FACE congress, 11-13 october 2023, Posieux

Microbial transfers across a food chain: from grassland to cheese using systemic and multidisciplinary approaches

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CONTEXT

Global issues

World demand

Climate change


Food and Agriculture Organization of the United Nations

MICROBIOME: THE MISSING LINK?

SCIENCE AND INNOVATION FOR HEALTH, CLIMATE AND SUSTAINABLE FOOD SYSTEMS

Social expectations

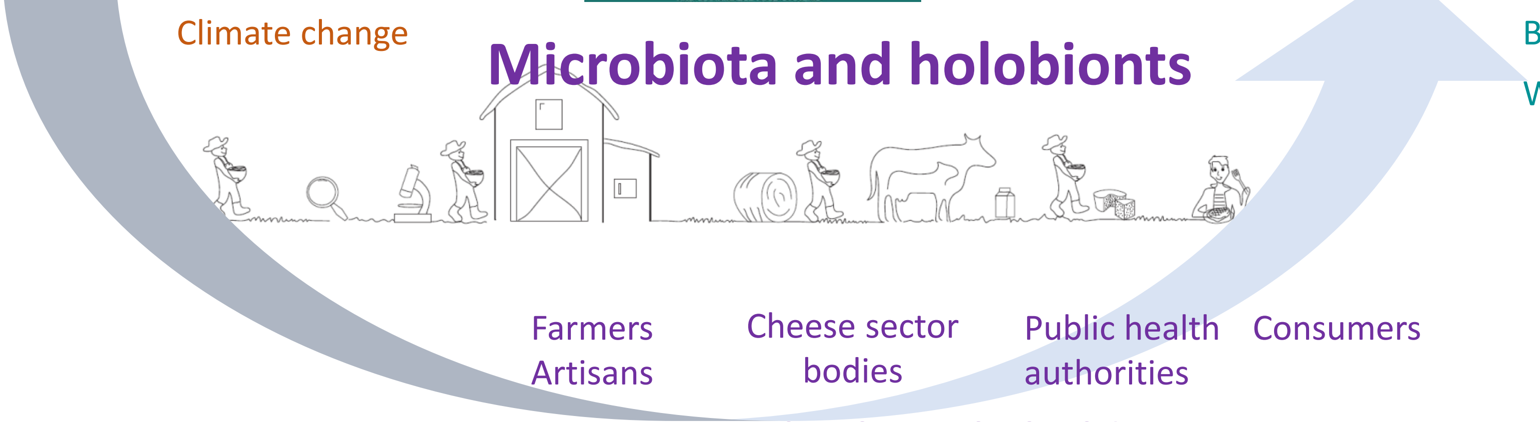
One Quality - One Health

Safe and secure food

Biodiversity

Wellfare

Microbiota and holobionts



Farmers
Artisans

Cheese sector bodies

Public health authorities

Consumers

Multiple stakeholders

Raw milk cheeses:

Terroirs

Know-how

Complex microbiota

Potential of microbial drivers ?

- ☐ performance, sustainability and resilience of food systems
- ☐ agro-ecological transitions in livestock systems

Scientific issues

Understanding the drivers of microbiota across the dairy system for safe, healthy and sustainable raw milk cheese

Outline of the presentation

- 1/ Milk's contribution to the establishment of cheese microbiota
- 2/ Microbial transfers across dairy systems
- 3/ Microbial transfers and safety management
- Perspectives

1/ Milk's contribution to the establishment of cheese microbiota

Contribution of milk to the microbial signature of French PDO cheeses

F. Irlinger (UMR SAYFOOD, INRAE, AgroParisTech, Saclay)
C. Delbès (UMRF, INRAE, UCA, VAS, Aurillac)

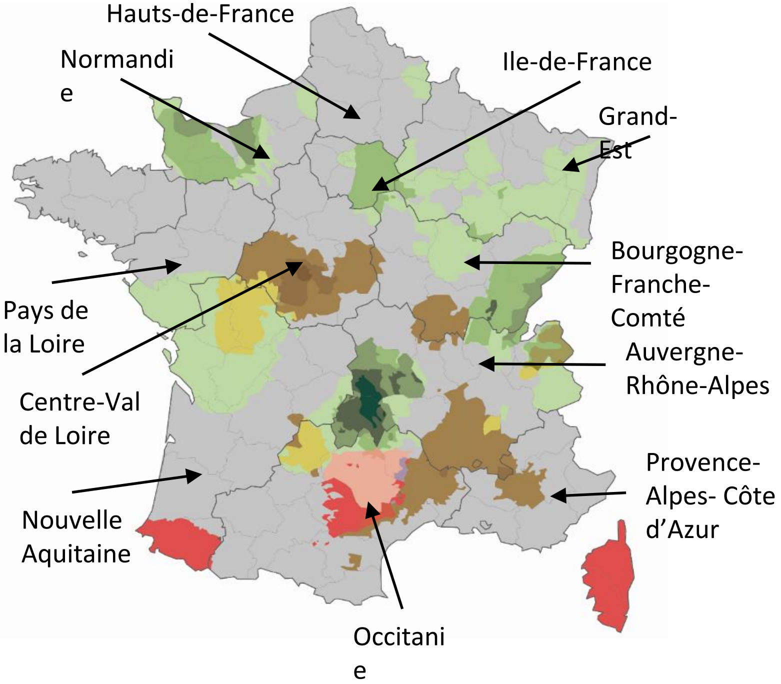
44 PDOs
7 cheese technological families

- ~ 10 ateliers/AOP
- **1200 cheeses** (core and rind)
- **370 milks**

Chosen to cover diversity in : Season, Feeding practices, milk processing, milk typeFarm size, Inoculation practices

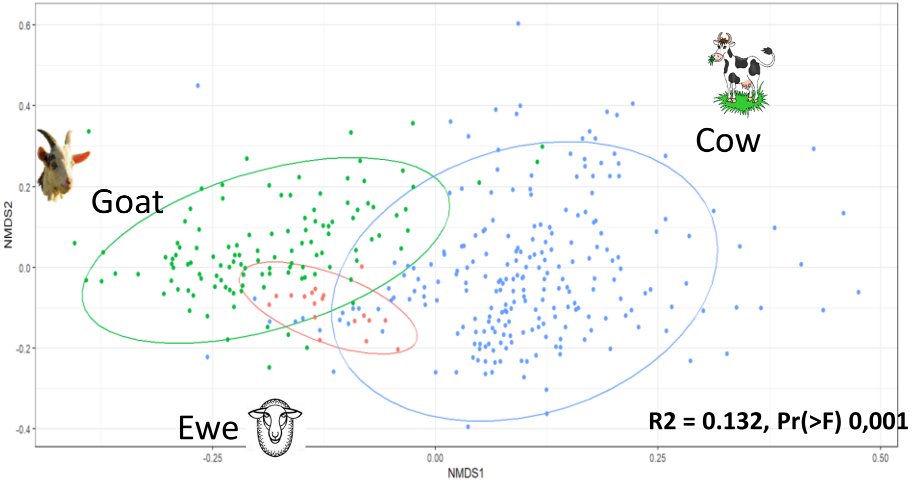
Fromages Croûtes et Cœurs

Crédit: V. RIBAUT / CNIEL



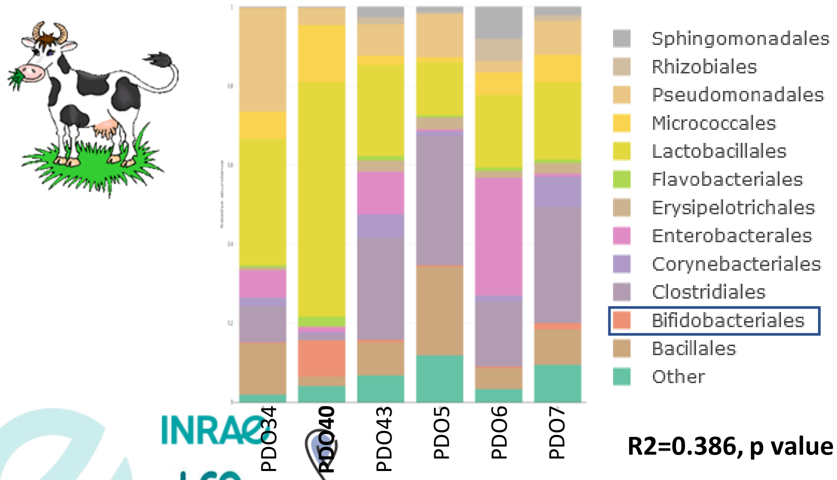
Drivers of milk microbiota composition

Milk Bacterial community beta-diversity (BC index, Total milks' samples: N=370; 3219 ASV sequences (>0.005%)



- In the 370 milks, 1230 bacterial species and 1367 fungal species (>0.005%) were identified.
- **Dairy ruminant species** is a major driver of milk bacterial microbiota composition.

Bacterial profiles of cow's milk from 6 PDOs, most influenced by PDO (N=63) (mean profiles at the order level)



R2=0.386, p value < 0.001

PDO is the second most important driver of the bacterial microbiota in milk.

2/ Microbial transfers across dairy systems

Potential transfer of microorganisms from cow teat surfaces to cheese

SCIENTIFIC REPORTS


OPEN

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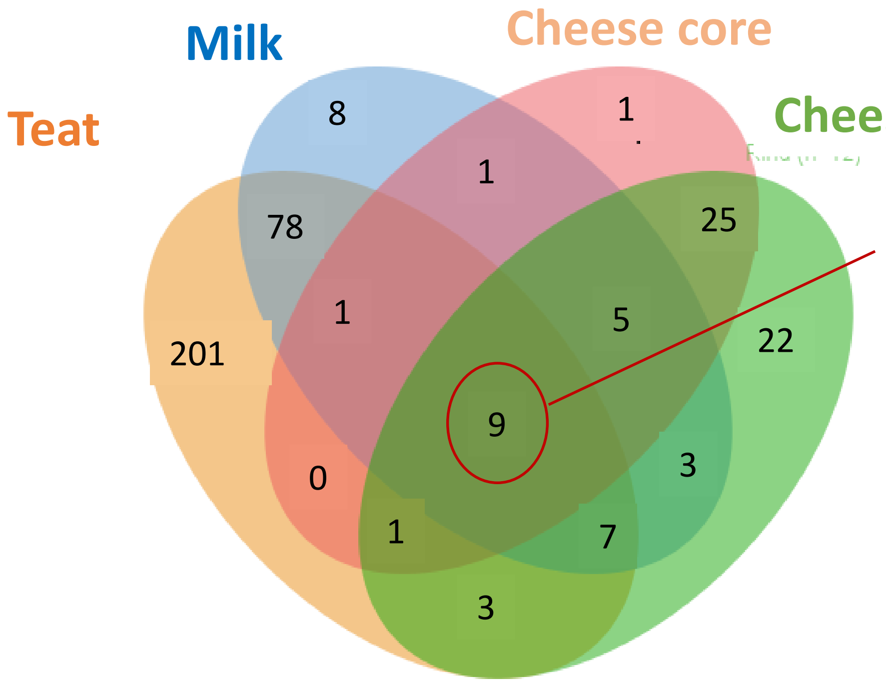
ed: 11 December 2017

Bacterial community assembly from cow teat skin to ripened cheeses is influenced by grazing systems

rétin[1,2], Bruno Martin[2], Etienne Rifa[1], Verdier-Metz Isabelle[1], Dominique Pomiès[2], rlay[2], Marie-Christine Montel[1] & Céline Delbès[1]

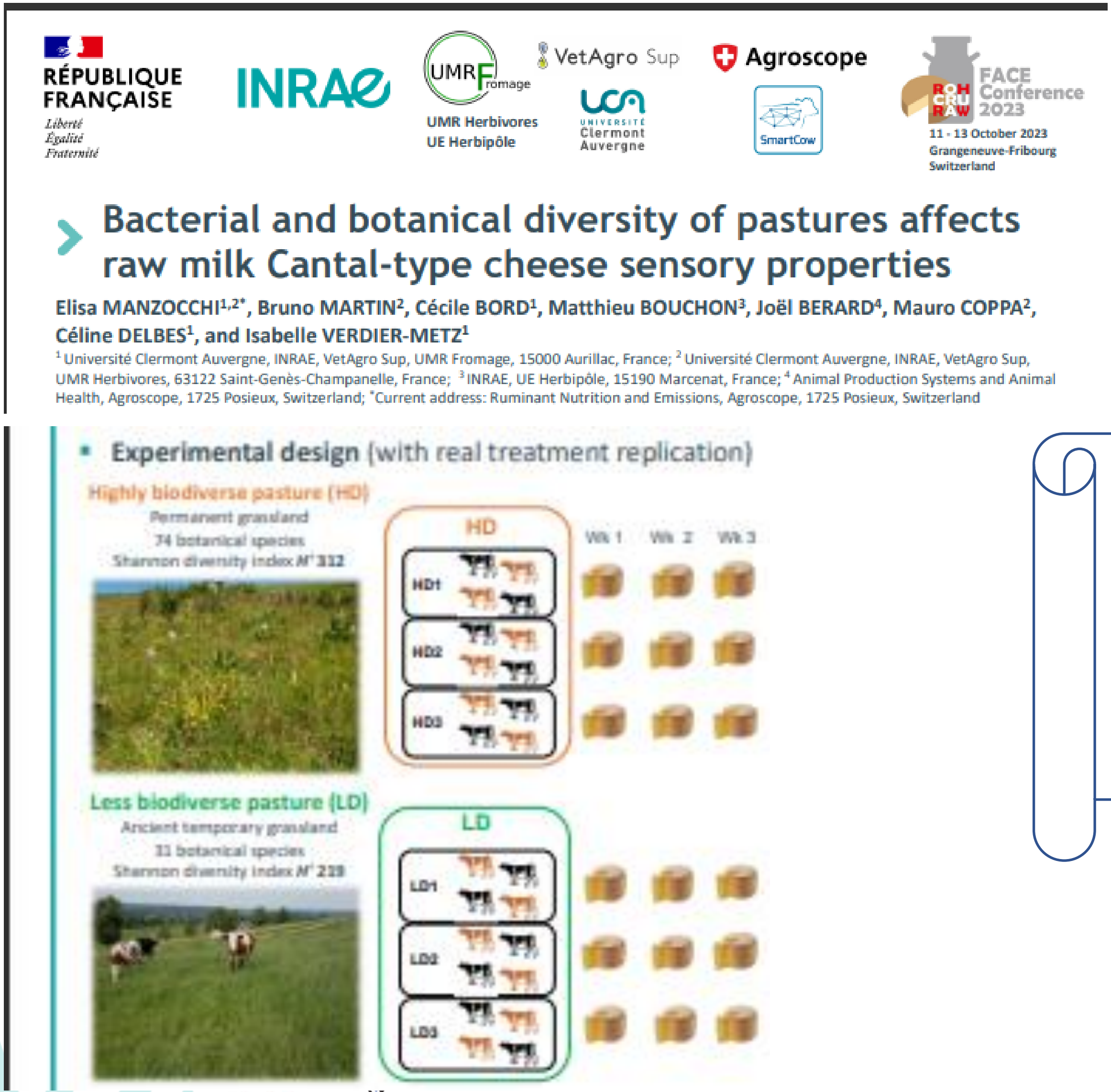


Lactococcus lactis
Brevibacterium sp
Lactococcus sp
Staphylococcus sp
Macrococcus caseolyticus
Streptococcus sp
Streptococcus dysgalactiae
Escherichia coli

- ☐ 85% of bacterial species present in milk are also detected on teat skin
- ☐ 27% of bacterial species present in cheese (core+rind) are also detected on teats, including bacteria potentially involved in flavour development (*B. linens, Staph. equorum*).

Teat skin: a potential reservoir of bacterial diversity for milk and cheese

Potential transfer of microorganisms between grass surface and dairy products



UMR Herbivores
UE Herbipôle

FACE Conference 2023
11 - 13 October 2023
Grangeneuve-Fribourg
Switzerland

Bacterial and botanical diversity of pastures affects raw milk Cantal-type cheese sensory properties

Elisa MANZOCCHI[1,2*], Bruno MARTIN[2], Cécile BORD[1], Matthieu BOUCHON[3], Joël BERARD[4], Mauro COPPA[2], Céline DELBES[1], and Isabelle VERDIER-METZ[1]

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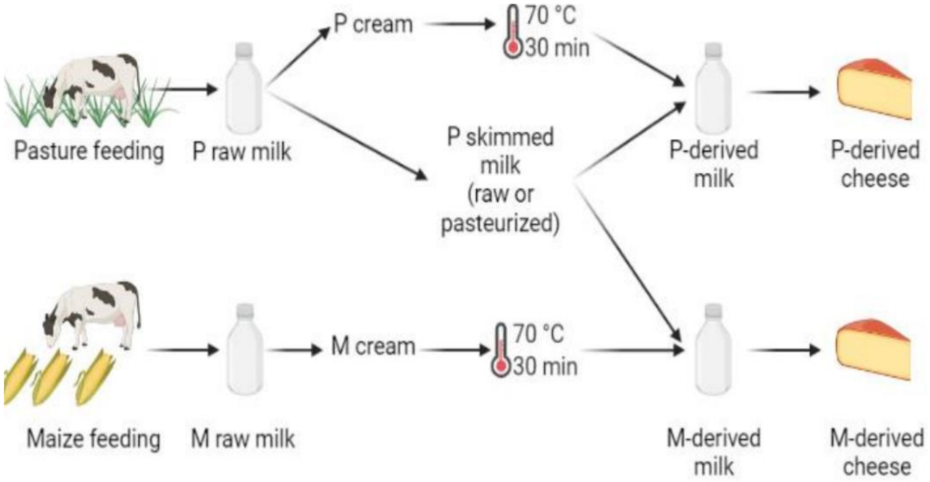
- **Experimental design** (with real treatment replication)

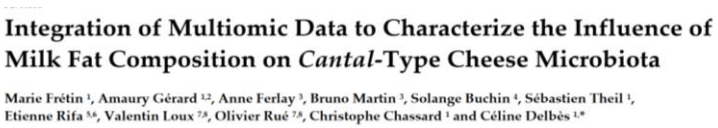
Highly biodiverse pasture (HD)
Permanent grassland
74 botanical species
Shannon diversity index H' 3.12

Less biodiverse pasture (LD)
Ancient temporary grassland
31 botanical species
Shannon diversity index H' 2.19

Poster by Elisa Manzocchi et al.

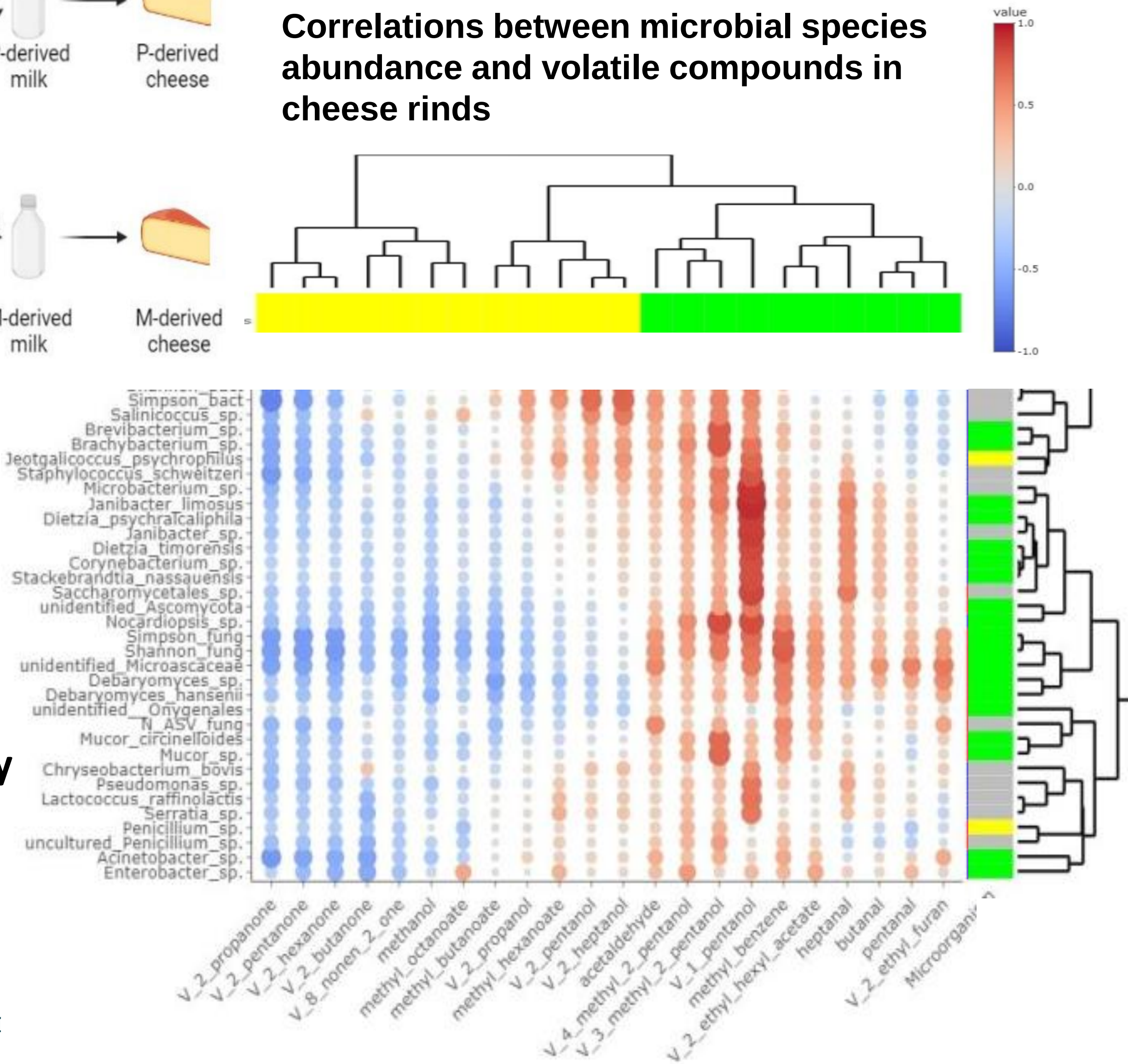
Influence of animal feeding on fat composition and microbiota in Cantal-type cheese




Integration of Multiomic Data to Characterize the Influence of Milk Fat Composition on *Cantal*-Type Cheese Microbiota

Marie Frétin [1], Amaury Gérard [1,2], Anne Ferlay [3], Bruno Martin [3], Solange Buchin [4], Sébastien Theil [1], Etienne Rifa [5,6], Valentin Loux [7,8], Olivier Rué [7,8], Christophe Chassard [1] and Céline Delbès [1,*]

Correlations between microbial species abundance and volatile compounds in cheese rinds



- Cream origin modulates :

- fatty acids and volatile compounds
- cheese microbiota (fungi as *Sporendonema casei* (ripening starter -> rind appearance)

→ How to match milk primary production conditions and properties of added ripening starters ?

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Impact of added “microbial solutions” on microbiota in the farm

Live yeast (LY) supplement

Dietary Live Yeast Supplementation Influence on Cow’s Milk, Teat and Bedding Microbiota in a Grass-Diet Dairy System

Isabelle Verdier-Metz [1,*], Céline Delbès [1], Matthieu Bouchon [2], Etienne Rifa [1], Sébastien Theil [1], Frédérique Chaucheyras-Durand [3,4], Eric Chevaux [3], Lysiane Dunière [3,4] and Christophe Chassard [1]

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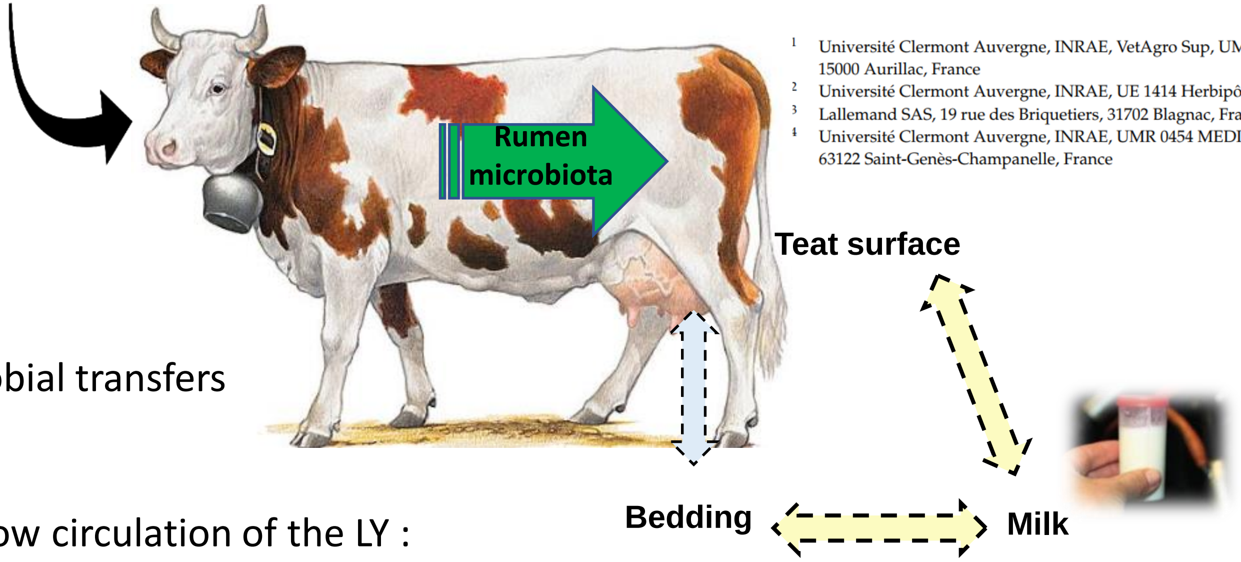
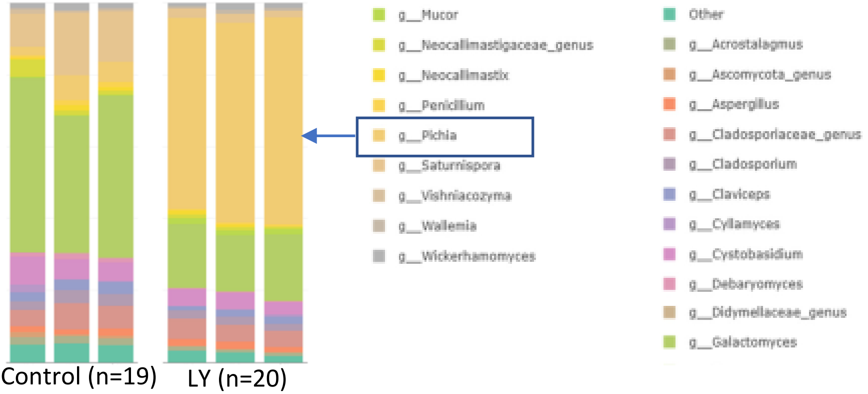


⇢ Microbial transfers

⇢
- Low circulation of the LY : sporadic detection on bedding and teats

- LY effects on the abundance of yeast species (*Pichia kudriavzevii*) on teat and bedding surfaces (p < 0,05)

Abundance of fungal genera on bedding surface after 4 months of LY supplementation



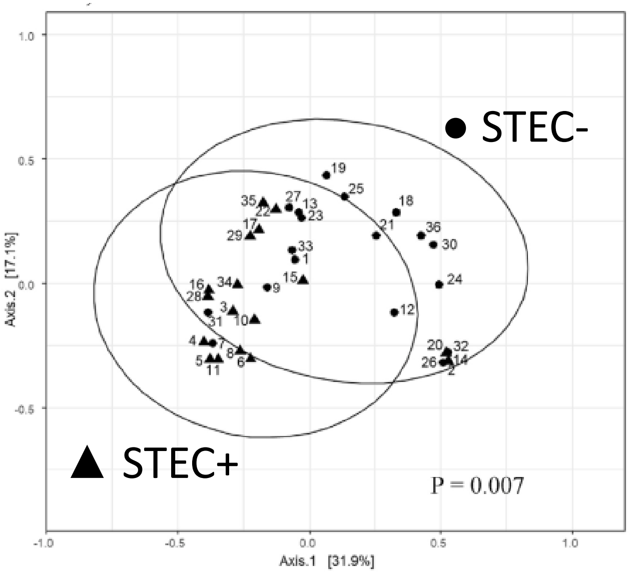
3/ Microbial transfers and safety management

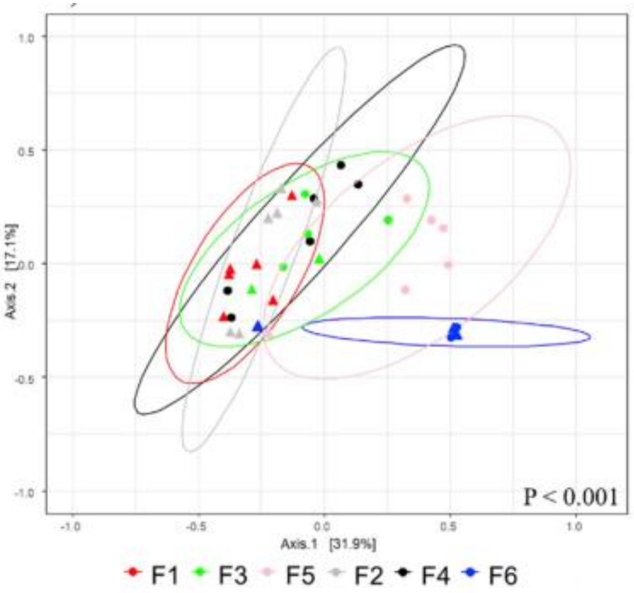
Impact of the milk microbiota on the growth of pathogens in cheese

Robustness and efficacy of an inhibitory consortium against *E. coli* O26:H11 in raw milk cheeses

Marie Frétin[a], Christophe Chassard[a], Céline Delbès[a], René Lavigne[a], Etienne Rifa[a], Sébastien Theil[a], Benoit Fernandez[b], Patrice Laforce[b], Cécile Callon[a,*]

[a] *Université Clermont Auvergne, INRAE, VetAgro Sup, UMR 545 Fromage, 20 Côte de Reyne, F-15000, Aurillac, France*
[b] *Lallemand Specialty Cultures SAS, 19 Rue des Briquetiers, 31 700, Blagnac, France*





Inoculation of *E. coli* O26:H11 (0.5 CFU/mL) in 36 raw milks from 6 farms

Comparison of bacterial communities in raw milk
→ Depending on the growth of *E. coli* O26:H11 in cheeses: high (▲ STEC+) or low (● STEC-)

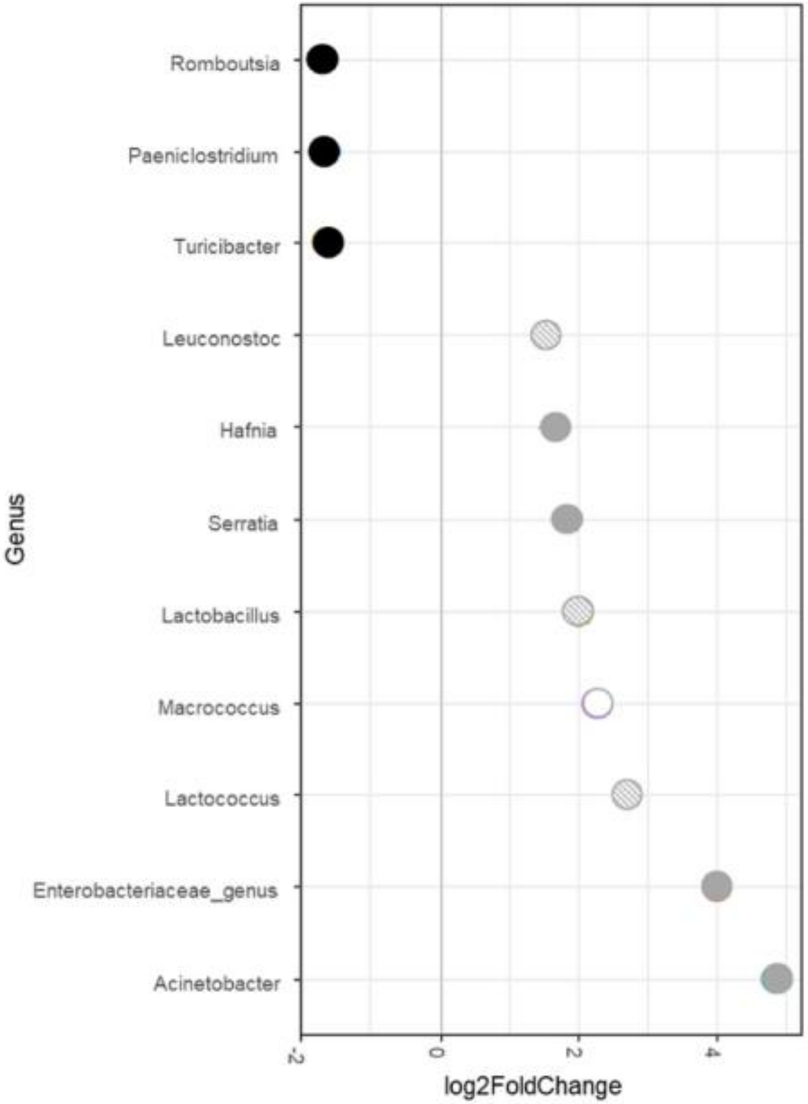
→ Depending on the farm

F1, F2	F4, F5
▲ STEC+	● STEC-
More intensive practices	

Bacterial genera in raw milk associated with low or high growth of *E. coli* O26:H11 in cheeses

Counts of *E. coli* O26:H11 in cheese

● > mediane (STEC+) ● < mediane (STEC-)



▨ Bactéries lactiques
○ Bactéries d'affinage
● Bactéries à Gram négatif

Genera associated with :

● the lowest growth of *E. coli* O26:H11 in cheeses:
Lactococcus, Lactobacillus and *Leuconostoc,*
Acinetobacter, Serratia and *Hafnia*
Macrococcus (ripening bacteria).

● the highest levels of *E. coli* O26:H11 in cheeses:
Romboutsia, Paeniclostridium, Turicibacter
= potential link with the intestinal microbiota

=> Marker of a greater fecal contamination and of the propensity of milk to allow STEC to grow in cheese

Multifactorial qualification of milk production systems in Saint-Nectaire PDO area for safety

- **Strategy:**
  - Compare farms with contrasting levels of safety of milk and cheese
  - Identify microbial, biotechnical and organizational indicators associated with the prevalence of pathogenic bacteria

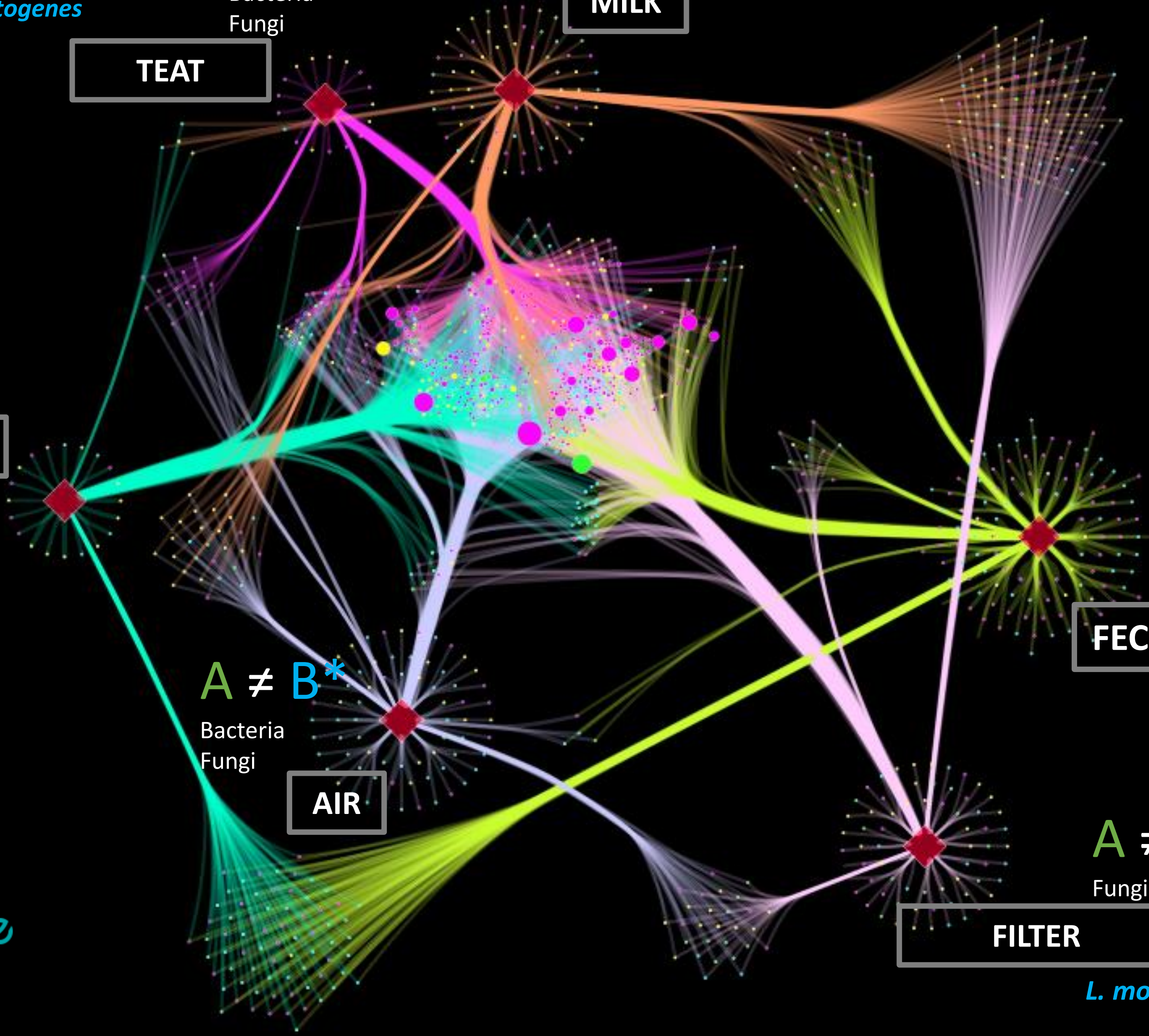
14 voluntary farmers

Among 198 farms
➔ 2011-2015 : 95 000 data

Class A
7 farms
No occurrence of *Listeria monocytogenes* nor *Salmonella*

Class B
7 farms
Recurrent *L. monocytogenes*
Sporadic *Salmonella*

New insight on the farm microbiota in winter



TEAT

L. monocytogenes

A ≠ B*

Bacteria
Fungi

MILK

Coag+ Staphylococci **

A ≠ B*

Fungi

BEDDING

A ≠ B*

Bacteria
Fungi

L. monocytogenes

FECES

A ≠ B*

Fungi

AIR

A ≠ B*

Bacteria
Fungi

FILTER

A ≠ B*

Fungi

L. monocytogenes

* p<0,05
** p<0,01

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Conclusions

- ❑ Farm management modifies the microbial balance in farm environments and milk

- ❑ Extensive, multidirectional sharing of microorganisms between farm environments and with milk

  - ➢ Relevance of systemic, transdisciplinary approaches at farm level

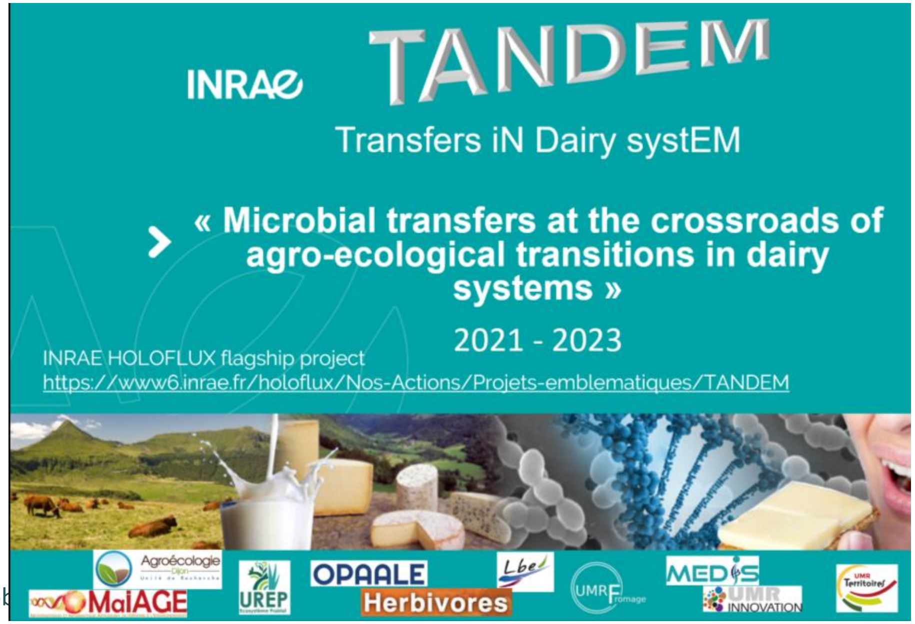
Perspectives

- ☐ Define the “One quality" of traditional cheeses (risks vs. health benefits, biodiversity, etc.).

- ☐ Understanding the role of microbiota in the adaptation of dairy systems to climate change?

➔ Which factors influence microbial diversity and microbial transfers ?

➔ To be explored under a wide range of dairy systems (farming practices, use of microbial inputs, etc.) and of geographical conditions.



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TANDEM

Transfers iN Dairy systEM

« Microbial transfers at the crossroads of agro-ecological transitions in dairy systems »

2021 - 2023

INRAE HOLOFLUX flagship project

https://www6.inrae.fr/holoflux/Nos-Actions/Projets-emblematiques/TANDEM

Thanks for your attention!

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