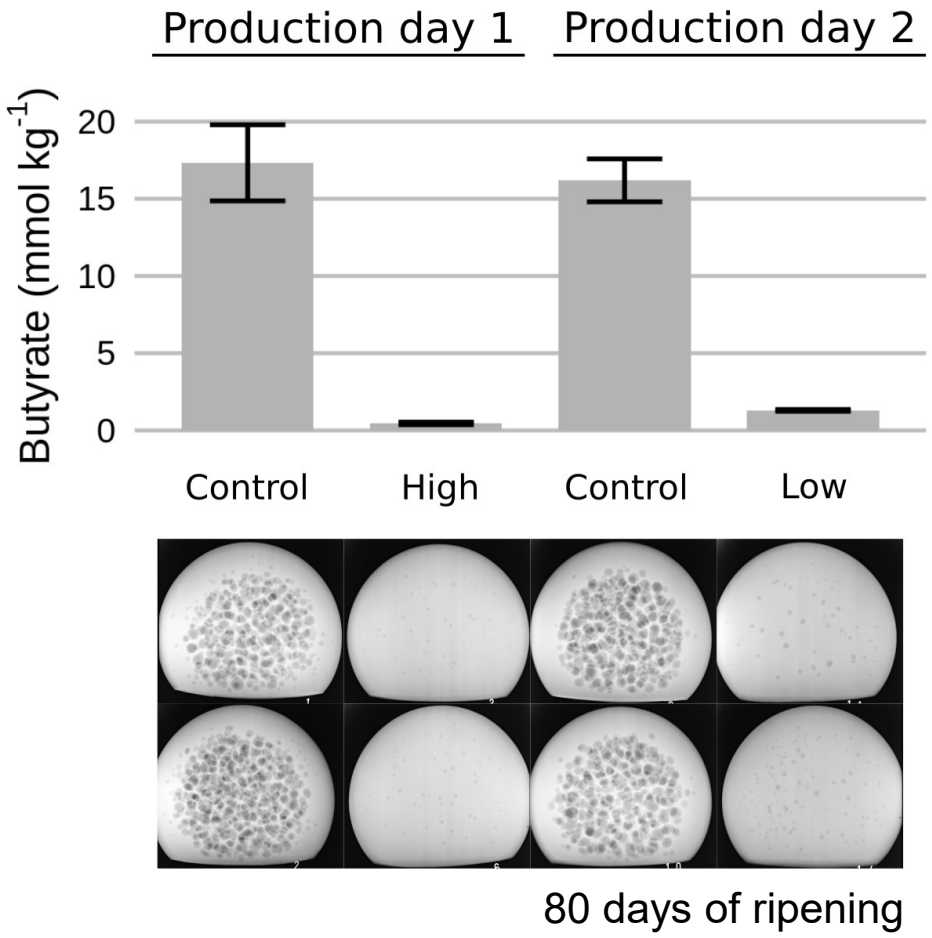


| Bacteriocin | Antibacterial activity against |
|---|---|
| Lactolisterin BU | *L. lactis, Lactobacillus casei, Listeria monocytogenes, S. aureus, Enterococcus faecalis, Bacillus spp. and Streptococcus* spp. |
| Lacticin Q | *Bacillus spp., Enterococcus spp., Lactobacillus spp., L. lactis, Leuconostoc mesenteroides, Listeria innocua and Staphylococcus aureus* |

Ryser et al, Manuscript in preparation
Fujita *et al*, 2007 - Lozo *et al*, 2017

# FAM17919 reduced the cheese late blowing caused by *C. tyrobutyricum*




Pilot-scale trials of red smear semi-hard cheese FAM17919 was added in 2 different concentrations:

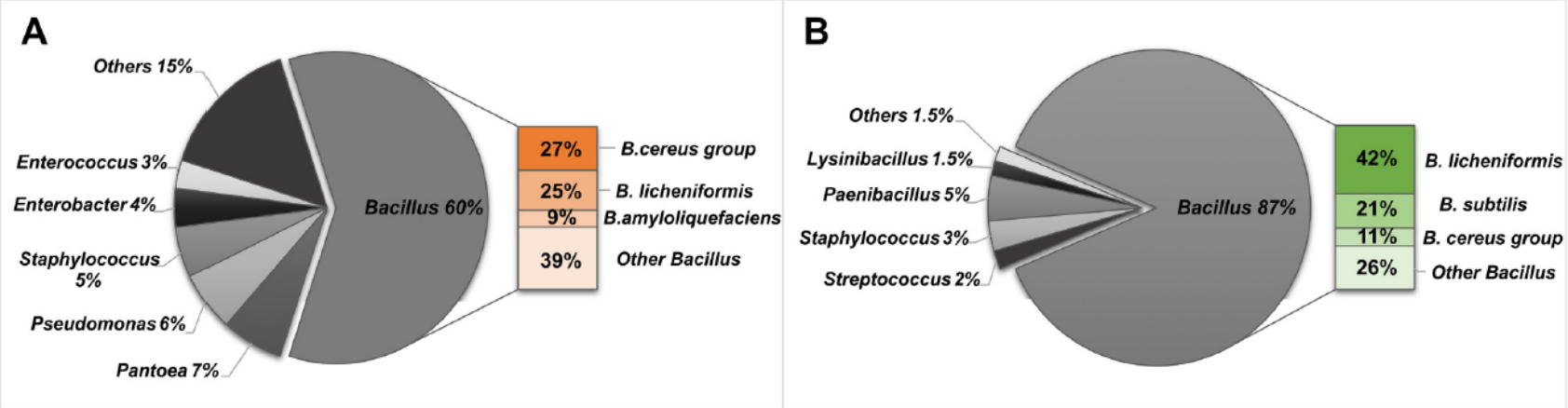
High: $10^6$ cfu/ml
Low: $3*10^5$ cfu/ml

→ FAM17919 **reduced butyric acid fermentation and late blowing** caused by *C. tyrobutyricum*.

Ryser et al, Manuscript in preparation

# Could we use these protective cultures in plant-based foods?

Contaminants in plant-based ingredients:

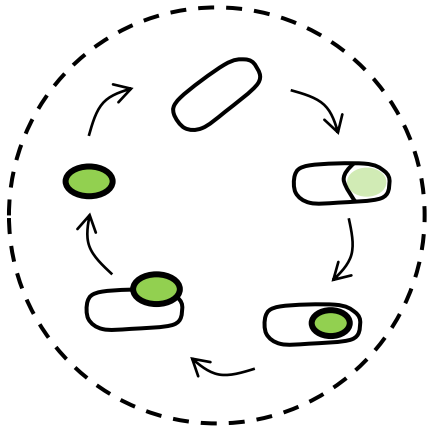



Analysis of 88 different ingredients including (pseudo-) cereals, pulses and drupes.

| Bacteriocin | Antibacterial activity against |
|---|---|
| Lactolisterin BU | *L. lactis, Lactobacillus casei, Listeria monocytogenes, S. aureus, Enterococcus faecalis, Bacillus* spp*., and Streptococcus spp* |
| Lacticin Q | *Bacillus* spp*., Enterococcus spp., Lactobacillus spp., L. lactis, Leuconostoc mesenteroides, Listeria innocua and Staphylococcus aureus* |
| Mesentericin | *Bacillus* spp., *Enterococcus faecalis*, *Lactobacillus* spp., *Listeria* spp., *Clostridia* spp., *Pediococcus pentosaceus*, *Staphylococcus aureus, Streptococcus thermophilus, Carnobacterium* spp. |

Todorov *et al*, 2004 - Fujita *et al*, 2007 - Lozo *et al*, 2017 - Kyrylenko *et al*, 2023

# *Bacillus* spp. in plant-based ingredients



**Spore forming bacteria!**

**Pasteurization** can damage the spore coat which causes germination of the bacterial cell.

## *Bacillus liqueniformis*

Production of surfactant lichenysin → causes food borne intoxication

From the isolates ca. 98% carry the *lchAA* gene

## *Bacillus cereus*

Production of enterotoxins and heat-stable emetic toxin Cereulide

*B. cereus isolates* toxicity genes: 9% *ces*, 28% *hbl* & 42% *cytk* & *nhe*

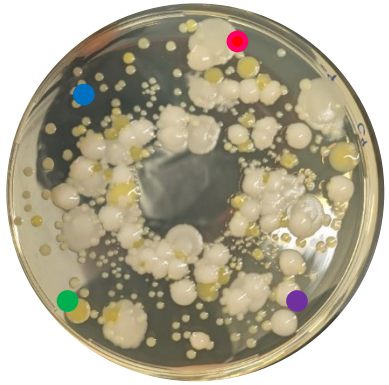
Prevalence of *B. cereus* in different foods:

- Vegetables 37.2%
- Pseudo- and cereals 41.4%
- Beans/Legumes 44.9%

Kyrylenko *et al*, 2023 & Rahnama *et al*, 2023

# Could we use these protective cultures in plant-based foods?

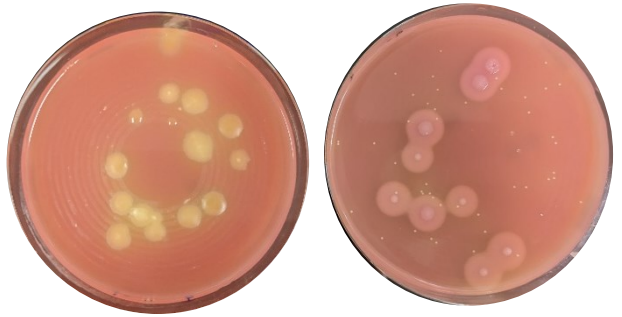
Lupine Matrix – spontaneous Fermentation:



- *Bacillus cereus*
- *Enterococcus mundtii*
- *Enterococcus casseliflavus*
- *Acetinobacter radioresistens*

Before heat treatment

Heated for 10 min at 80°C

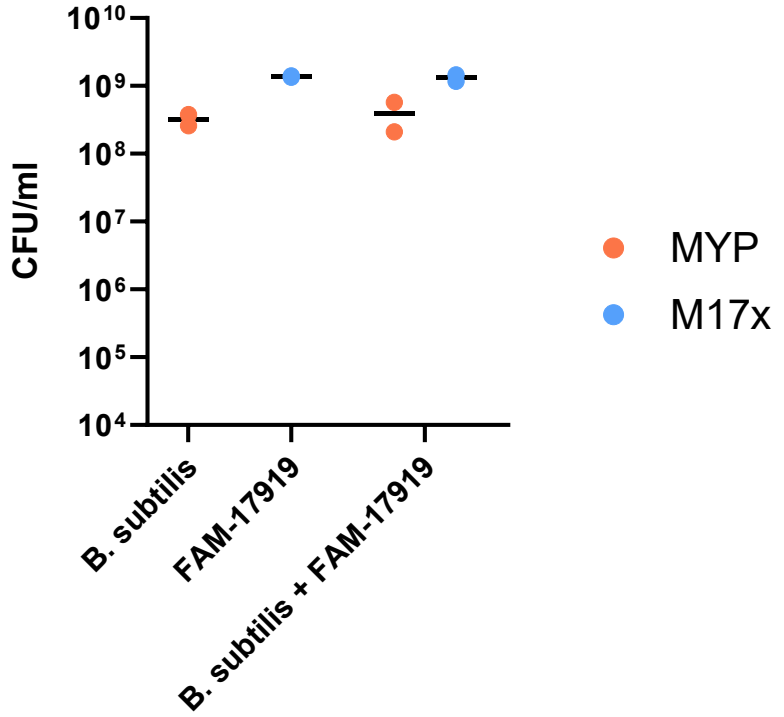


*B. subtilis* *B. cereus*

# Could we use these protective cultures in plant-based foods?

***Lactoccous lactis*** strain FAM17919
vs
***Bacillus subtilis*** in Lupin matrix

*Bacillus subtilis* FAM17919 *Bacillus subtilis* + FAM17919




No effect, due to aerobic growth?
→ *Leuconostoc mesenteroides* is a better candidate?

# Conclusions and Outlook

- **Anti-costridial effect** of *Lactococcus lactis* FAM17919 bacteriocins reduced butyric acid fermentation and late blowing in cheese.
- **Facility-dependent antagonism** against ***Listeria*** with the aid of Mesentericin + *L. mesenteroides* strains.
- **Plant-based ingredients** and products could also benefit from protective cultures, we just need to find suitable candidates.
- LAB can be use as **protective cultures** to prevent food spoilage and food borne diseases

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# Thank you for your attention

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