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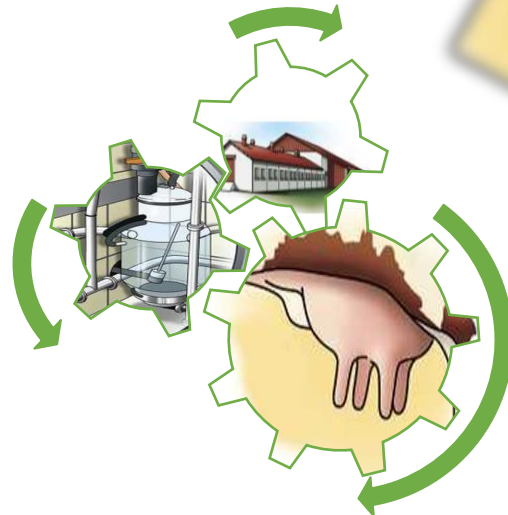
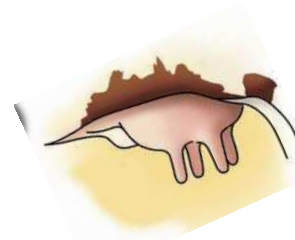
Autochthonous lactic acid bacteria from  
milk: why is it necessary to preserve it  
and how could it be possible?

Las bacterias ácido lácticas autóctonas  
de la leche: por qué y cómo pueden  
preservarse.





# How to protect microbial biodiversity of raw milk ?



INTERNATIONAL  
SCIENTIFIC CONFERENCE  
ON RAW MILK

*H.Tormo, 23 Octobre 2018*



# MILK MICROBIOTA : ACTORS OF TRADITIONNAL CHEESEMAKING

300 species of bacteria  
74 species of yeasts

Strains diversity  
Lake of knowledge

Microbiota of milk and cheese

Elimination of pathogenic microorganisms



*Tasting pleasure*

*Health*

*Protection against pathogenic  
microorganism*

Good sanitary conditions

***How to protect microbial biodiversity ?***



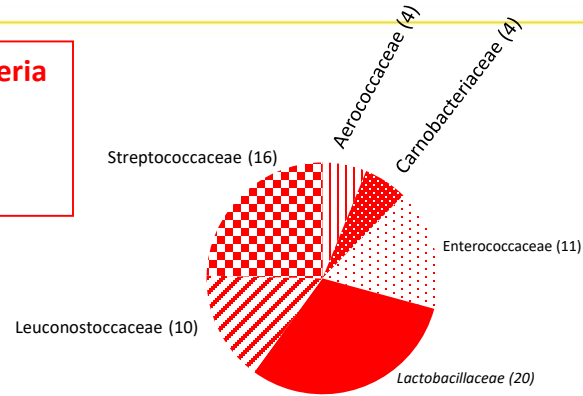
INTERNATIONAL  
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ON RAW MILK



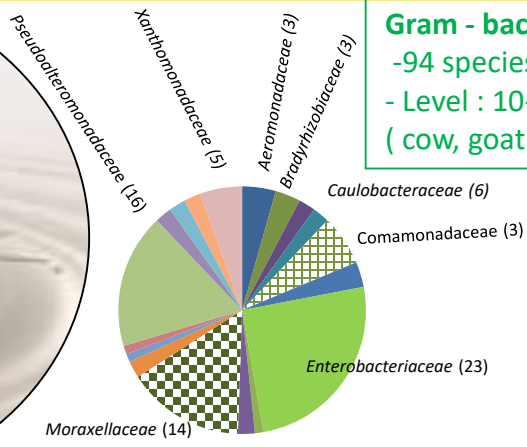
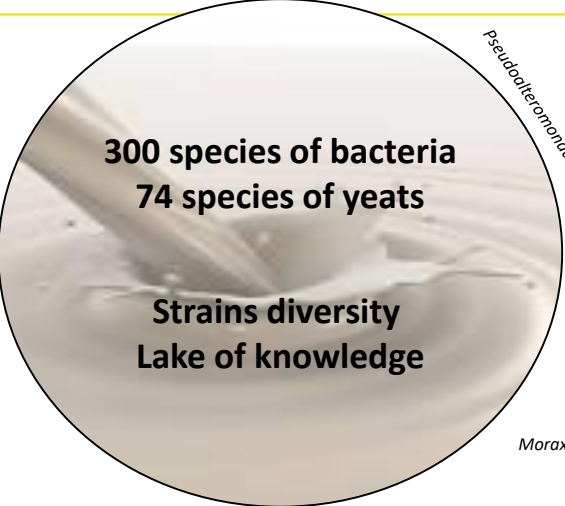


# MILK MICROBIOTA

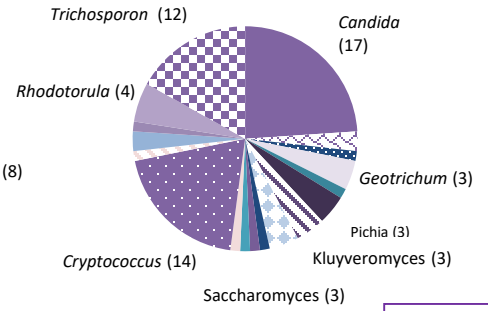
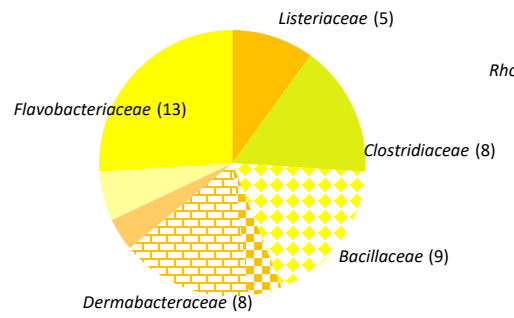
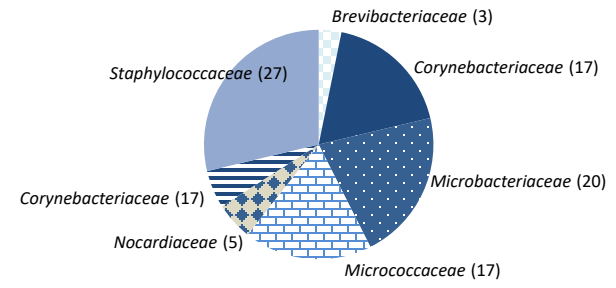
**Lactic acid bacteria**  
 - 65 species  
 - Level : 10-100 cfu/ml



**Gram - bacteria**  
 - 94 species  
 - Level : 10-1000 cfu/ml ( cow, goat)



( ) number of specie/ genera



**Ripening bacteria**  
 - 94 species  
 - Niveaux : 100-1000 cfu/ml ( cow, goat)

**Milk microbiota= 40 species of microorganisms**

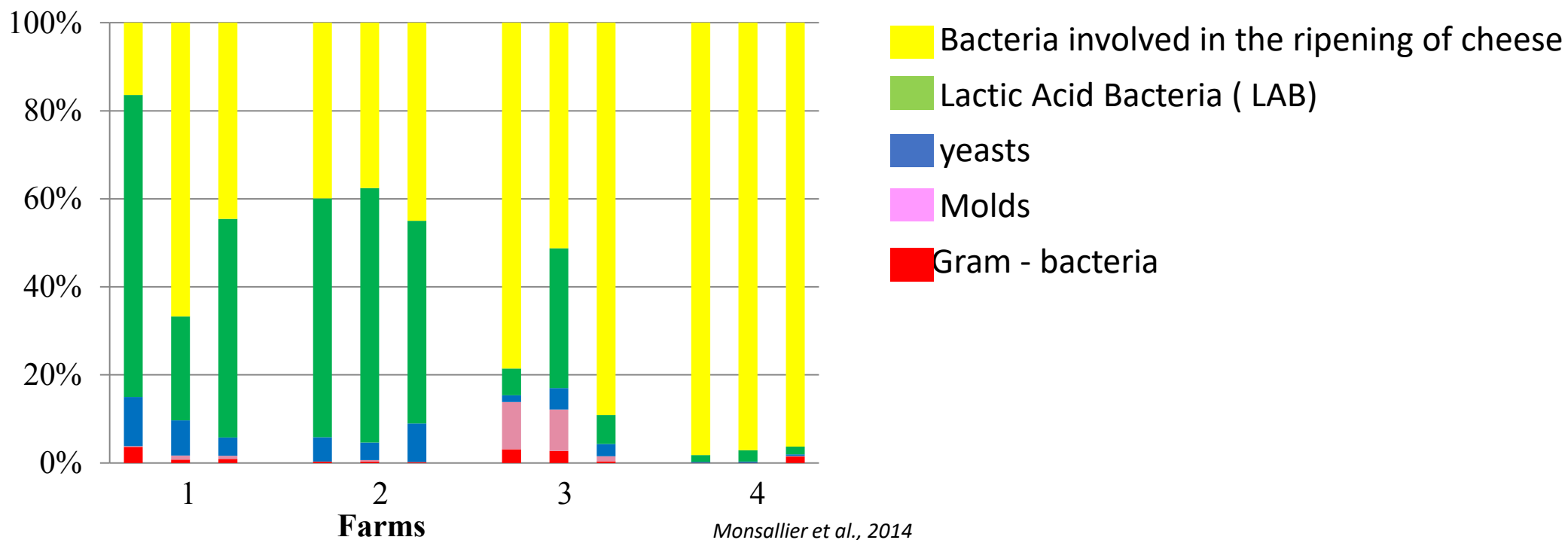
**yeasts**  
 - 71 species  
 - Level :10-100 cfu/ml ( cow milk)

**Each milk from farm has its own microbial balance and diversity**





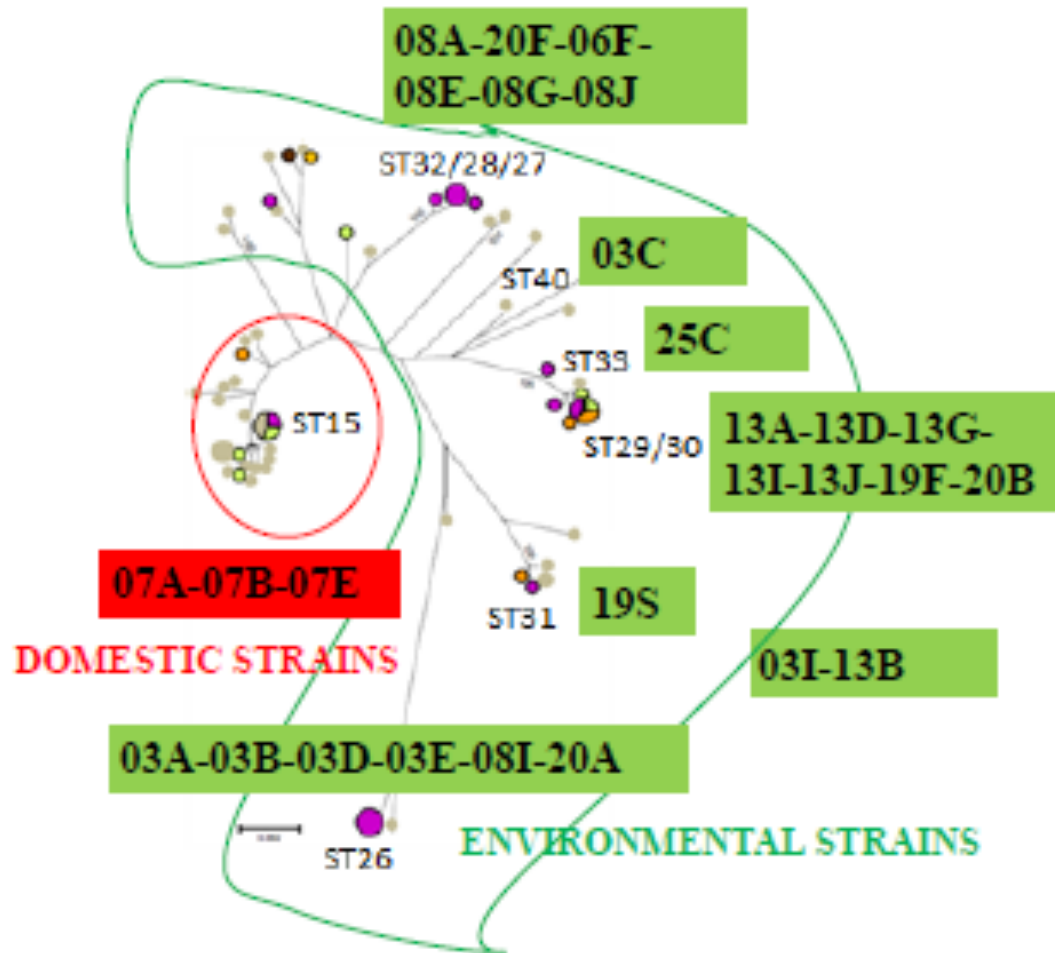
## SPECIFIC MICROBIAL BALANCE OF MILK FROM 4 FARMS



- The microbial balance differ from farms
- Stability of microbial balance (~ 2-3 months, same season).

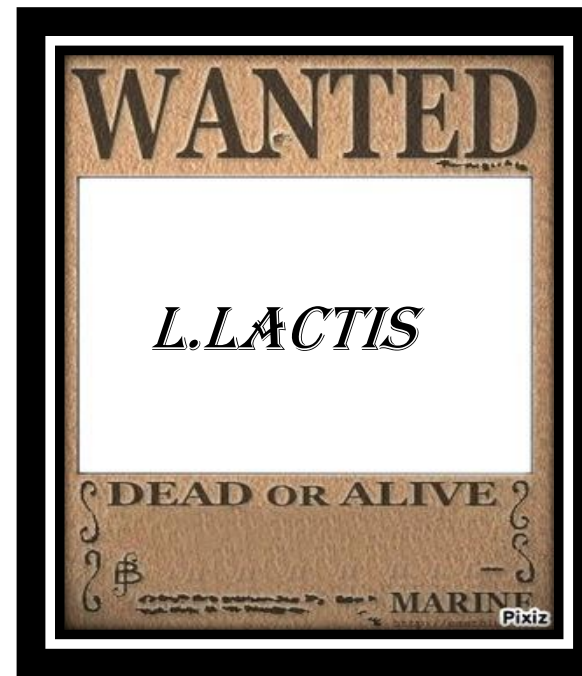


# COMPARISON OF DNA SEQUENCE OF *L. lactis* STRAINS ISOLATED FROM GOAT MILK FARMS



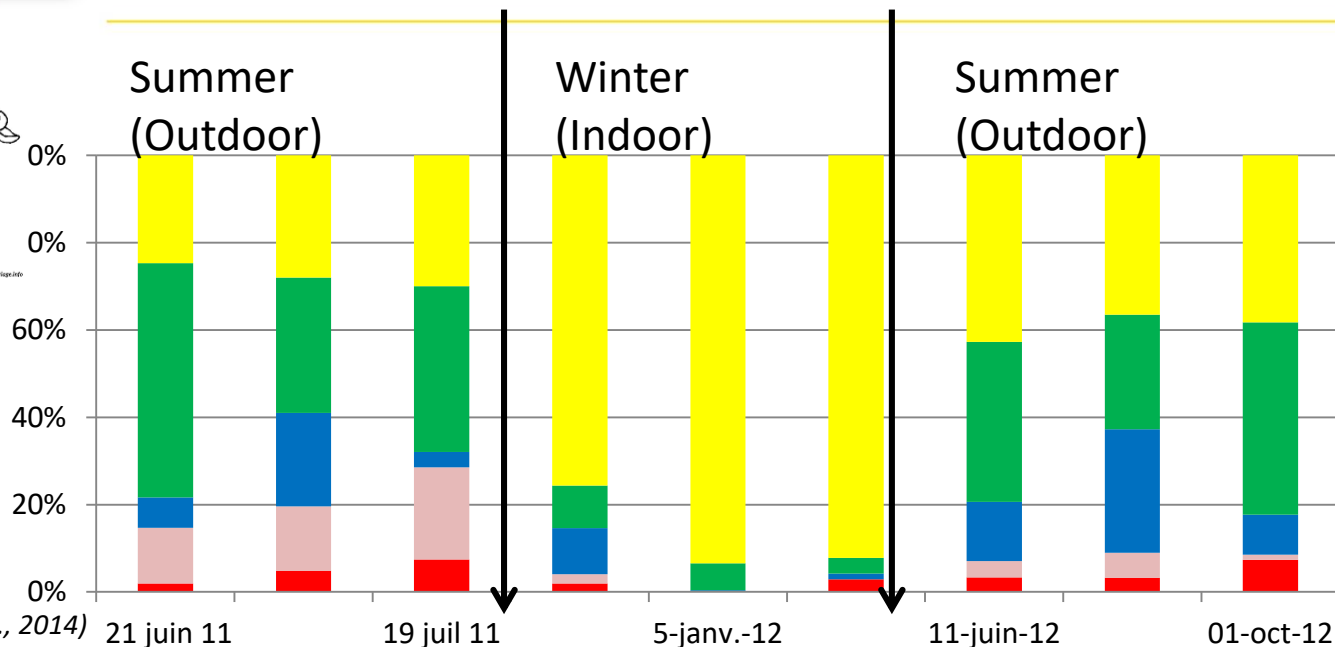
- ❖ Great diversity of *L.lactis lactis* strains
- ❖ ST MLST similar to environmental samples
- ❖ Technological and healthy interests of strains

( Caillaud et al., 2019 ; Couderc et al., 2019)



Picture 1 : Phylogenetic tree from concatenated sequences of the six loci of MLST [3] Passerini et al., 2010

# EVOLUTION OF MICROBIAL GROUPS IN MILK DURING THE LACTATION – INDOOR/OUTDOOR \* LACTATION



## Outdoor (May)

+++ Soil associated :

*Pseudomonas, Acinetobacter  
Lactococcus  
Tumebacillus*

Doyle et al., 2017

## Indoor ( February)

+++ hot- gut associated :

*Eremococcus, Ruminococcus,  
Prevotella, uncultured  
Corynebacteriales bacterium,  
and Ruminococcaceae Incertae  
Sedis.*

Winter

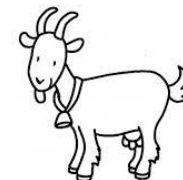
Majority of Staphylococcus and Actinobacteria

(Tormo et al., 2011)

*L.lactis* and pseudomonas ( Tormo et al., 2011, Callon et al., 2007)

Enterobacter

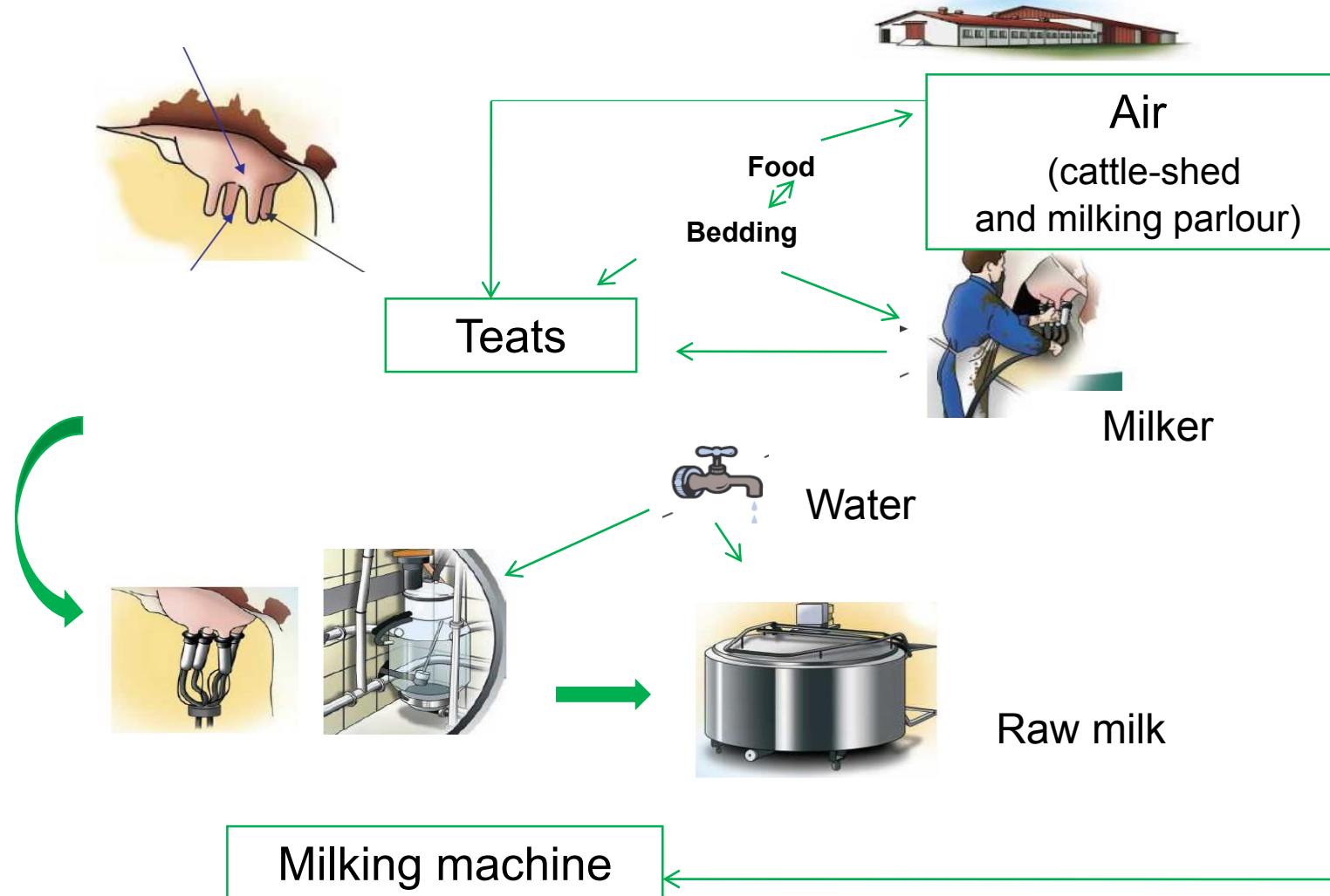
Automn



Staphylococcus,  
Chryseobacterium indologenes,  
Acinetobacter baumannii, Corynebacteria  
and yeasts

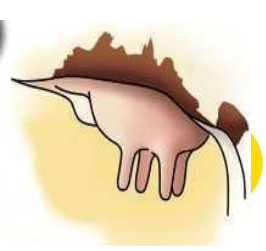
(Callon et al., 2007)

# MICROBIAL TRANSFER FROM ENVIRONMENTAL SOURCES TO RAW MILK

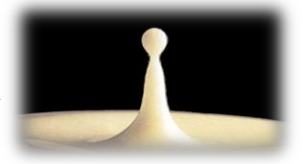


(Bouton *et al.*, 2010)

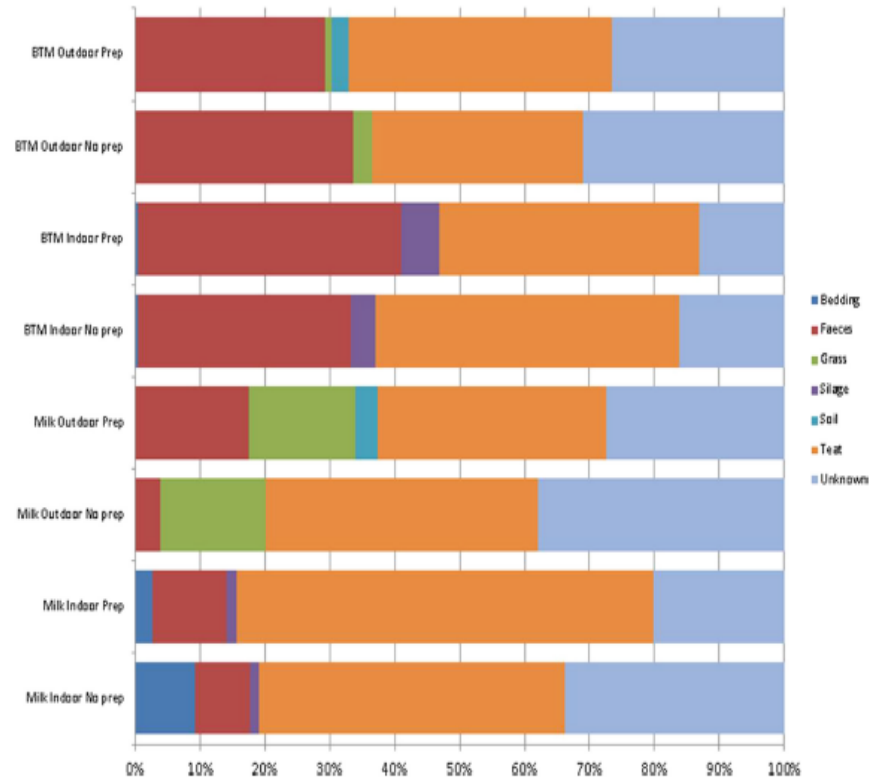




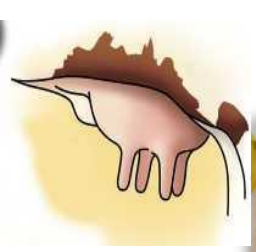
# FLOW OF MICROORGANISM FROM ENVIRONMENT TO MILK



- ❖ Bacteria from teat, feces and grass could be a major sources of bacteria in milk
- ❖ The seasonal housing influence the microbiote of the milk

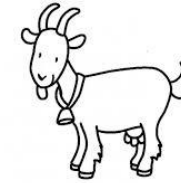
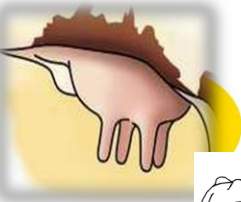


Percentages of inferred sources of contamination in BTM and individual milk samples



# THE TEAT RESERVOIR : ITS COMPOSITION AND THE PRACTICES THAT INFLUENCE IT

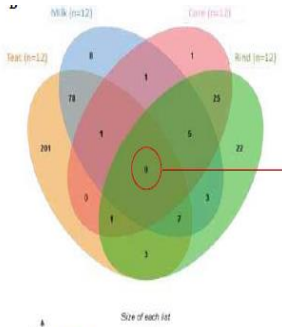
# FLOW OF MICROORGANISM : TEAT SKIN to MILK and CHEESE



	Teat skin (n=12)	Raw milk (n=12)	Cheese core (n=12)	Cheese rind (n=12)
<i>Firmicutes</i>	56.5%	41.3%	99.8%	73.3%
<i>Actinobacteria</i>	39.5%	30.7%	0.02%	26.5%
<i>Proteobacteria</i>	4.02%	27.1%	0.10%	0.18%

(Fretin, 2017)

Commun taxa between  
teat/milk/cheese (Fretin, 2017)



**Lactobacillus casei/paracasei**  
**Staphylococcus haemolyticus**  
 /petrasii  
**staphylococcus**  
 Lactococcus lactis  
 Lactococcus spp  
 Brevibacterium linens  
 Macroccoccus caseolyticus  
 Streptococcus porcinus/uberis  
 Streptococcus dysgalactiae

Commun taxa between teat/milk  
(Bouton et al., 2007 ; Vacheyrou et al., 2011)

**Lactobacillus paracasei/plantarum/paraplantarum**  
**Staphylococcus**  
 Propionibacterium spp.  
 Corynebacterium  
 Bacillus  
 Acinetobacter  
 Pseudomonas



**G<sup>+</sup> C<sup>+</sup>**

**LAB & NSLAB: Enterococci,**  
*Lactobacillus plantarum/casei.,*  
*L. lactis*  
*Leuconostocs spp.*  
*Pediococcus spp.*

(Tormo et al., 2011)



Cow teat skin serves as a potential source of microorganisms found in milk and in raw milk cheeses  
 Teat could be a source of LAB & NSLAB and other bacteria involved in the ripening of cheese



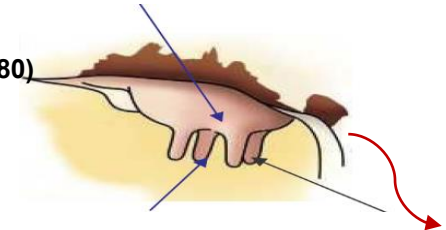
# FLOW OF MICROORGANISM : FEED/BEDDING FROM TEAT AND MILK

GRASS  
HAY  
FLOUR

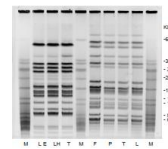


TBC (0.65) – Gram – (0.72)  
Yeats (0.78) – LAB (0.65)  
**Bacteria involved in the ripening of cheeses (0.80)**

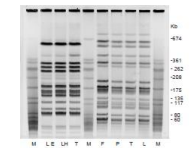
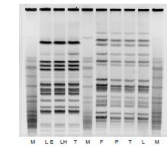
Gram – (0.73)  
LAB (0.61)  
**Bacteria involved in the ripening of cheeses(0.85)**



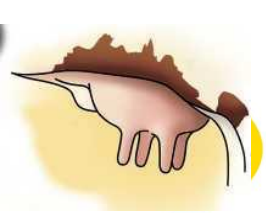
*Lactobacillus plantarum/paraplantarum*



*Lactobacillus plantarum/paraplantarum*



*Correlations between levels of microorganism in straw, rubber mat, and teat skin (Floracq, 2014) (n=30) and flow of lactobacillus (Bouton et al., 2007)*



# WHAT VARIATION EXISTS IN THE MICROORGANISMS FOUND ON TEAT SURFACES ?

## 1. SEASON

Winter vs Summer

- ❖ 4 times higher MAB and Halophilic microorganisms <sup>1</sup>
- ❖ Difference in major species <sup>2</sup>
- ❖ Great difference in Animal's Environment

## 2. Bedding

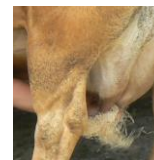
- ❖ Nature of bedding : abundance : Saw dust > straw > rubber mat <sup>1,3</sup>
- ❖ Density  
Heterofermentative lactobacilli higher in tied-up barns vs free stall barns <sup>5</sup>
- ❖ Soiled bedding : higher levels of enterocci <sup>6</sup>

## 3. Milking practices

Predipping : Reduction of level ( Gram- )

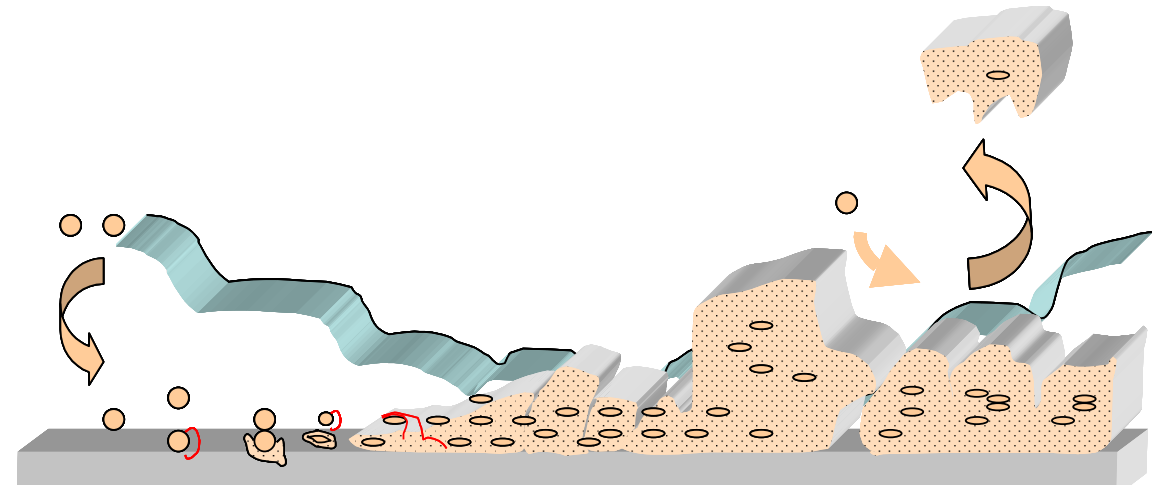
HIGHER LEVEL : wooden wool or udder towels > postdipping > Predipping

Predipping : lower frequency of *Lactococcus lactis*, *Microbacterium*, *Staphylococcus saprophyticus* et *Acinetobacter*



# THE MILKING MACHINE (MM)

## ITS COMPOSITION AND THE PRACTICES THAT INFLUENCE IT

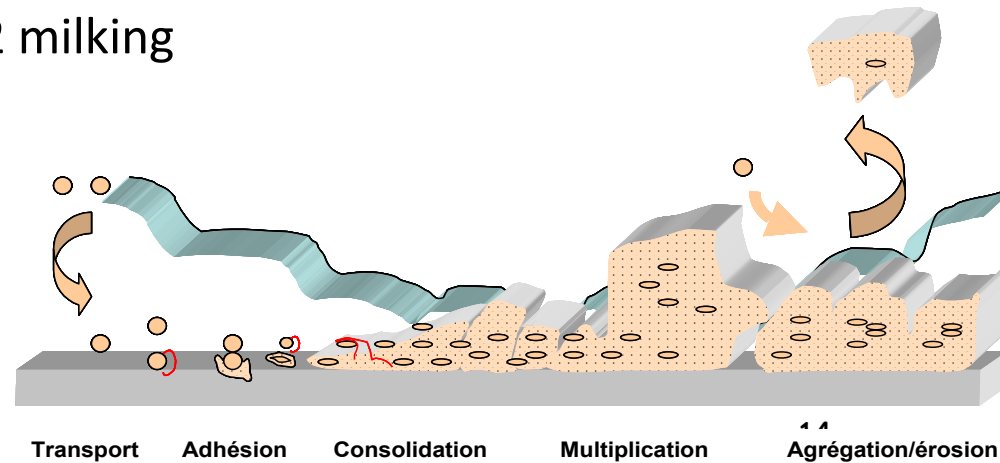


# THE MILKING MACHINE

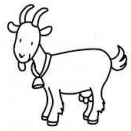
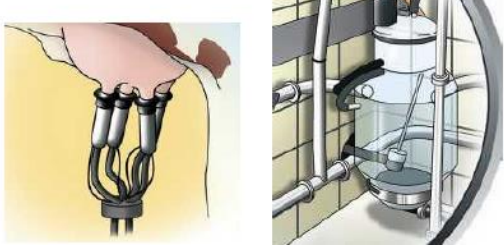


Milking machine : IN FAVOUR OF BIOFILM

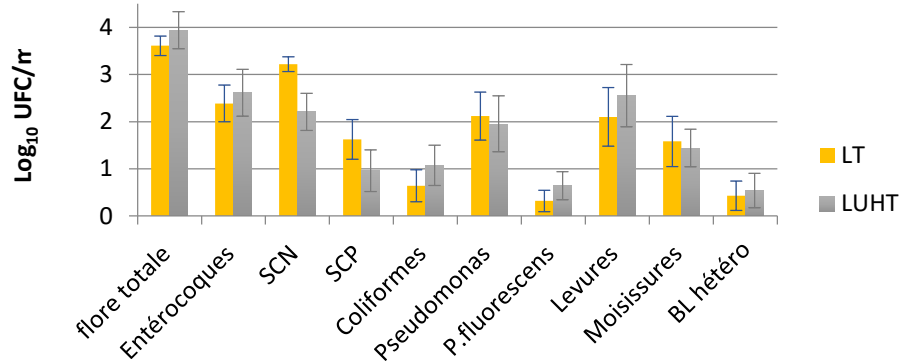
- Maintain of microorganismes beetween 2 milking
- Expulsion in milk



# MICROORGANISM THAT MAY BE MOBILISED IN THE MM



Level of microorganisms in milk (raw goat milk and UHT milk through MM) UHT (n=12 farms)

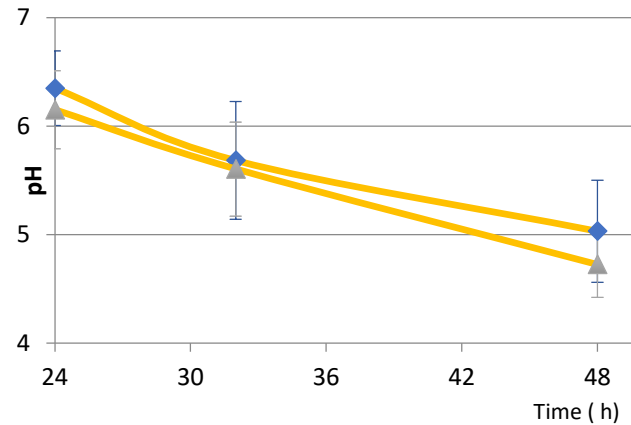


❖ Correlation between level of pseudomonas spp. UHT milk vs raw goat Milk <sup>1</sup>



- ❖ Low levels of microorganism in the MM <sup>3</sup>
- ❖ Coliforms and Pseudomonas = Microorganism of interest in cheesemaking <sup>4</sup>
- ❖ *L.lactis* <sup>3</sup>

Acidification profile of raw goat milk and UHT milk (n=12 farms)



**Identification of LAB in UHT milk**

*L.Lactis*  
 Enterococcus spp  
 Heterofermentative facultative lactobacillus

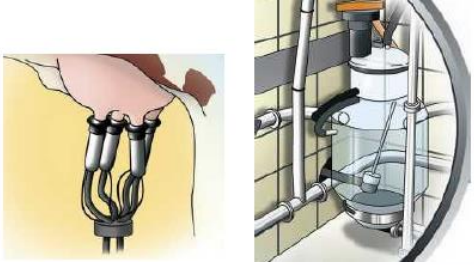
- ❖ Correlation : pH<sub>24, 48h</sub> UHT milk vs raw goat Milk <sup>1</sup>
- ❖ MM: 70% of the contribution of the pH<sub>24, 48h</sub> of the milk vs 30% by the teats <sup>2</sup>

**Milk may be inoculated by the MM : Pseudomonas and LAB**

<sup>1</sup> F. Darthia, 2013 ; <sup>2</sup> Laithier et al, 2012; <sup>3</sup> Michel et al., 2006 ; <sup>4</sup> Mallet, 2012







# DESIGN AND CLEANING PRACTICES OF THE MM

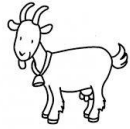
		Cleaning practices	General trend on levels of microorganism
		Defects in maintenance of the MM or in cleaning procedures (cow)	↗
		T of cleaning > recommendations (cow/ goat)	↘
		Defect in rinse (cow/ goat)	↘
		Rotation in each milking alkaline/acid products (cow/ goat)	Trend on increase of pseudomonas and coliforms
		No Chlorine	↗ No modification ( Cow) (Goat)

Materials/design	General trend on levels of microorganims
High level of pipelines, bends ... (cow, goat)	(pseudomonas) ↗
High proportion of stainless steel glass... (goat)	↘
Silicone liners (goat)	↘

Laithier et al., 2012; Mallet, 2012; Michel et al., 2006; Microflore du lait cru » 2011, collective Work of RMT fromages de terroirs ; Tormo et al., 2011

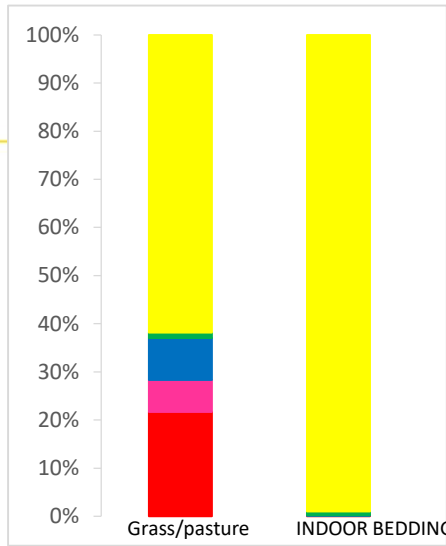
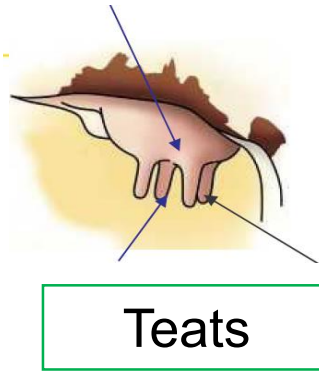
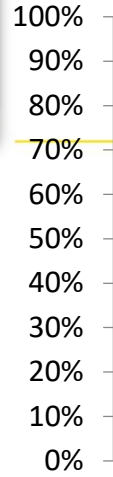


# CLEANING PRACTICES OF THE MM & PSEUDOMONAS



Milking practices	Low level of Pseudomonas ( 1,9-2,4 log CFU /ml) n=23	High level of pseudomonas ( 2,7-4 log à UFC /ml) n=11
Cleaning of the milking parlour	Dry	Wet
T water end of cleaning	+	- (<36 °C)
Age of MM	-	+
Cleaning of the MM	+++	
Overdosage of cleaning products	-	+
Residue of milk in the MM	-	+

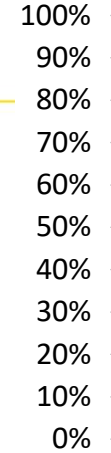
# MICROBIAL TRANSFER



**Air**  
(cattle-shed and milking parlour)



**Milker**

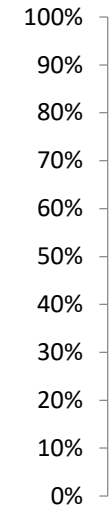
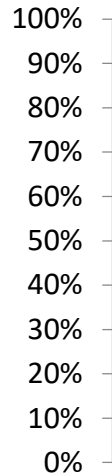


**Water**



**Raw milk**

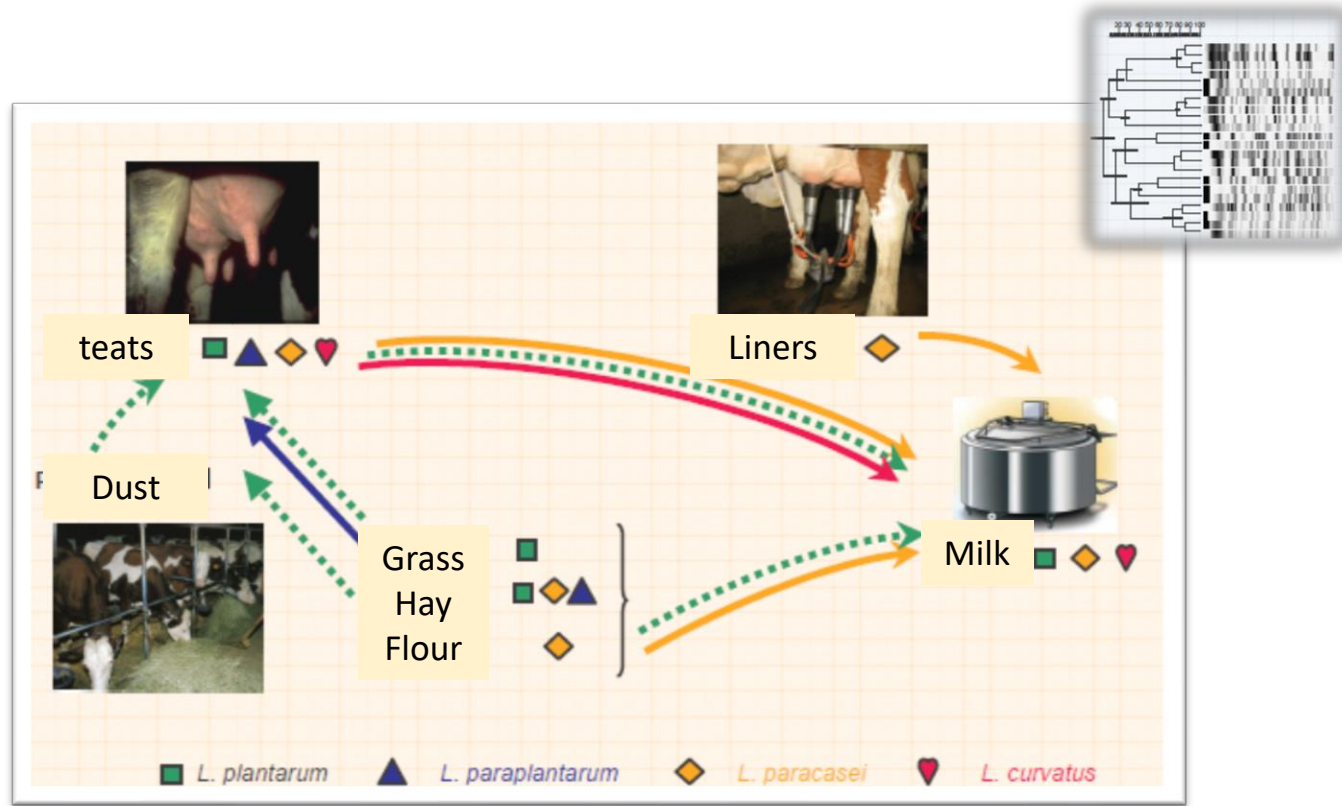
**Milking machine**





# Possible implication of LAB from environmental sources in milk contamination?

## The case of Lactobacillus strains in cow milk



Bouton, 2007



# Possible implication of LAB from environmental sources in milk contamination?

## The case in goat milk



*L. lactis lactis*

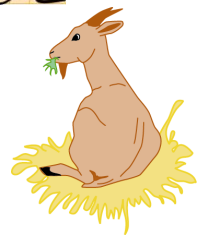


*L. lactis*  
*L. plantarum, casei*  
*L. citreum*  
*P. pentosaceus*



**G+C+**  
*L. Lactis*  
*L. plantarum, casei*  
*L. citreum*

Air in the milking parlour



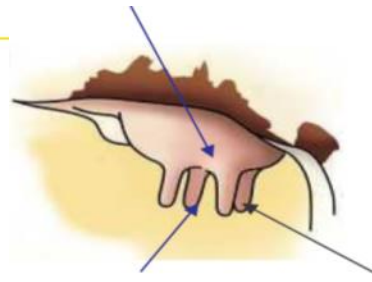
Bedding with straw





# PRESERVATION OF MICROBIAL COMMUNITY

Practices of teats care less intensive



Bedding and milking parlour

Bedding with straw or grass  
No separation



Teats



Rubber Liners



Milking Machine



No long storage (4-6°C)

Raw milk

For a microbial biodiversity of milk



Simplicity of the conception of the MM  
Practice of cleaning less intensive (T, product)  
Rinse correctly



# Preservation /improvement of 'good' microbial communities of milk in the cheesemaking

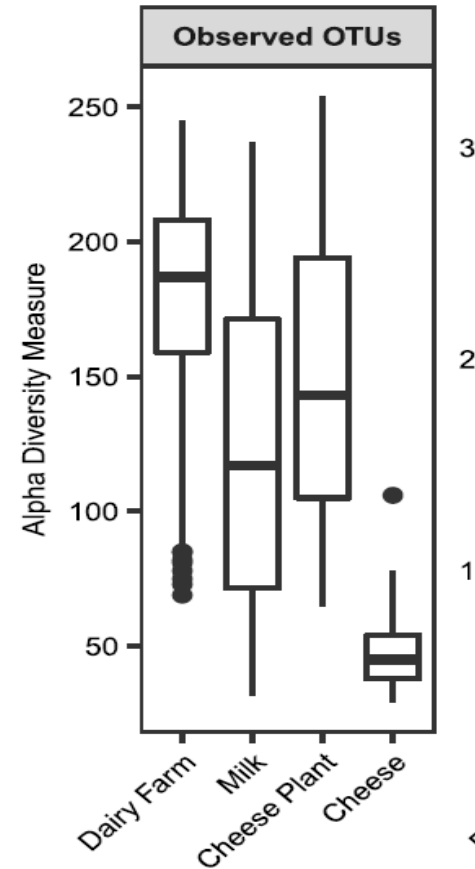
From dairy farm to cheese

Number of OTU decreases

But :

- Common genera
- Facility resident strains in rind cheese surfaces

(Falardeau, 2019; Fretin, 2017)



Source : Falardeau, 2019



# Preservation /improvement of 'good' microbial communities of milk in the cheesemaking

No long time storage of milk

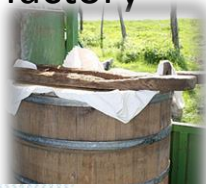


Backslopping /Undefined starters



The wooden tools

THE VAT  
A starter factory



The wooden tools

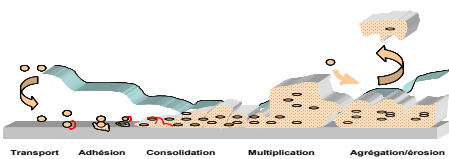
THE SHELVES  
A Ripening factory



Choice of Technology parameters

**TIME !!!!**

'Reasoned' cleaning procedure



Terracotta





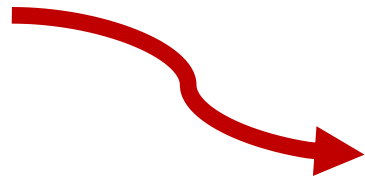


# Raw milk and traditional products made with raw milk

## What's the challenge ?

**Traditional systems are a source of unvaluable microbial diversity (typicity, health ) and crucial for rural and developing countries**

You have to continue to explore these microbial systems and the flow  
From animal housing to cheese



Milking practices, housing animals  
undefined starters/back slopping  
Traditionnal tools for cheese making

Even more we have now – Omic Tools !!!!

and Together its better !  
Confrontation of approach  
More Data/more 'strong'  
Shared culture

