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Agroscope

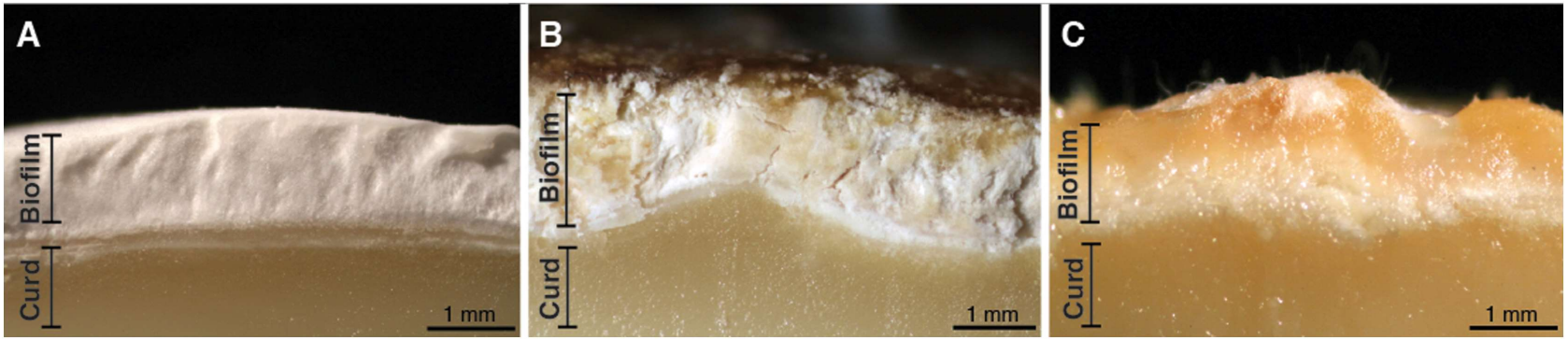
Cheese Smear

or the Ancestral Cultivation of a Beneficial Biofilm

Emmanuelle Arias-Roth

May 6th, 2022, S12 - Biofilm Formation by Food-Associated Bacteria – Friend or Foe?

Introduction to Cheese Smear



- Surface-ripened
  - Mold (inoculated/natural)
  - Bacteria (=Smear cheese)
    - Wooden Shelves
    - Brushes
    - Hard work / robots

- Reduce water loss
- ↑ pH of the cheese core
  - ↓ lactate, ↑ NH_3
- Aroma (sulfur compounds)
- Antagonistic properties
  - Molds, pathogens,…

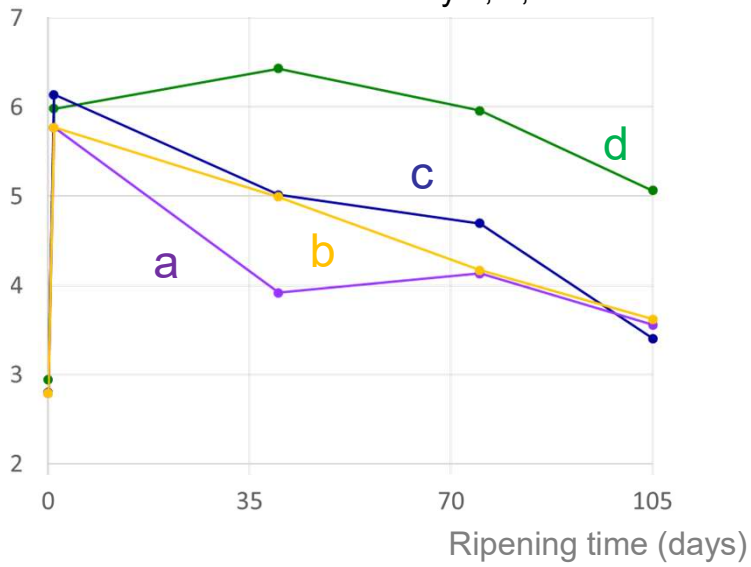
Antagonistic behavior

Montel et al., 2014

Traditional cheeses: Rich and diverse microbiota with associated benefits

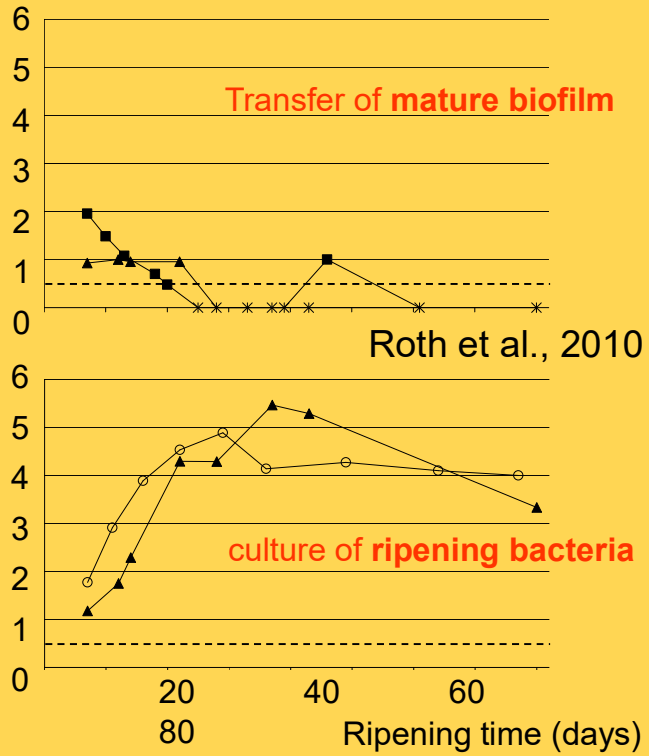
Cheeses	Soft red smeared								Raclette		Saint-Nectaire		
Consortia name*	N.RI	N.RII	N.KII	N.6	N.11	N.24	S.FC12	S.Elm S.Alm	S.ECF	S.ECFS	N.TR15	S.TR15	S.AB
Reference	Maoz et al. (2003), Mayr et al. (2004)			Bleicher et al. (2010a, 2010b)			Monnet et al. (2010a)	Imran et al. (2010, 2013)	Roth et al. (2010, 2011)		Retureau et al. (2010), Callon et al. (2014)		Callon et al. (2011b)
Hypothesis on nature of inhibition	nd	nd			Bact.		nd		Nut.	Nut.	Acids	Acids	Acids

Survival of *E. coli* (Log [cfu g^{-1}])
within biofilms from dairy a, b, c and d

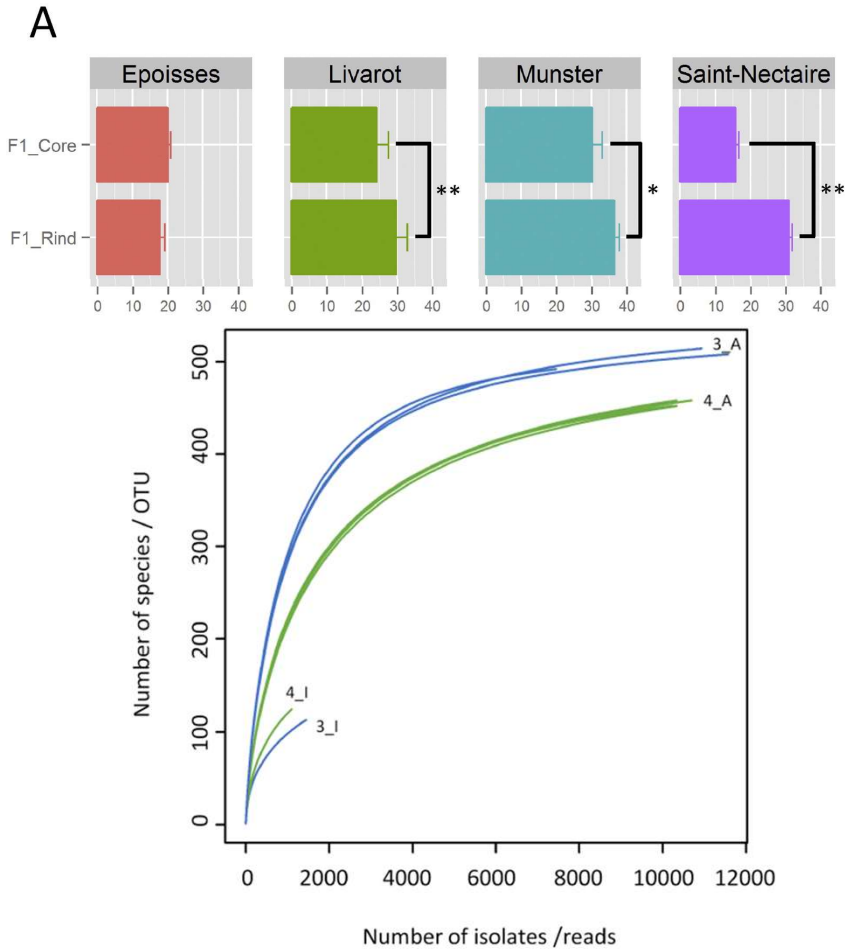


Arias-Roth et al., 2020, unpublished

***Listeria* cell count (Log [cfu cm^{-2}])**

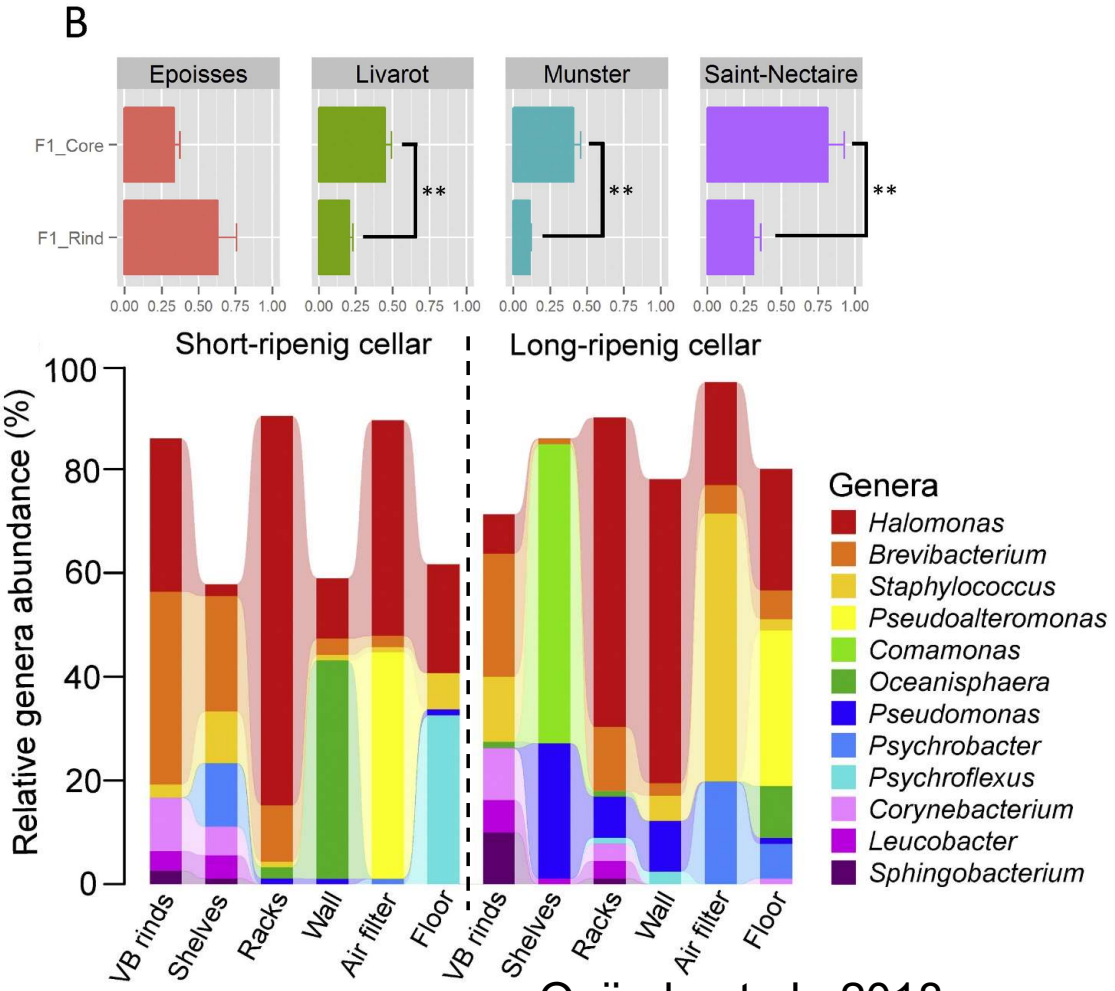


Biodiversity of smear with NGS



Breitenwieser et al., 2021

High Biodiversity Within Raw Milk Microbiota



Quijada et al., 2018

Autochthonous facility-specific microbiota

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Research focus on thermized soft smear cheese

- Is the antagonistic behavior of the microbiome facility-dependent?
- Can we detect the biotic drivers for this phenotype
- Challenge tests
- Hypothesis on the nature of inhibition
- Safety assessment of the species/strains for use as a protective culture

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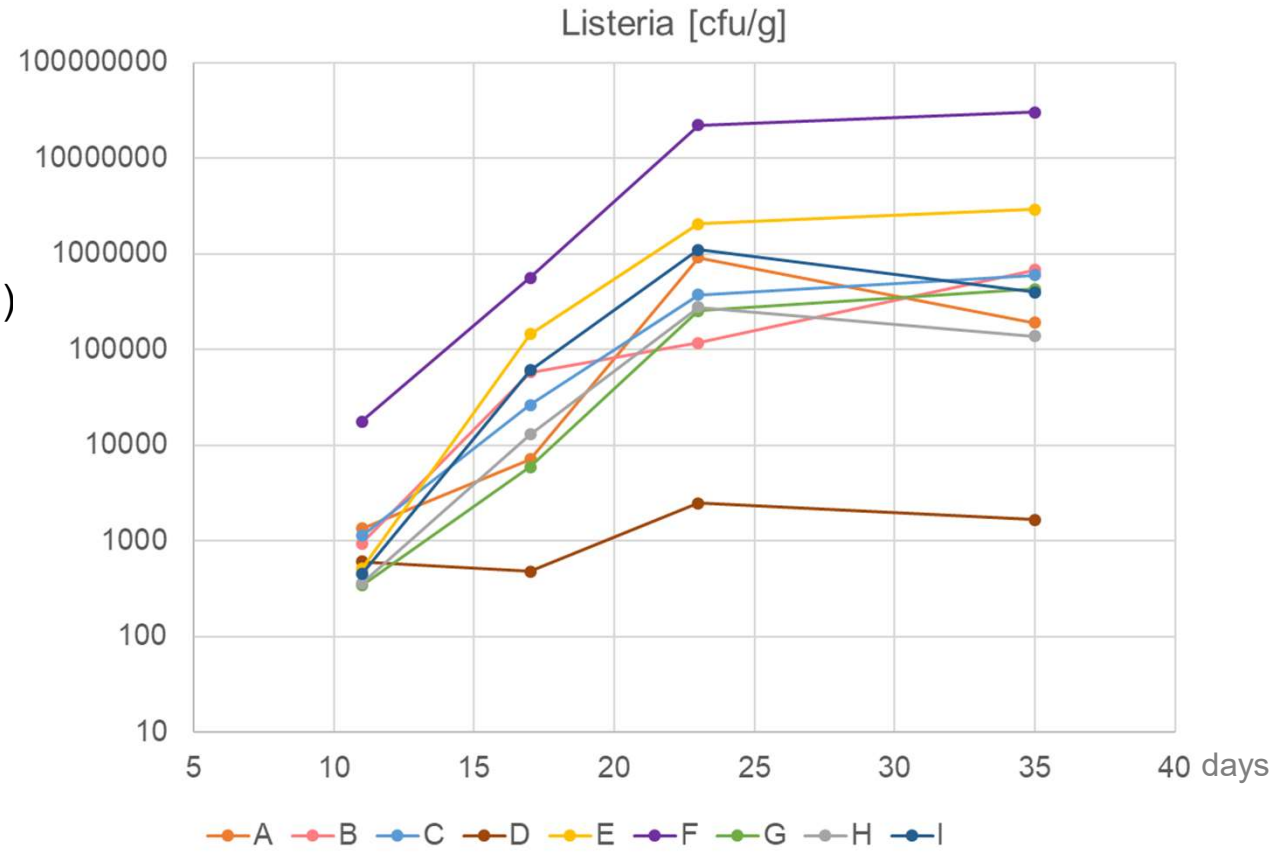
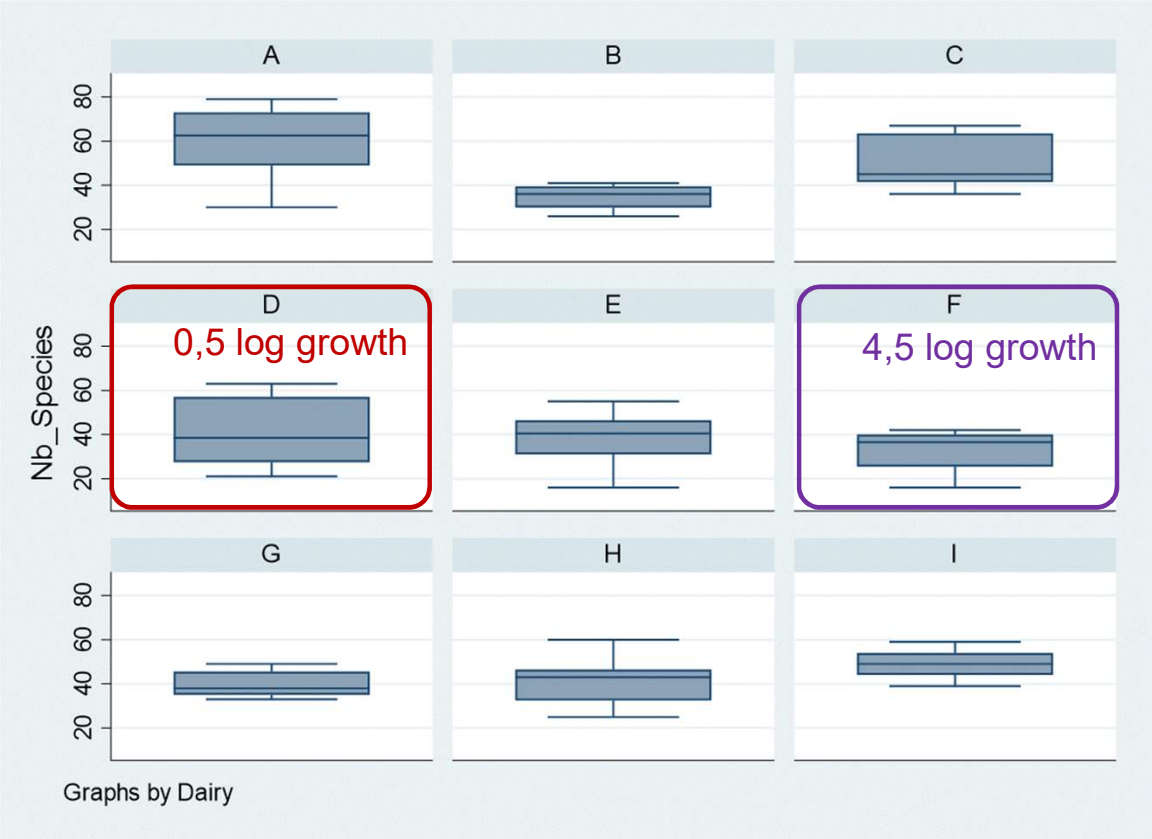
The antagonistic behavior is facility-dependent

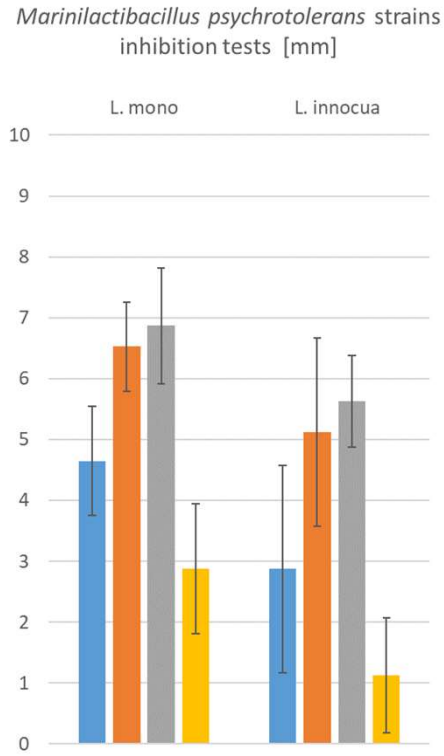
- 1-day old cheeses produced in 9 dairies
- Ripened in experimental cellar under identical abiotic conditions
  - avoiding cross-contamination
  - addition of *Listeria innocua* (50'000 cfu/ml)



Can we detect the biotic drivers?

Microbiome, isolates, *in vitro* phenotypes, WGS



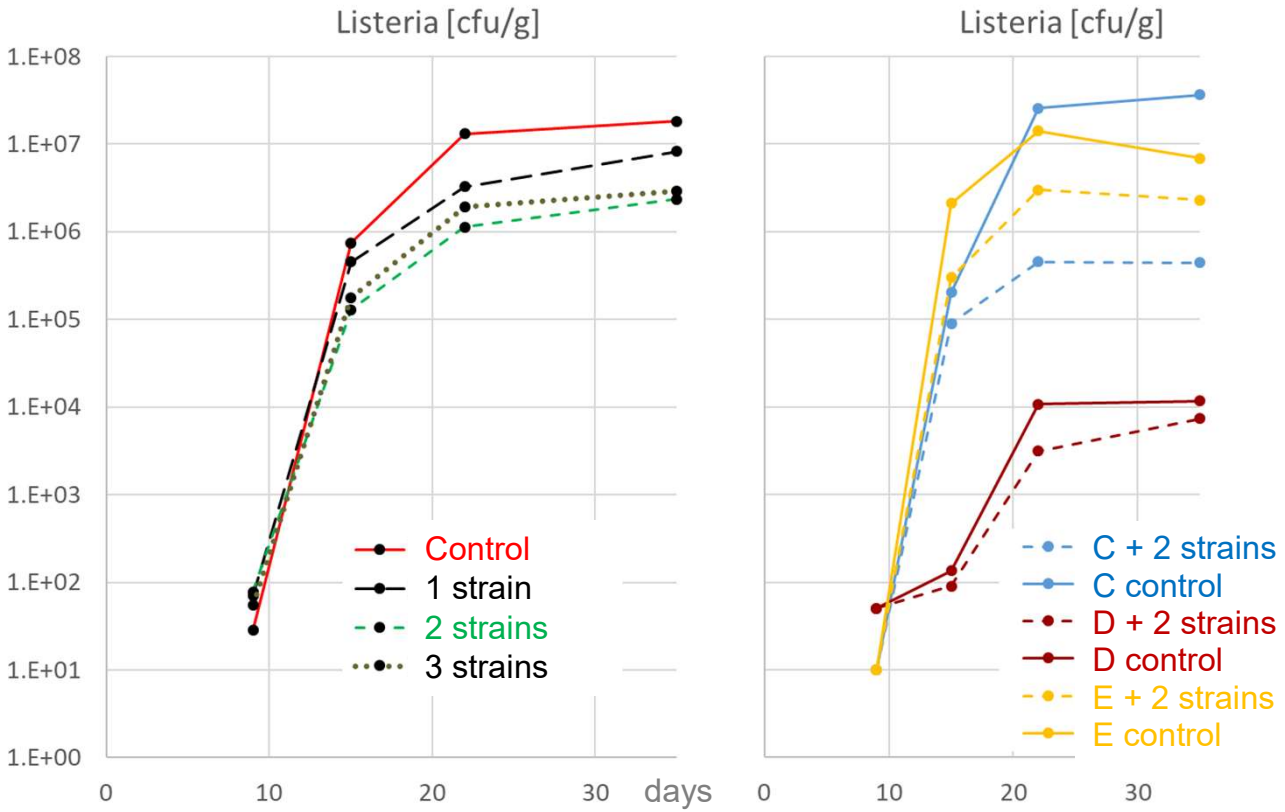


- Selection of strains for challenge tests
  - *Marinilactibacillus psychrotolerans*
  - *Ruoffia tabacinasalis*
  - *Desemzia sp.*
  - *Leuconostoc mesenteroides*

Challenge tests

- 1-day old cheeses produced in 6 dairies
- 10'000 Listeria per ml smear water
- Protective culture for surface application
  - *Marinilactibacillus psychrotolerans*
  - *Ruoffia tabacinasalis*
  - *Desemzia sp.*

- 0.5 Listeria per ml milk
- 1st protective culture for milk application
  - *Leuconostoc mesenteroides*
    - Mesentericin Y
- 2nd protective culture for surface application
  - *Marinilactibacillus psychrotolerans*
  - *Ruoffia tabacinasalis*



1: milk application	none		10^3 cfu/ml		10^4 cfu/ml	
2: surface application	none	10^7 cfu/ml	none	10^7 cfu/ml	none	10^7 cfu/ml
% positive (4 replicates)	100	100	50	0	25	0

Hypotheses on the nature of inhibition

- Carbon sources utilisation
  - Biolog data

Muchaamba et al., 2019

Carbon Source Utilization in Strains Responsible for Listeriosis Outbreaks

PM01, PM02	*Listeria monocytogenes*								*Ruoffia tabacinasalis*	*Marinilactibacillus psychrotolerans*
	LL195	N2306	N16-0044	N14-0435	EGDe	Lm3136	Lm3163	N11-1515		
D-Tagatose	-	-	-	-	-	+	+	-	-	+
D-Trehalose	+	+	+	+	+	+	+	+	+	-/+
D-Mannose	+	+	+	+	+	+	+	+	+	-/+
D-Galactose	-	-	-	-	-	-	-	-	-	-/+
L-lactic acid	-	-	-	-	-	-	-	-	+	-/+
Citric acid	-	-	-	-	-	-	-	-	-/+	-

- Essential nutrients
  - iron (siderophore production, citrate utilisation)
  - manganese scavenging
- Bacteriocin
  - *in vitro* inhibition : mesentericin Y105
  - *in silico*: other gene cluster present

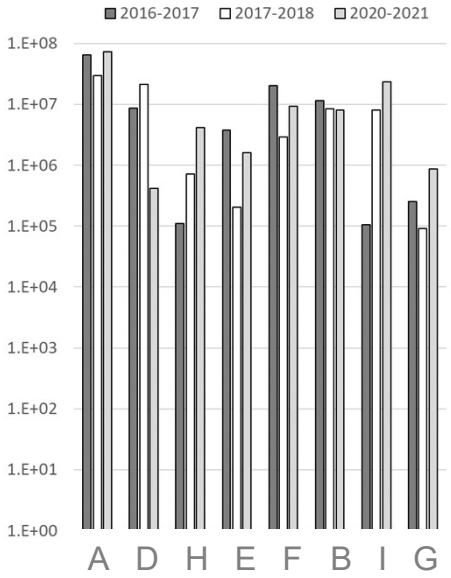
Leuconostoc mesenteroides strains

- Motility, biofilm vs dispersion through fungal highways
  - *in silico: Listeria, Marinilactibacillus psychrotolerans*

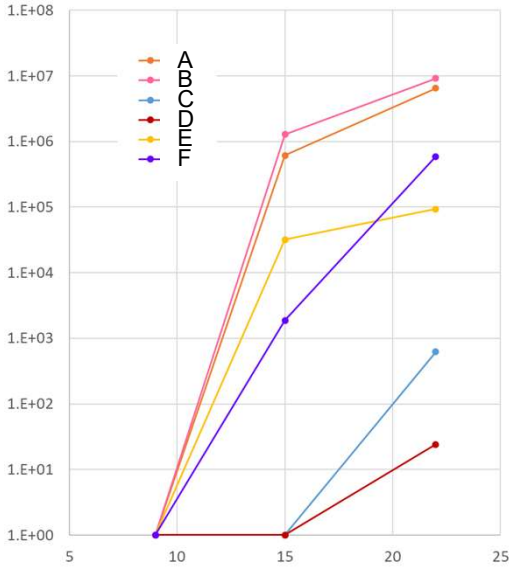
Safety assessment of the species/strains
for use as a protective culture

- No ABR genes
- No virulence genes
- Amount stays in the same range as in regular products (qPCR *Ruoffia tabacinasalis*)

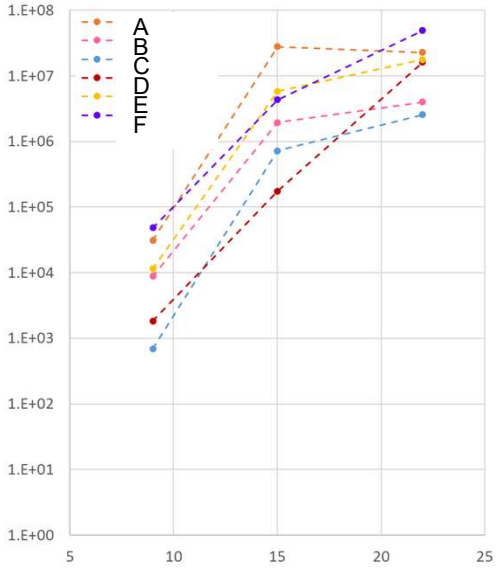
regular products



control cheeses

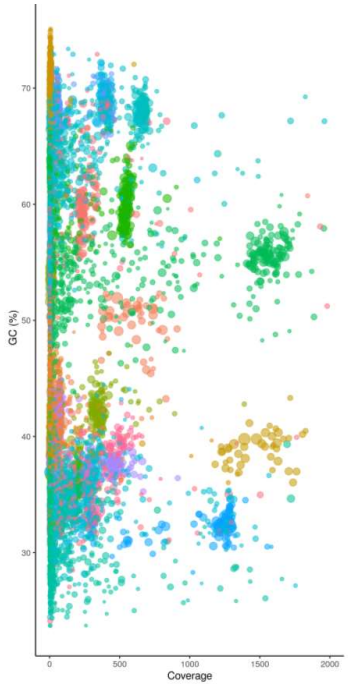


Cheeses with protective culture



Future research activities

- Use of metagenomic data
  - functional potential of uncultured species
    - Implication in the inhibition
- Other cheese types / pathogens
- Replication of smear in fermenters
- New process for smear ripening
















Thank you for your attention

Emmanuelle Arias-Roth

emmanuelle.arias@agroscope.admin.ch

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