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Targeted Protective Cultures for Cheese

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8 June 2026

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Pathogens in cheese

The usual suspects

- *Listeria monocytogenes*
- Shiga toxin producing *Escherichia coli* (STEC)
- *Staphylococcus aureus*
- *Salmonella* spp.

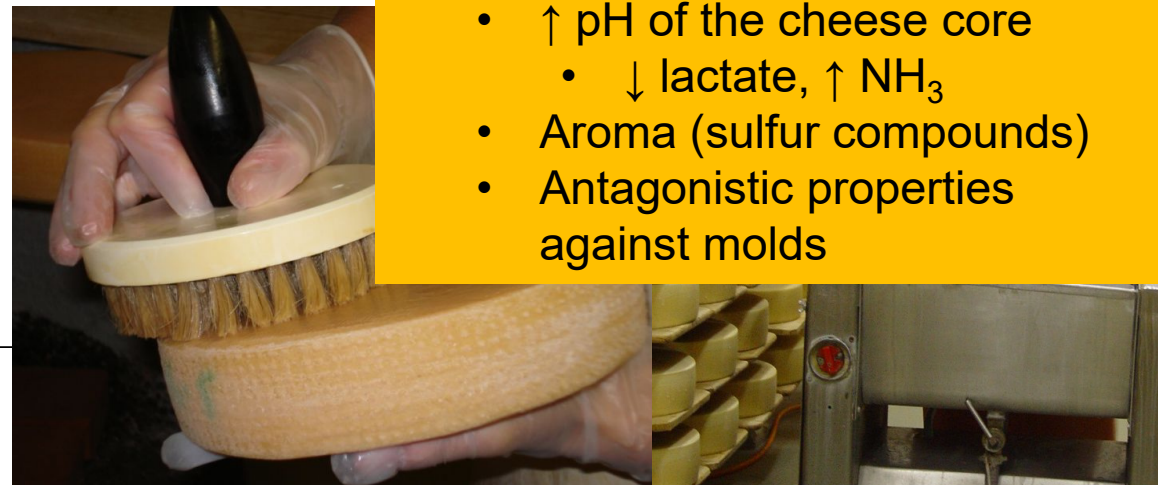


Introduction to Cheese Smear



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

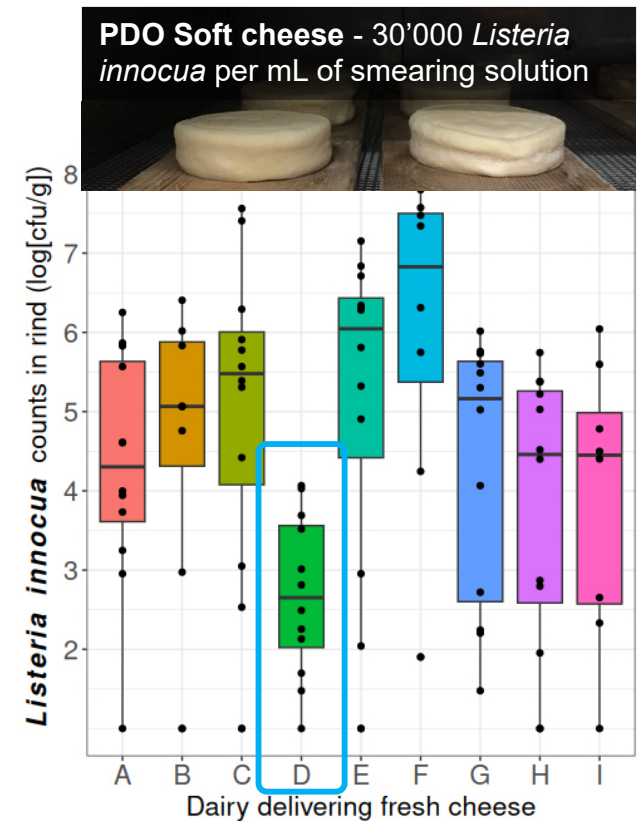
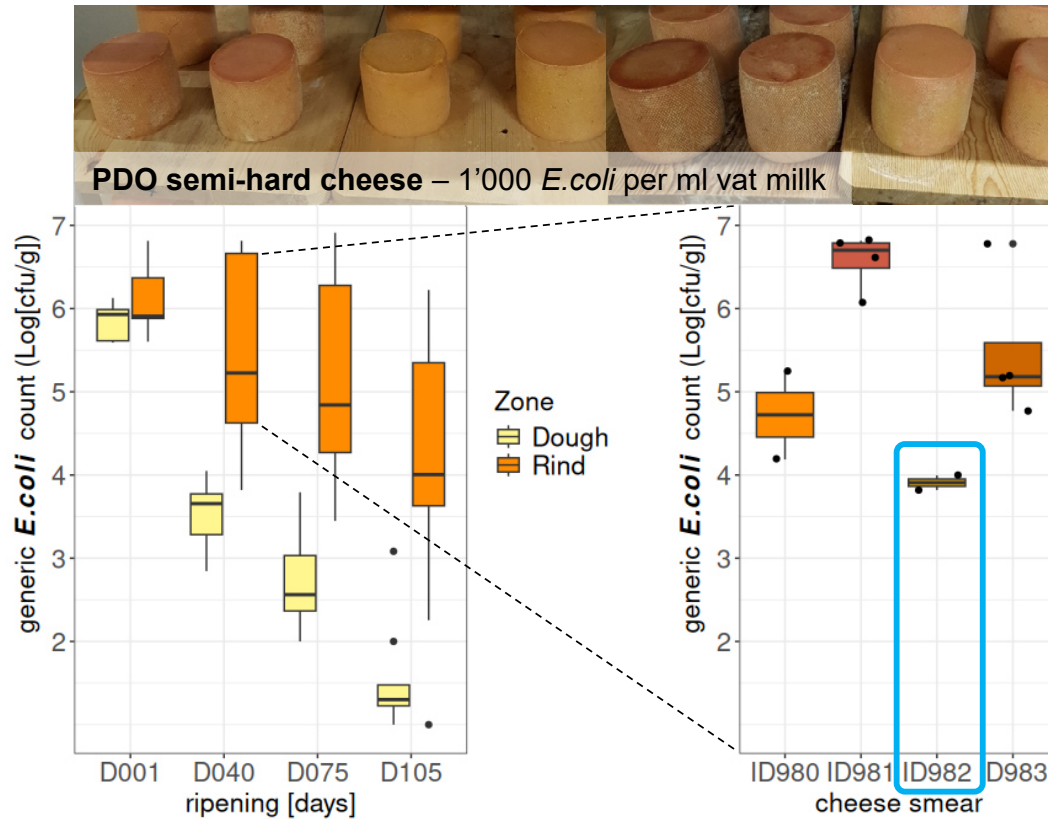
- Mostly smear cheeses in CH
- Functions of smear
 - Reduces water loss
 - \uparrow pH of the cheese core
 - \downarrow lactate, \uparrow NH_3
 - Aroma (sulfur compounds)
 - Antagonistic properties against molds





Does smearing promote the survival / growth of pathogens?

<https://doi.org/10.3390/foods13213473>



Mostly Yes... and sometimes No

→ which **abiotic and biotic factors** determine antagonistic behaviour?



Bacterial biodiversity by shotgun metagenomics

Abundances in PDO semi-hard smear cheese



	classification	ID980	ID981	ID982	ID983
Actinomycetota 24 species	Ancrocorticia_populi	0.8	1.8	0.1	0.0
	Flaviflexus_ciconiae	0.1	0.7	0.0	1.4
	Actinomycetaceae_gJB111	0.1	0.2	1.8	0.0
	Actinomycetaceae_sJB111sp900	0.3	0.3	1.3	0.0
	Ruania_s	0.0	0.0	0.5	0.0
	Brevibacterium_aurantiacum	16.4	20.4	15.5	7.0
	Brevibacterium_yomogidenseA	3.9	4.3	15.4	17.3
	Brachybacterium_sp014400405	5.1	1.9	0.9	8.2
	Brachybacterium_sp030457675	0.6	0.8	0.3	0.6
	Agrococcus_casei	13.8	6.6	10.3	10.7
	Canibacter_s	9.0	2.8	0.6	7.4
	Gulosibacter_s	0.0	0.0	0.3	0.0
	Gulosibacter_sp900163695	1.2	0.0	0.0	0.0
	Leucobacter_s	0.0	0.3	0.5	0.0
	Microbacterium_s	1.3	0.6	3.3	0.0
	Microbacterium_gubbeenense	2.1	1.8	2.2	0.6
	Microbacterium_sp900163665	5.9	2.9	0.3	0.0
	MicrococcaceaeCmP2_s	4.8	5.3	0.7	7.4
	Yaniella_sp030453185	0.0	0.7	0.0	1.5
	Corynebacterium_casei	13.6	14.5	6.7	14.9
	Corynebacterium_faecigallinarum	12.1	17.2	2.4	10.4
	Corynebacterium_variable	1.4	6.7	3.2	0.0
	Dietzia_s	0.0	0.0	0.7	0.0
	Aviropionibacterium_s	0.0	0.0	0.9	0.0

	classification	ID980	ID981	ID982	ID983
Bacillota 8 species	Ruoffia_tabacinasalis	0.7	0.8	0.4	1.5
	Alkalibacterium_gilvum	2.4	2.0	0.2	2.3
	Atopostipes_s	0.0	0.3	0.0	0.5
	Marinilactibacillus_psychrotolerans	0.0	0.1	0.2	0.0
	Vagococcus_s	0.0	0.2	0.0	0.0
	Staphylococcus_equorum	0.9	2.5	0.3	0.4
	Anaeromonas_s	0.1	0.8	0.1	1.3
	TissierellaA_s	0.0	0.0	0.0	0.2
Bacteroidota 2 species	Psychroflexus_halocasei	0.1	0.3	0.0	0.1
	Sphingobacterium_s	0.7	0.4	21.9	0.6
Pseudomonadota 7 species	Advenella_sp002810445	0.0	0.1	4.3	0.0
	Oceanisphaera_s	0.2	0.0	0.0	0.7
	Vreelandella_s	0.7	1.4	2.3	2.9
	Psychrobacter_sp014861395	0.0	0.0	0.8	0.0
	Psychrobacter_sp030457785	1.5	1.1	0.2	2.0
	Marinobacter_s	0.1	0.4	0.0	0.0
	LuteimonasD_s	0.0	0.0	1.3	0.0

- 41 species detected in the smear from 4 dairies
 - Only 25 out of 41 are cultivable
- → up to 12 interesting species to investigate in the antagonistic smear from dairy ID982



Ripening of real size smear cheeses experimental cellar



- ✓ to investigate real conditions / complex microbiota
- ✗ to test:
 - multiple factors & levels
 - interactions
 - pathogens class 2/3
- ✓ to confirm solutions

Can we stick
with the cheese matrix
whilst increasing
the throughput?

Cheese miniaturization technology

Soft cheese



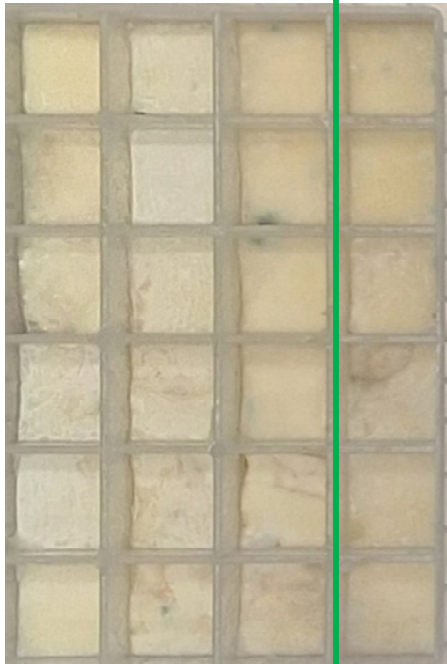


Ripening model using Deepwell plates

Soft cheese



12 days



- 1 row = 4-6 miniature cheeses = 1 replicate
 - Homogeneous microbiota
 - rubbed with same cotton swab every 2-3 days
- Standardized ripening conditions across rows and Deepwell plates
 - smearing solution (ml), relative humidity
- Enables challenge tests with pathogens
 - e.g. *Listeria monocytogenes*
- No cross contaminations between rows (n=8)



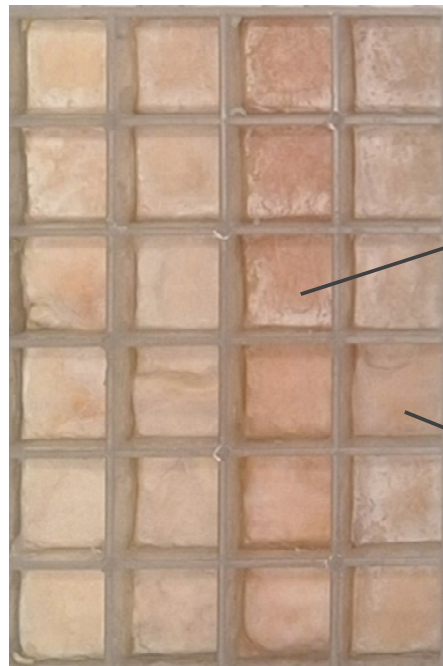
Ripening model using Deepwell plates

Soft cheese

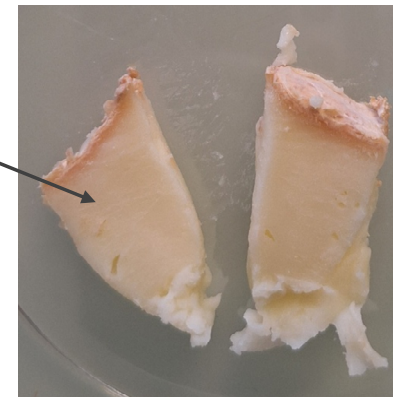
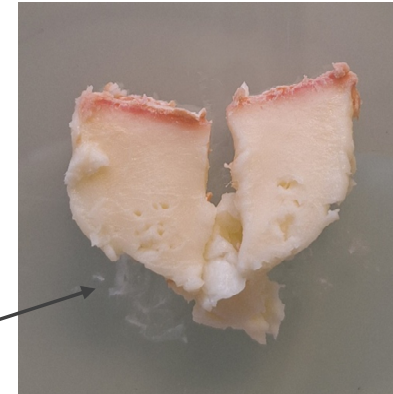
12 days



23 days



26 days

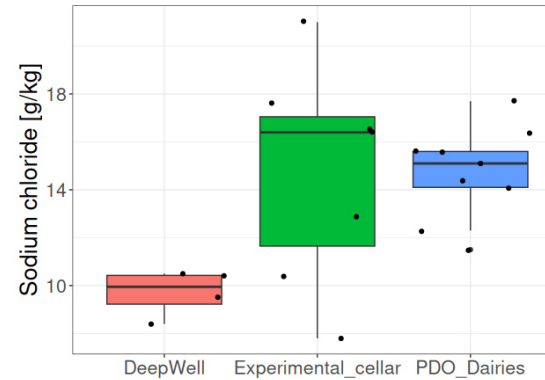
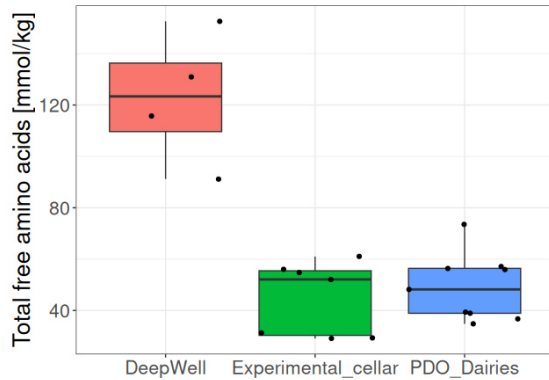
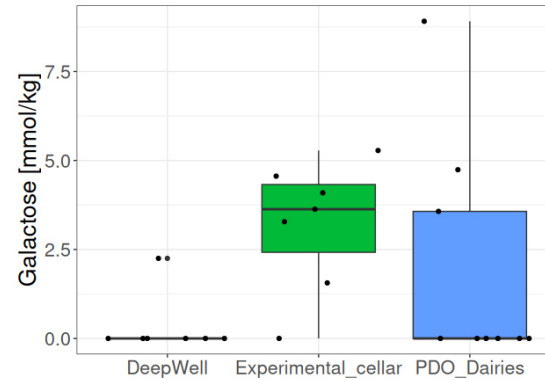
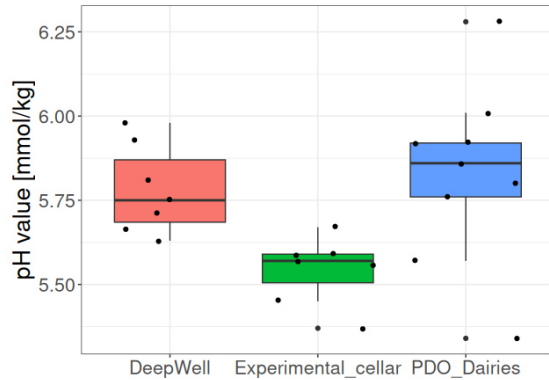




Performance of ripening model miniature VS real size cheeses

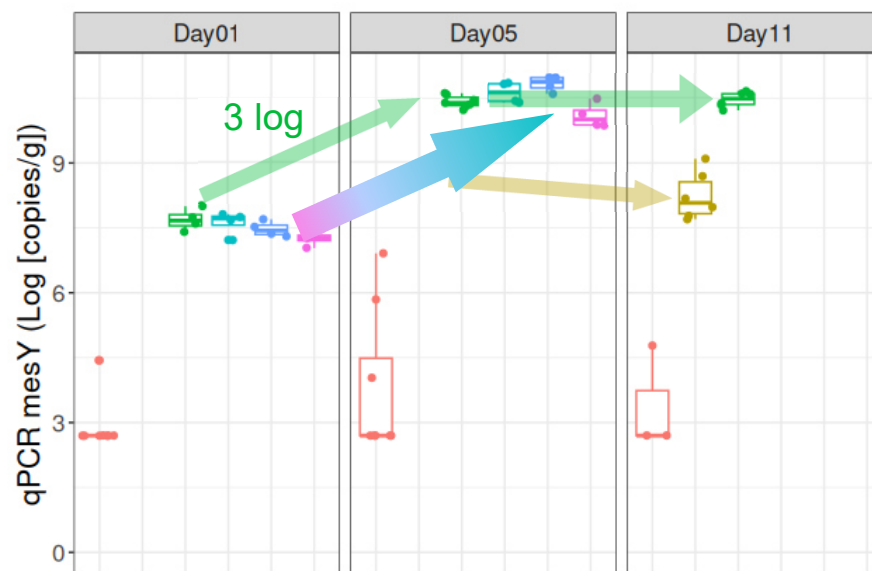
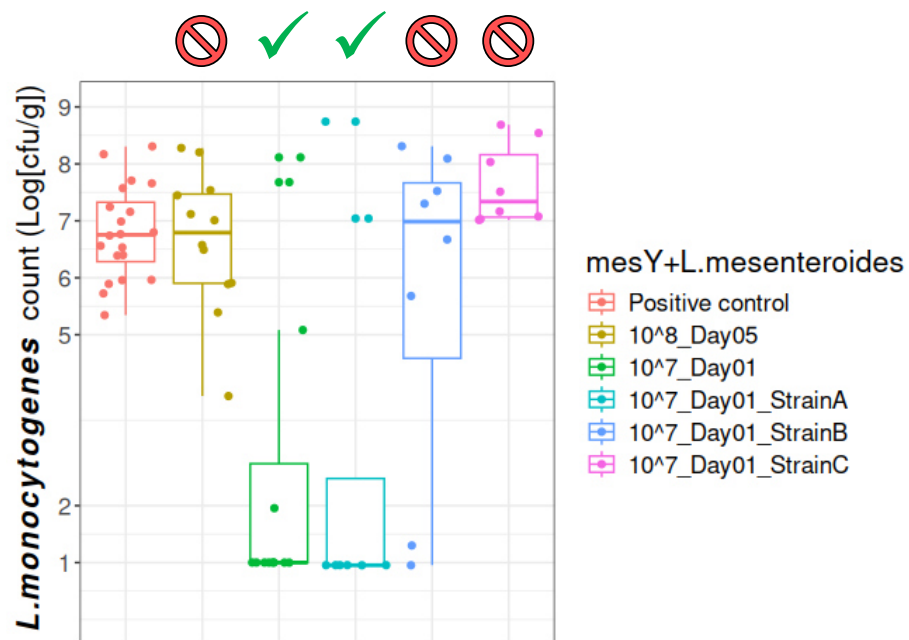


Ripening_facility
■ DeepWell
■ Experimental_cellar
■ PDO_Dairies






Optimization of protective culture application / timing / strain selection



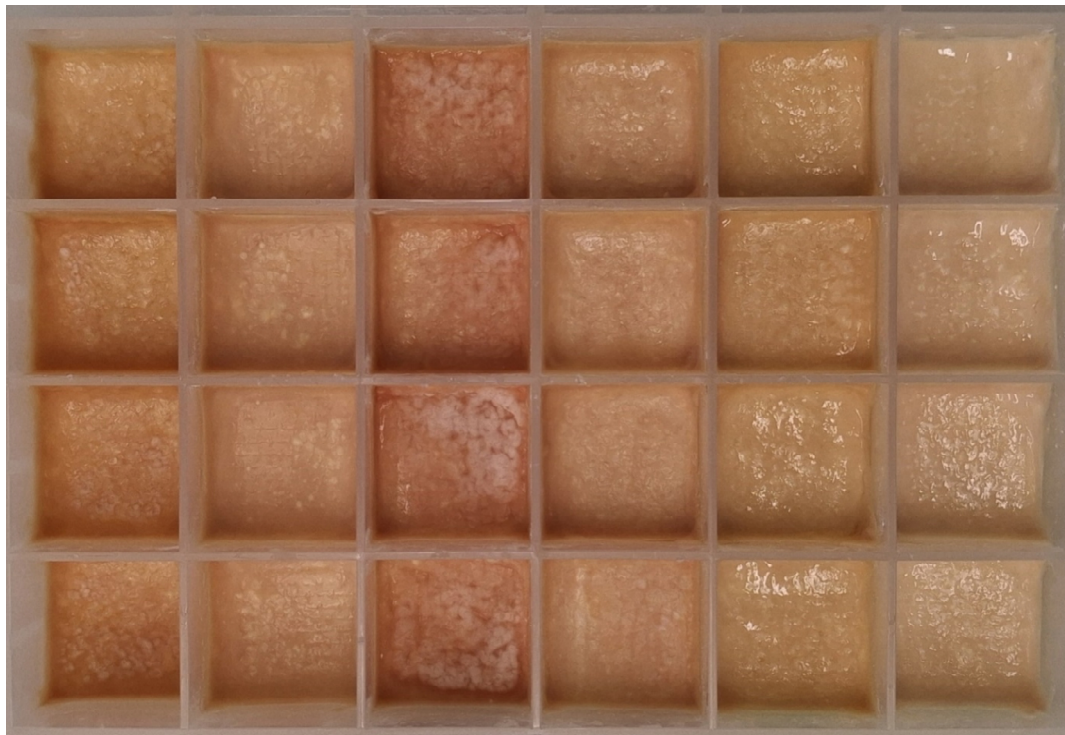
Optimization of mesentericin producing *Leuconostoc mesenteroides*

- an early application is mandatory to enable *in situ* growth and activity
- strain selection: *in vitro* inhibition  *in situ* inhibition



Ripening model

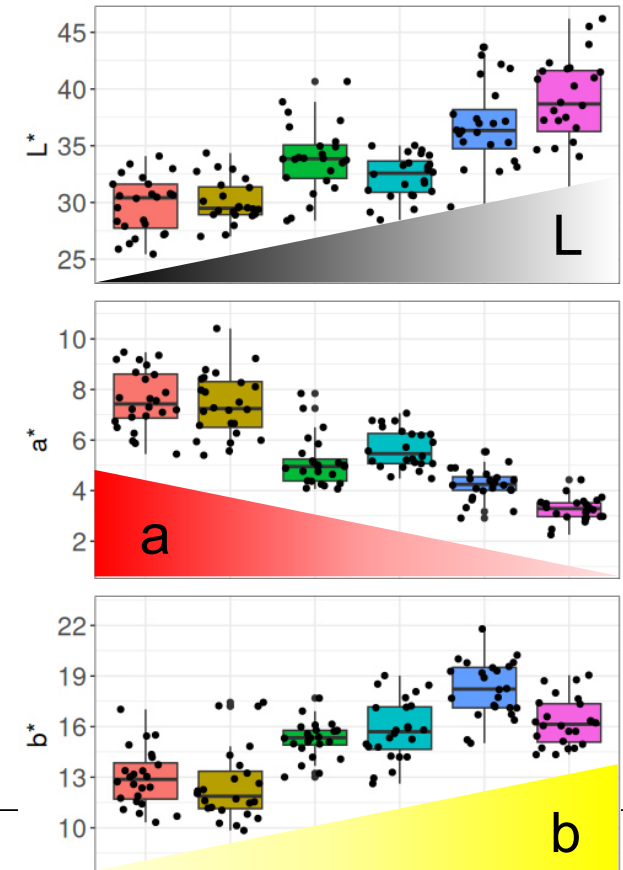
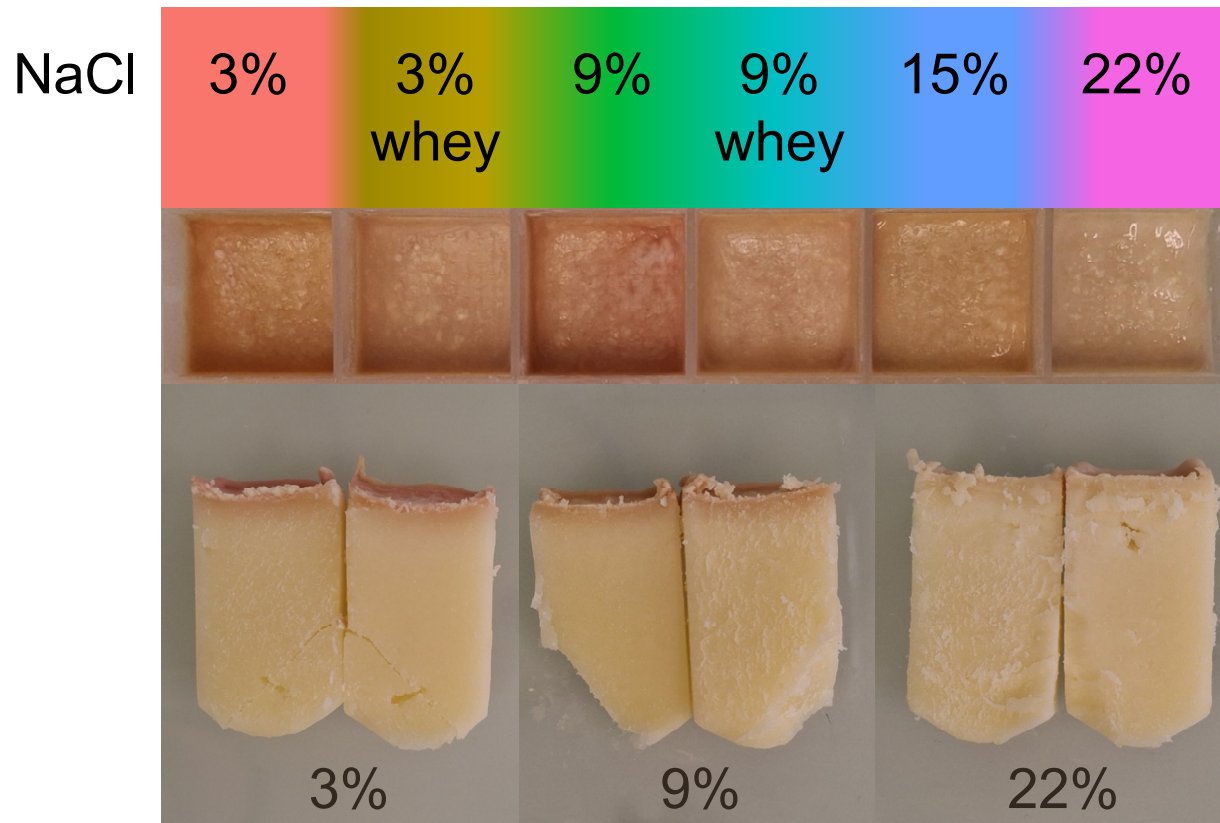
Hard cheese after 7 weeks





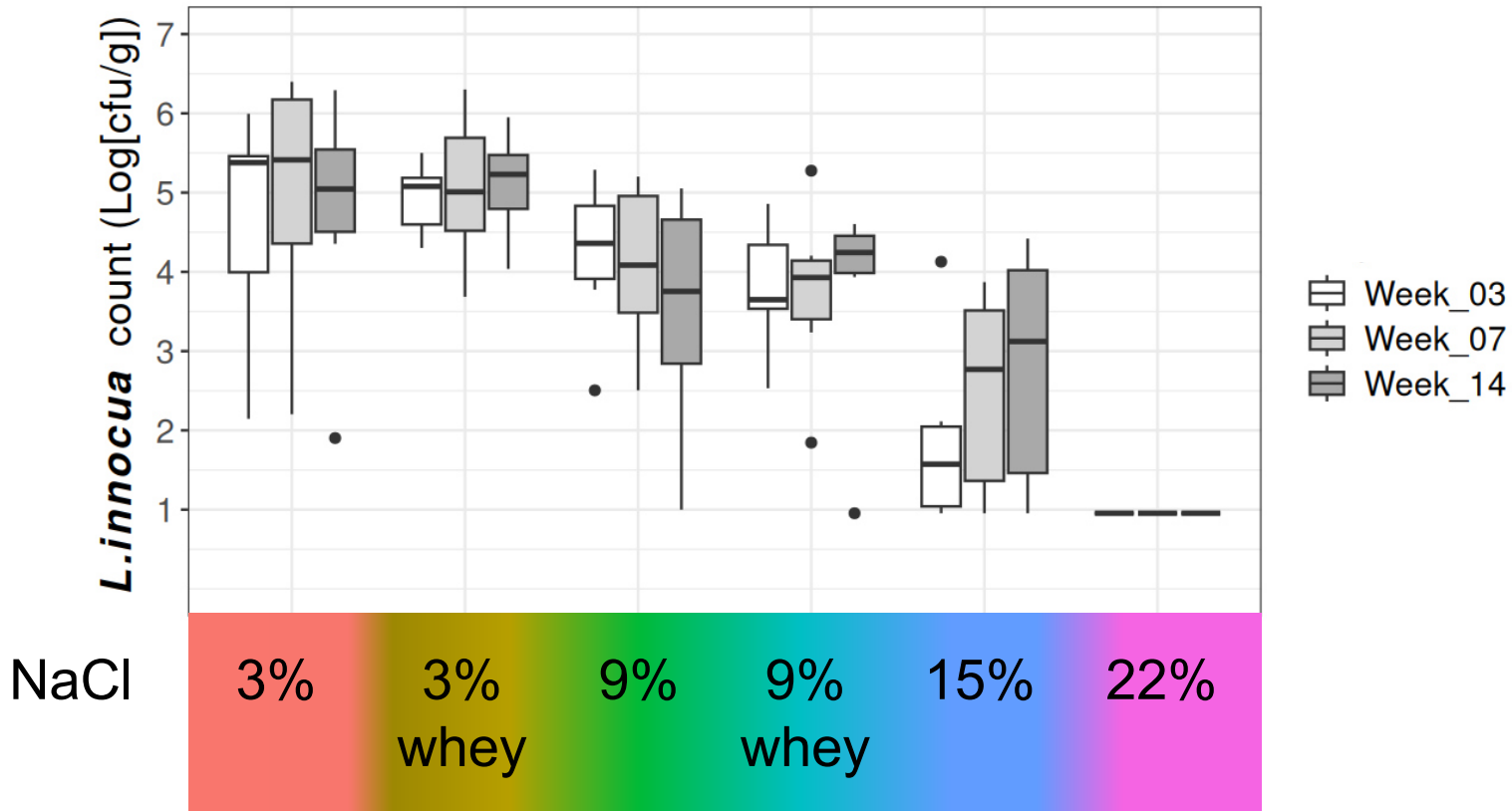
Ripening model

Hard cheese after 7 weeks





Abiotic parameter in smearing solution influences food safety



- Salt > 15% ✓
- Fermented whey ⚡



Conclusions and perspectives

Ripening models using Deepwell plates

- A new tool for:
 - Disentangling the influence of biotic and abiotic factors
 - Challenge tests using raw material from artisanal / industrial dairies
 - ...investigate interactions
 - ...develop solutions combining multiple hurdles

Development of protective cultures based on ecological competition

- Study of complex smears (metagenomes, *in situ* antagonistic properties)
- Characterization of strains (WGS, phenotypes)
- Design of synthetic consortia (5-10 strains)
- Replication and *in situ* testing of synthetic consortia



Acknowledgments

AGROSCOPE

Food Microbial Systems

- Cheese Quality, Cultures and Terroir
 - *Remo Schmidt*
- Risk Evaluation and Risk Mitigation
 - *Lena Fritsch*
- Microbiological Food Safety
 - *Jörg Hummerjohann & Team*
- Applied Processing Technology
 - *Florian Loosli, John Haldemann*
 - *Helena Stoffers & Team*
- Biotechnology
 - *Ueli von Ah & Team*
- Production of Cultures
 - *Christoph Kohn & Team*

Methods Development and Analytics

- Biochemistry of Milk and Microorganisms
 - *Stefan Irmeler & Team*
- Fermentation Organisms
 - *Alexandra Roetschi*
 - *Hélène Berthoud & Team*
- Molecular Ecology
 - *Florian Gschwend*

Collaborations

University of Bern

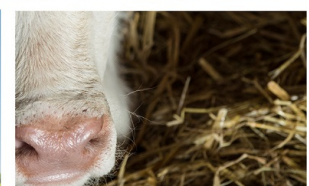
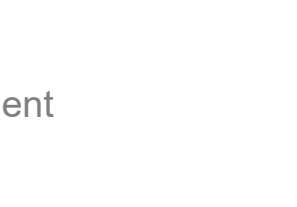
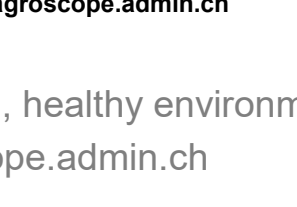
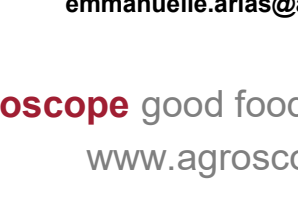
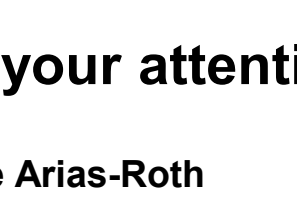
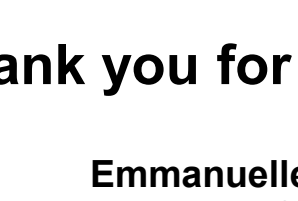
- *Joana Carlevaro-Fita*

University of Veterinary Medicine Budapest

- *Lívía Darnay*

Dairies and Cheese Trade Associations

- *Vacherin Mont-d'Or AOP*
- *Sándor Tamás Sajtmanufaktúra*



Thank you for your attention

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