Effect of post dipping treatment after milking on teat and milk microbiota

Isabelle Verdier-Metz, Marie-Chloé Boudon, F. Fournier, Philippe Pradel,
Françoise Monsallier, Marie-Christine M.-C. Montel

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Unité de Recherches Fromagères

Aurillac
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UEMA INRA Marcenat

Effect of post dipping treatment after milking on teat and milk microbiota

réseau
Fromages de Terroirs

FLORAcQ

Isebelle Verdier-Metz, Marie-Chloe Boudon, Florence Fournier, Philippe

In the context of a French program « FlorAcQ

Get high quality raw milk cheese by favoring microbial groups having an interest fas early as possible from the milk production

Sociological approach

Technical approach

- ➤ Actor behaviour face new strategy in milk production taking into account microbial biodiversity

- ➤ Diagnostic tool of breeding practices
- ➤ Microbial analysis
- ➤ **Get knowledge about sources of microbes and effect of practices on milk quality**

Education

Share common knowledge ...bial ecology

Objectif

Get knowledge on the effect of <u>post dipping treatment</u> on teat and milk microbiota

Why to study teat treatment?

❑ The milk microbiota is obviously strongly influenced by the overall management system of the farm. which makes it **difficult to identify the influence of a single practice**

But

❑ Teat surface is **a potential** direct source of micro-organisms for farm milk (Vacheyroux et al., 2011; Verdier-Metz et al. 2011)

❑ Teat microbial count depend on age of animal and cleaning practices (Monsallier et al., 2012)

❑ Teat care and washing. as well as disinfection of the milking equipment are of primary importance for milk microbiota (Julien et al.. 2008; Mallet et al.. 2012; Michel et al.. 2001; Tormo et al.. 2011;Verdier-Metz et al.. 2009).

Protection of teat by post dipping treatments?

❑ Technicians and producers are very confident with post dipping treatment

❑ 8 different products for 14 farms in Cantal area

Lactic acid
7%

Lactic acid +
Salicylic acid 7%

Unknown 7%

Propane 2-ol
7%

Chlorexidine 7%

Iodine products
44%

Cosmetic 7%

Essential oils +
fruity acids
14%
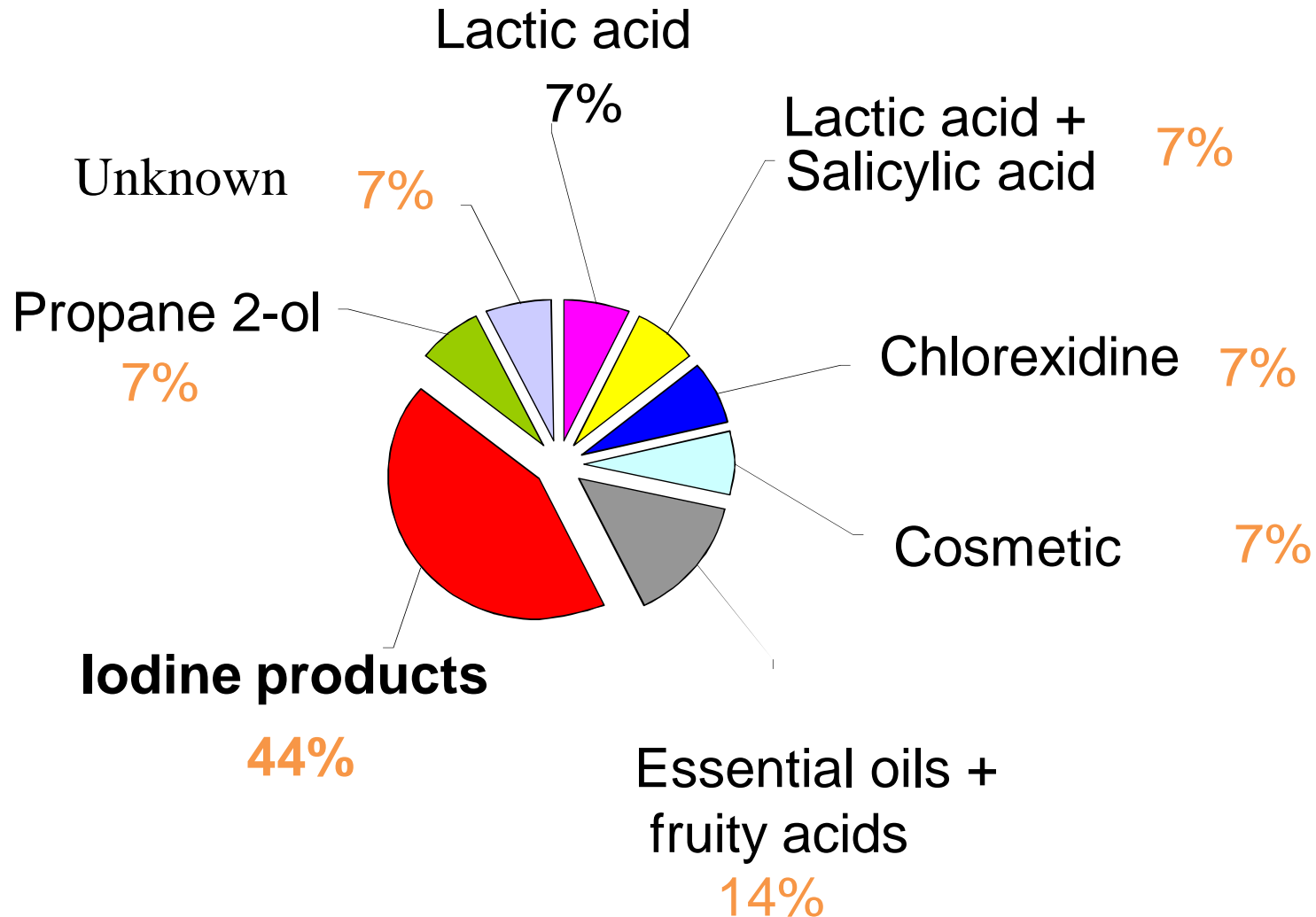
Study at INRA experimental farm UEMA Marcenat in Massif central to compare the microbiota of teat and milk according to 3 different post dipping treatments :

1. **Iodinated product**
2. **Glycerol**
3. **No treatment**

Experimental Design in INRA farm

75 Dairy cows divided in 3 homogeneous groups:

- 10% primipareaous
- Same ratio of Montbeliarde and Holstein
- Same calving date
- Same feedings of animals but in the same group different feedings

I	**G**	**N**
Iodinated product (usual in this farm) →Disinfecting action	**85% glycerol product** →Regenerating power of lipid layer	→No product

Somatic cell count every week
Occurrence of mastitis
Milk and teat samplings through all lactation

2 experiments during two lactation years
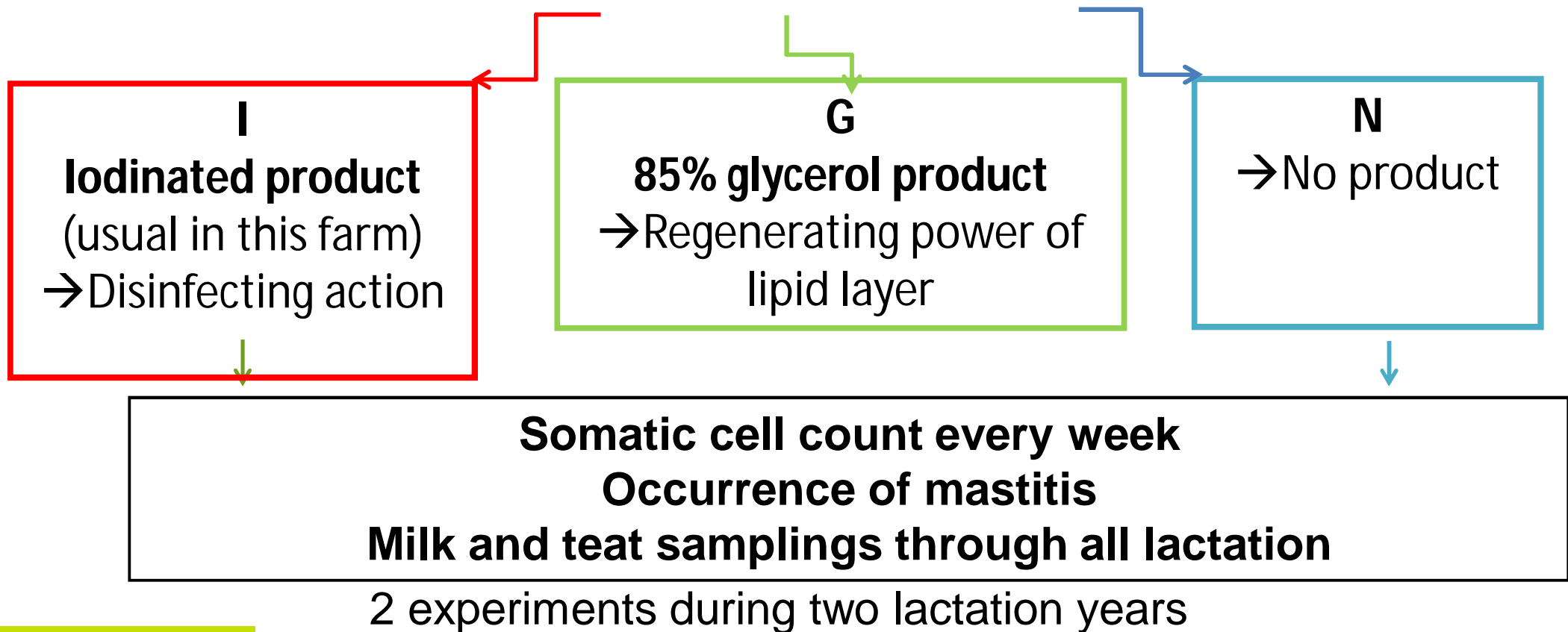
Sampling for microbial analysis

Sampling once a month throughout the whole lactation

At the surface of 4 teats BEFORE milking and teat preparation

Individiual wet wipe

Individual milk of each cow

For each lot, for milk and « teat surface juice » : mix at equal volume the individual samples

Microbial analyses

Gram + catalase +bacteria

Some named « Ripening bacteria »

CRBM*

Total microflora

PCA

Gram negative bacteria

PCA modified

Enumeration on culture media
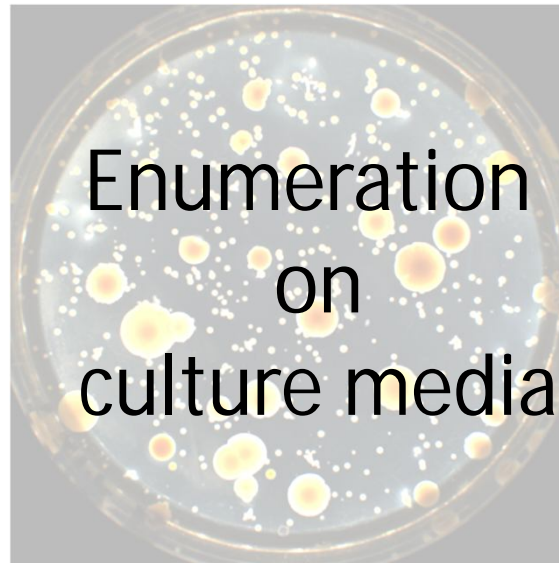
Lactic acid bacteria

MRS. FH. SB

Mesophilic acid lactic bacteria
Enterococcus
Heterofermentatif facultatif Lactobacillus

Yeasts and Moulds

OGA

Pick up colonies on CRBM, 2 PCA medium

382 teat isolates

380 milk isolates

Identification by 16S DNAr sequencing

* Selectivity of this medium studied in Floracq project

Cows with somatic cell count >400 000 during lactation period (2 year experiments)

Number of cows

Legend: ■ Iodine treatment ■ Glycerol treatment ■ NO treatment

	I	G	N
mastitis	6	7	5
Sub-clinical mastitis	6	4	1

month after lactation
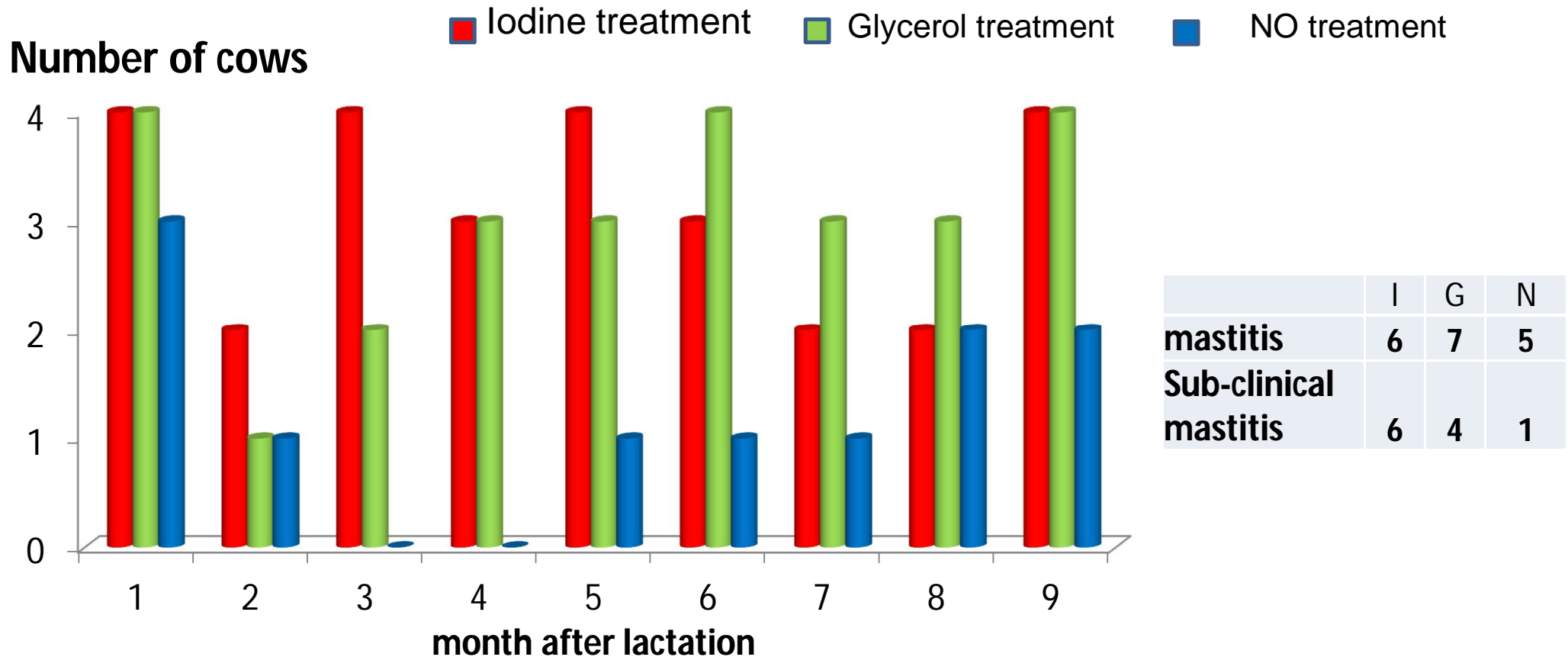
❑ Number of cows with **CSS**>400 000 similar in the three groups : maximum 4/group , less in group without treatment
❑ Trends to lower number of mastitis in group without treatment

Evolution of teat's microbial count during one lactation period

■ Iodine treatment ▲ Glycerol treatment ◆ NO treatment

Bacteria on PCA medium

Gram+ catalase+ bacteria

Gram negative bateria

Lactic acid bacteria
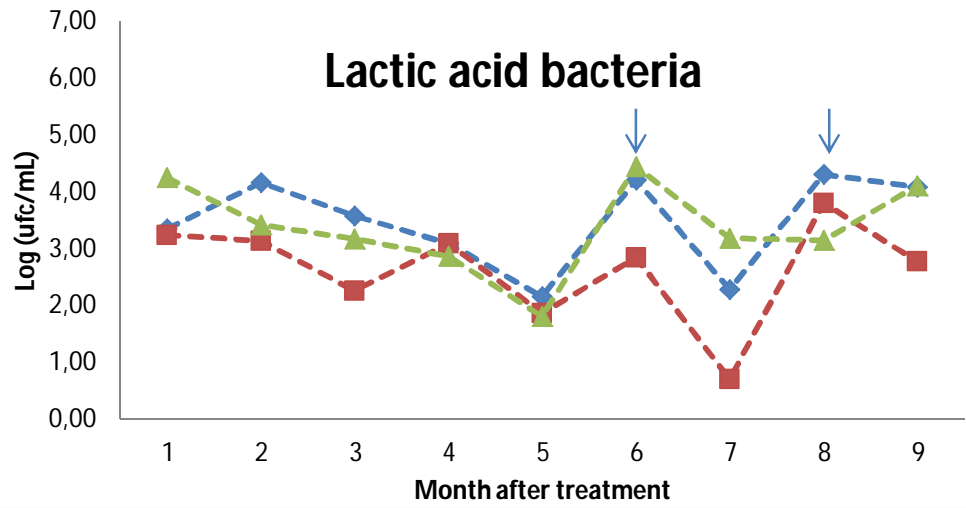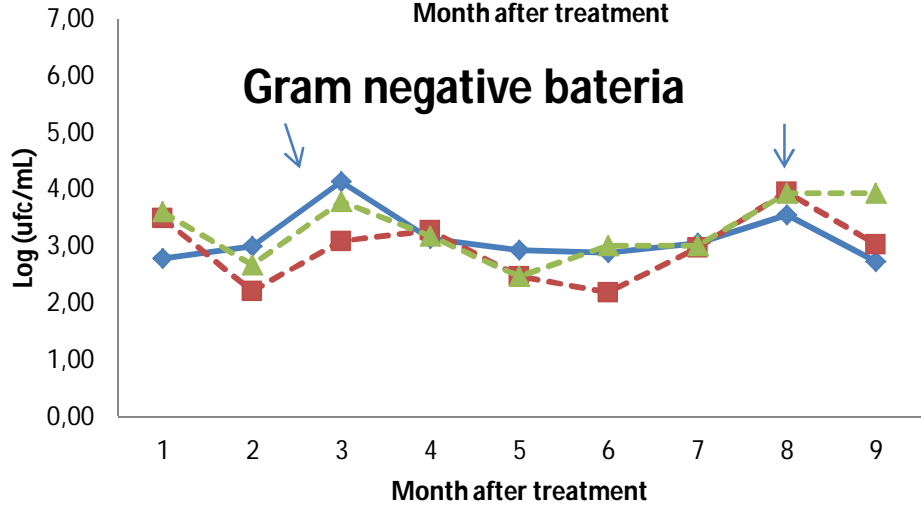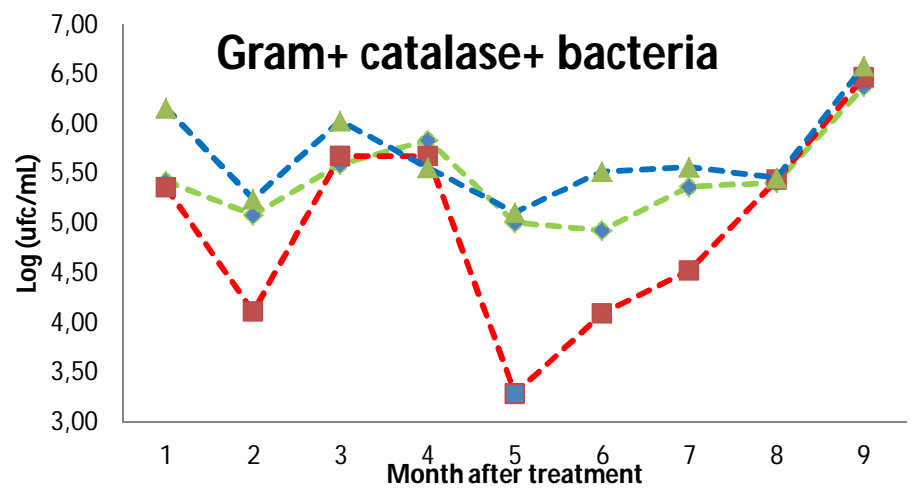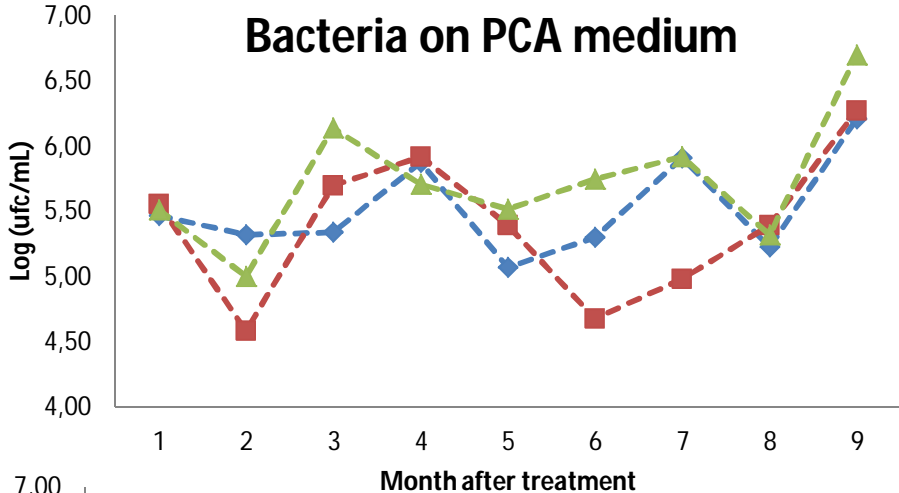
❑ Greater variation in G+C+ bacteria level and bacteria on PCA medium with iodine treatment
❑ Variation in level of Gram negative bacteria and LAB according to sampling

Comparison of « **teat** surface juice » microbial count between the 3 treatment's group

(cfu/ml juice)	Iodinated	Glycerol	Nothing	S	
Microflora on PCA	5.89	5.96	**6.27**	***	Δ<1Log
Gram+ catalase+ bacteria	5.34	5.49	**5.84**	**	
Gram negative bacteria	3.44	3.27	3.32	ns	
Lactic acid bacteria	3.70	3.91	3.83	ns	
Enterococcus	1.30	1.45	1.34	ns	
Lactobacillus	1.97	1.96	1.95	ns	
Moulds	1.96	2.08	2.09	ns	
Yeasts	1.91	1.74	2.03	ns	

- ❑ **Higher G+C+ bacteria (dominant population) count on teat without treatment than with iodinated product or glycerol treatment (not due to increase during treatment)**
- ❑ **Other microbial groups at lower level than G+C+ bacteria and similar whatever the treatment**

Evolution of milk microbial count during one lactation period

■ Iodine treatment ▲ Glycerol treatment ◆ NO treatment

Bacteria on PCA medium

Gram+ catalase+ bacteria

Gram negative bacteria

Lactic acid bacteria
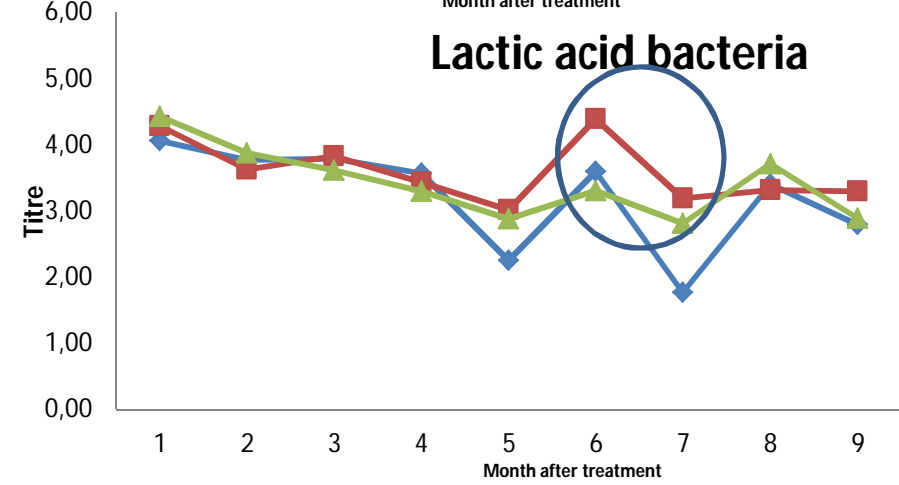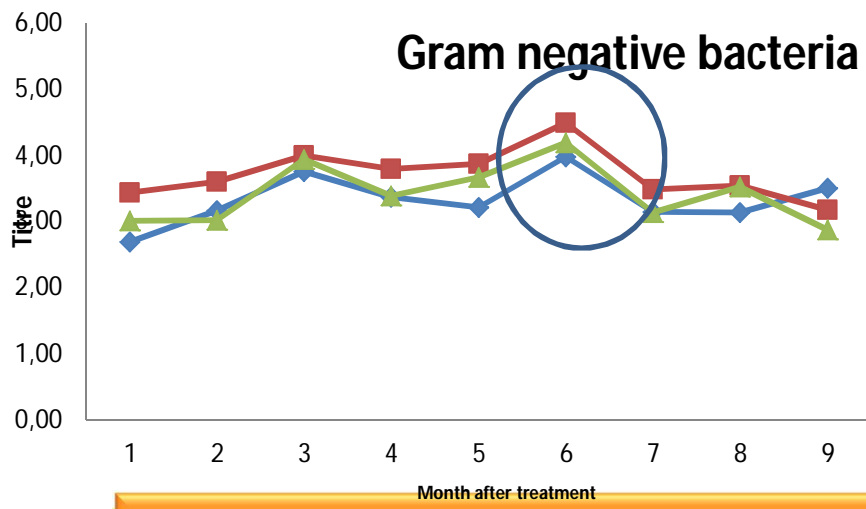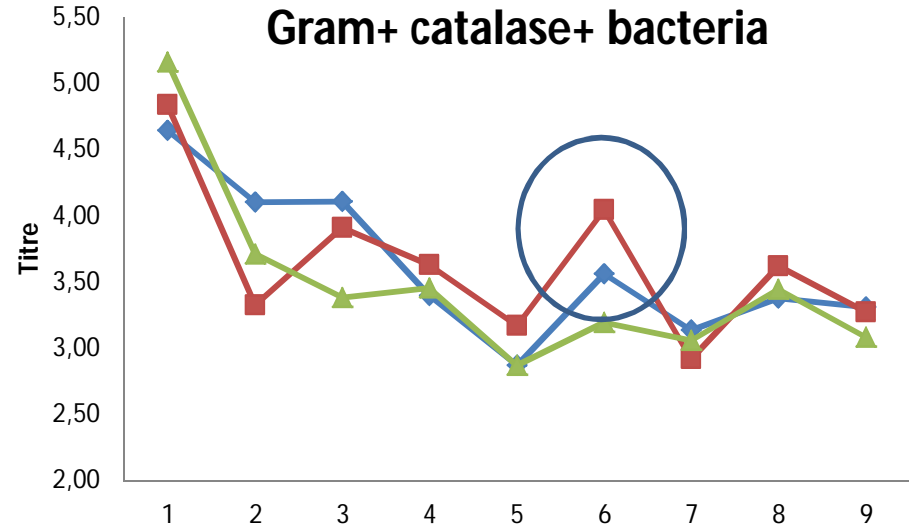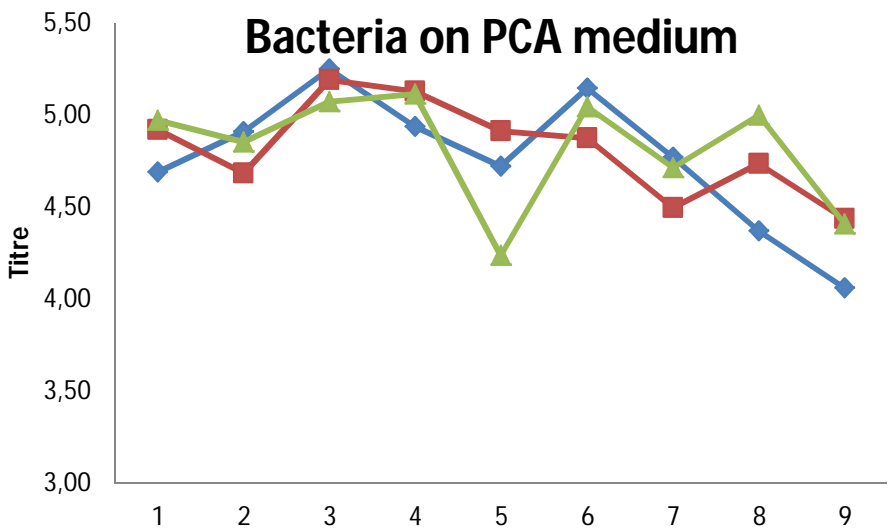
❑ More variation according to the date of sampling than to treatment :
  ❑ higher count of +C+ bacteria at sampling1

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Comparison of **milk** microbial count between the 3 treatment's group

	Iodinated	Glycerol	Nothing	S
Microflora flora	4.75	4.84	4.84	ns
Gram positive + catalase+ bacteria	3.47	3.53	3.53	ns
Gram negative bacteria	3.22	3.32	3.18	ns
Lactic acid bacteria	3.45	3.55	3.52	ns
Enterococcus	0.71	1.02	0.75	ns
Lactobacillus	2.95	3.05	2.96	ns
Moulds	2.83	2.89	2.80	ns
Yeasts	2.88	2.97	2.92	ns

❑ **No dominant microbial group : same proportion of G+C+ bacteria, LAB and Gram-**

❑ **For each microbial group, the level was similar for the 3 treatments**

Approach of microbial diversity by identification of isolat on different media

One lactation period

Diversity in Gram + catalase + bacteria in » teat surface juice » after 6 or 8 months of post dipping treatment

6 month (Spring)

Legend:
- Microbacterium
- Corynebacterium
- Clavibacter
- Kocuria
- Dietzia
- Agrococcus
- Cellulomonas
- Paenibacillus
- Micrococcus sp.
- Macroccus
- Arthrobacter
- Plantibacter
- Bacillus
- Staphylococcus

8 month (summer)

Legend:
- Micrococcus sp.
- Jeotgalicoccus
- Curtobacterium
- Dietzia
- Macroccus
- Agrococcus
- Plantibacter
- Kocuria
- Arthrobacter
- Staphylococcus
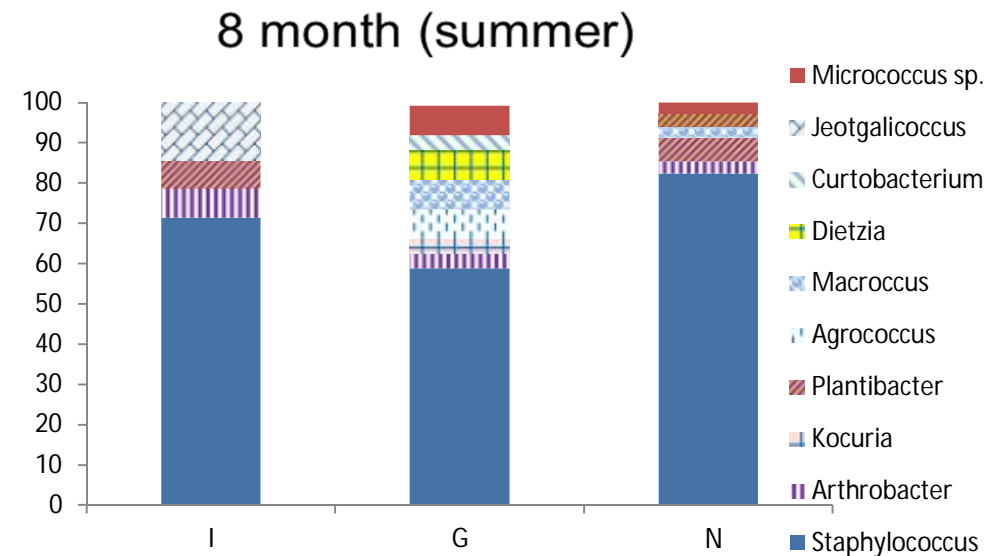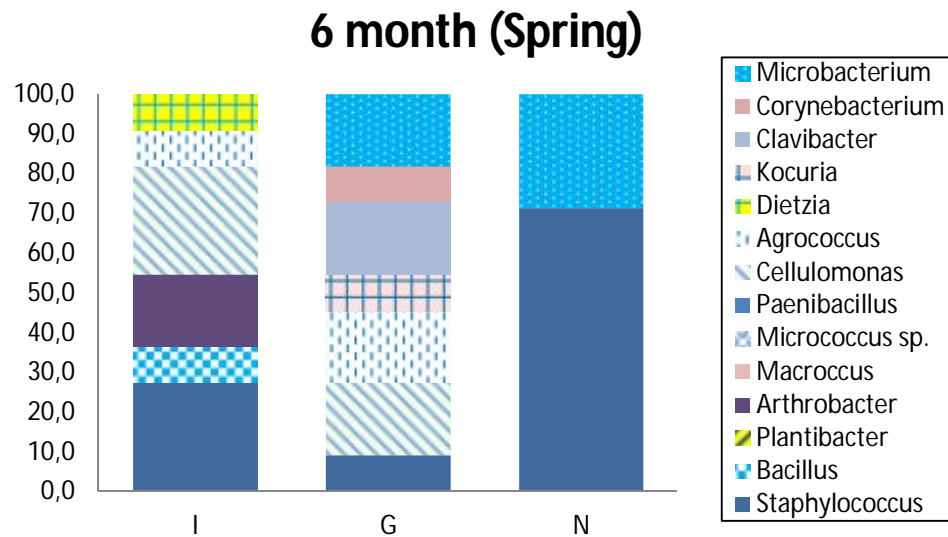
❑ Dominant Gram + Catalase+ genera were not the same at month 6 and 8 of the experiment (one lactation)

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Gram+ catalase+ bacteria in « teat surface juice » after 8 months of treatment (%isolats)

	I	G	N
Staphylococcus	**59**	**53**	**77**
Bacillus	**18**	**10**	**8**
Arthrobacter	**6**	**3**	**3**
Macroccus	0	7	3
Micrococcus sp.	0	7	3
Agrococcus	0	7	0
Dietzia	0	7	0
Curtobacterium	0	3	0
Kocuria	0	3	0
Jeotgalicoccus	**12**	0	0
Plantibacter	6	0	6

❑ Whatever the treatement *Staphylococcus, Bacillus, Arthrobacter* were the dominant Gram + catalase+ genera

❑ Higher diversity in « teat surface juice » with glycerol treatment

❑ *Jeotgaliococcus* one of the dominant genera with Iodinated products

Diversity in Gram +catalase + bacteria in milk after 6 or 8 months of post dipping treatment

6 month

8 month (Summer)

Legend (6 month):
- uncultured bacterial clone
- Kocuria
- Corynebacterium
- Brevibacterium
- Deinococcus
- Brachybacterium
- Microbacterium

Legend (8 month):
- Arthrobacter
- Rothia
- Brachybacterium
- unidentyfied
- Propioniciclava
- Rhodococcus
- Kocuria
- Microbacterium
- Staphylococcus
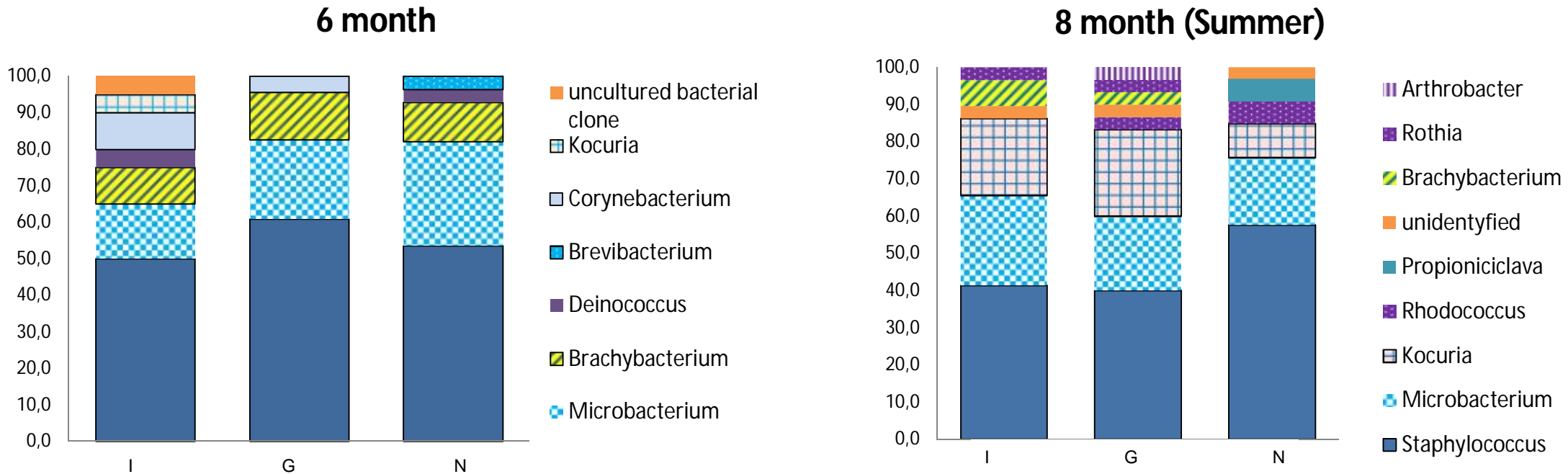
❑ More stability in the dominant population in milk than on « teat juice »
❑ Whatever the treatment *Staphylococcus*, *Microbacterium*, *Kocuria* (at month 8), *Brachybacterium, Corynebacterium* were in the dominant population in milk

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Gram+ catalase+ bacteria milk after 8 months of treatment (%isolats)

	I	G	N
Staphylococcus	41	40	58
Microbacterium	24	20	18
Kocuria	21	23	9
Rothia	3	3	3
Plantibacter	0	0	6
Propioniciclava	7	3	0
Brachybacterium	3	3	0
unidentyfied	0	3	6

- Whatever the treatment *Staphylococcus, Microbacteirum, Kocuria , Rothia* were the dominant Gram + Catalase+ genera
- Trend to the dominance of S*taphylococcu*s without treatment and less *Kocuria*
- Nearly same number of genus for the 3 treatments

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Comparison of « teat surface juice » and milk Gram+ catalase+ after 8 months of treatment (%isolats)

	Teat I	Teat G	Teat N		Milk I	Milk G	Milk N
Staphylococcus	59	53	76	Staphylococcus	41	40	58
Bacillus	18	10	8	Bacillus			
Microbacterium				Microbacterium	24	20	18
Kocuria		3		Kocuria	21	23	9
Arthrobacter	6	3	3	Arthrobacter	0	3	0
Rothia				Rothia	3	3	3
Macroccus	0	7	3	Macroccus			
Micrococcus sp.	0	7	3	Micrococcus sp.			
Agrococcus	0	7	0	Agrococcus			
Dietzia	0	7	0	Dietzia			
Curtobacterium	0	3	0	Curtobacterium			
Jeotgalicoccus	12	0	0	Jeotgalicoccus			
Plantibacter	6	0	5	Plantibacter	0	0	6
Propioniciclava				Propioniciclava	7	3	0
Brachybacterium				Brachybacterium	3	3	0
unidentyfied				unidentyfied	0	3	6

❑ *Bacillus, Jeotgalicoccus* and other genera found on teat with glycerol tretment not found in the corresponding milk

❑ *Microbacterium, Rhotia, Kocuria* dominant in milk not found in the dominant population on teat

Dominant Gram +catalase +bacteria on « teat surface juice » and milk whatever the treatment and sampling date

(frequency % among isolats	Teat	Milk	cheese*
Staphylococcus	**XXX**	**XXX**	X
Jeotgalicoccus *sp , psychrophilus, coquinae*	**XX**	X	?
Arthrobacter sp, bergeri, gandavensis	**XX**	X	X
Corynebacterium sp, casei	X	**X**	X
Microbacterium sp, lacticum, oxydans	X	**XX**	X
Micrococcus sp.	X	(X)	X
Kocuria rhizophila, carniphila	(X)	**XX**	X
Brachybacterium	(X)	**X**	X
Curtobacterium flaccumfaciens	(X)	(X)	X
Plantibacter	(X)	(X)	?
Brevibacterium	(X)	(X)	X

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Are genera detected on teat present in milk?

	Teat	Milk present study	Milk *	cheese*
Agrococcus	X	?	?	X
Bacillus	X	?	X	X
Dietzia	X	?	X	?
Macrococcus	(X)	?	?	X
Dezemsia	(X)	?	?	?
Trichococcus	(X)	?	X	?
Salinicoccus	(X)	?	X	?
Clavibacter	(X)	?	X	?
Exiguobacterium	(X)	?	X	?
Cellulomonas	(X)	?	?	?
Citrococcus	(X)	?	?	?
Planococcus	(X)	?	?	?

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Staphylococcus diversity in « teat surface juice » and milk

	Teat	Milk	Cheese (Interest)
S. aureus	X	X*	**No**
S. devriesei	**X**	**X**	**?**
S. hominis		**X***	**?**
S. haemolyticus	**X**	**X***	**?**
S. saprophyticus /S.xylosus	*	**X***	yes
S. sciuri subsp. carnaticus	*	**X***	X
S. sp.	X	**X***	?
S. succinus	*	**X***	X
S. pasteuri	*	**X**	X
S. vitulinus	*	**X***	yes
S. equorum	X	**X***	yes

- ❑ Important to quantify *Staphylococcus* at species level to determine if they are really useful ripening bacteria
- ❑ Undesirable *Staphylococcus* in milk, not dominant on teat surface juice

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* Found from literature data

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Dominant Gram negative bacteria genera whatever the treatment and sampling date (%isolats)

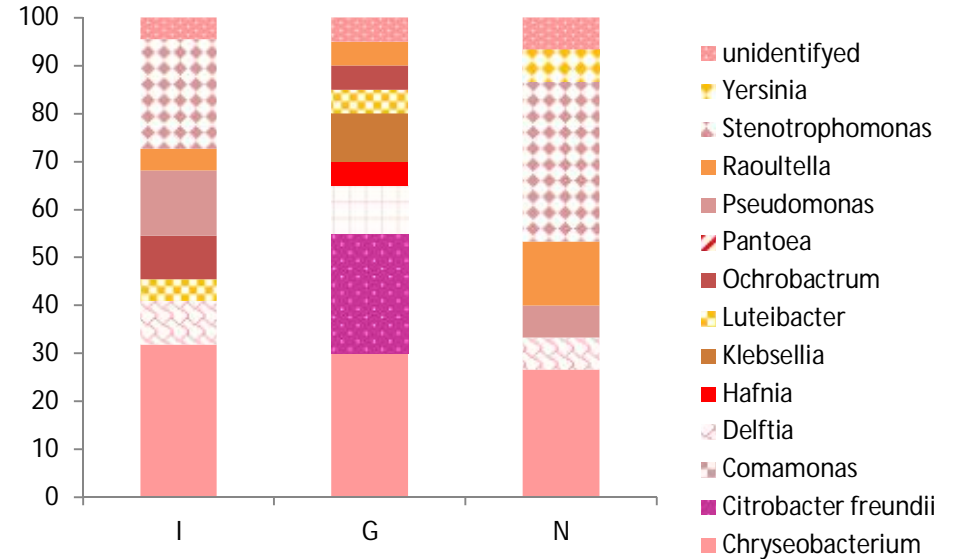|	Teat	Milk		Teat	Milk
Stenotrophomonas*	**17**	**20**	**_Chryseobacterium_**	*	**27***
Pseudomonas*	**17**	**15**	_Luteibacter_		6
Pantoea*	21	3	_Pseudomonas_	*	3*
Enterobacter*	8	2	_Rahnella_	*	3
Aminobacter	4		_Raoultella_		3*
Erwinia	4		_Serratia_	*	2*
Escherichia	13	*	_Yersinia_	*	2*
Acinetobacter	17	*	_Citrobacter_		4*
			Delftia		3
			Hafnia		3*
Found from literature data			_Klebsellia_		2*
			Ochrobactrum		3

❑ More Gram negative genera diversity in milk than in teat surface juice
❑ Some genera found in milk may have other origin than teats
❑ For teat surface juice, there was no difference according to treatment

Gram negative population in milk

Month 6

Month 8

Legend (Month 6):
- unidentifyed
- Yersinia
- Stenotrophomonas
- Raoultella
- Pseudomonas
- Pantoea
- Ochrobactrum
- Luteibacter
- Klebsellia
- Hafnia
- Delftia
- Comamonas
- Chryseobacterium

Legend (Month 8):
- unidentifyed
- Yersinia
- Stenotrophomonas
- Raoultella
- Pseudomonas
- Pantoea
- Ochrobactrum
- Luteibacter
- Klebsellia
- Hafnia
- Delftia
- Comamonas
- Citrobacter freundii
- Chryseobacterium
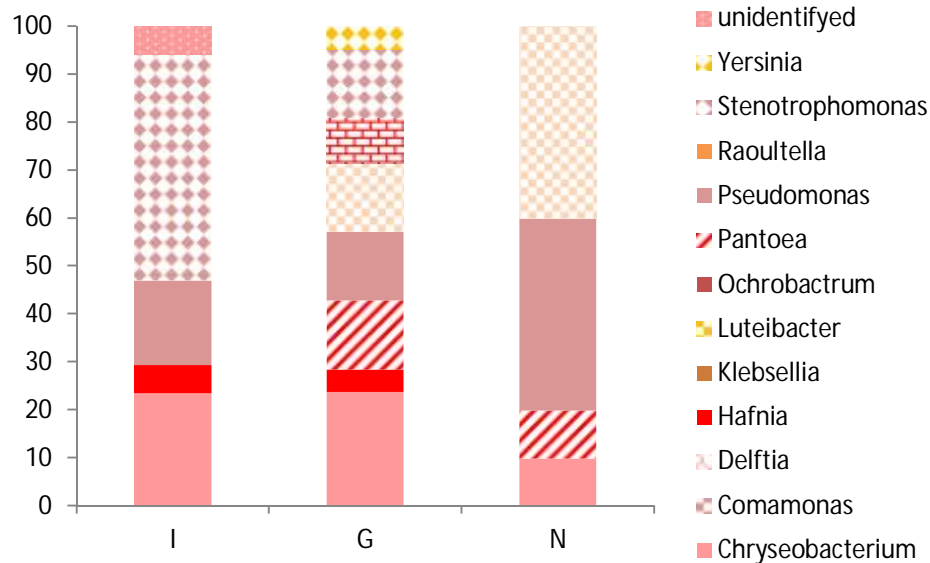
❑ Whatever the treatment, dominance of *Chryseobacterium*
❑ Occurrence of Gram negative species varied more according to sampling period than to treatment

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Frequency of lactic acid bacteria (%isolats)

	Teat	Milk
Aerococcus	**62**	**16**
Enterococcus	22	**19**
*Lactobacillus **casei**, brevis*	6	**34**
Lactococcus	2	**25**
Leuconostoc	4	4
Streptococcus	2	2
Pediococcus	1	0
Paenibacillus	2	

❑ On teat whatever the treatment dominance of ***Aerococcus*** and ***Enterococcus***
❑ In milk: dominance of ***Lactobacillus,*** *Lactococcus, Enterococcus*

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Pay attention to :

- ❑ Teat sampling with wipe :
  - ○ Is it the same effciency to recover micro-organisms than the milking machine?

- ❑ Experimental design in experimental farm with limited number of cow
  - ○ Need to check the results at a large scale but experiment difficult to settle

- ❑ Great diversity of Gram positive catalase positive bacteria in teat juice and change according to the sampling date
  - ○ Difficult to compare the G+C+ bacteria profil according to treatment

- ❑ Stability of G+C+ genera profil in milk but variability in Gram negatif bacteria

- ❑ Microbial profil dependant on microbial analysis performed
  - ○ Need to combine counting on media and identification of isolates
  - ○ Analysis of teat and milk microbiota by highthroughput sequencing is in progress

Preliminary inputs

❑ In the herd studied, **<u>no dramatic effect</u>** of post dipping treatment on SSC, mastitis occurrence, count in different microbial group on teat and especially in milk, no awful bacterial balance without treatment

❑ <u>No spectacular positive effect </u>of post dipping treatment to increase level of bacteria having a technological interest (lactic acid bacteria, ripening bacteria)and to modify significantly the bacterial balance in milk

    o Glycerol treatment may be interesting to have more diversity in G+C+ bacteria

    o No post dipping treatment may favor *Staphylococcus*

❑ *Staphylococcus* quantification at species level (desirable and undesirable ones) should be better considered

Questions for further studies

❑ Is it important to act on the microbiota of the teat skin to increase microbial diversity in milk or cheese?
  - o Teat reservoir of Gram + catalase + bacteria (including ripening bacteria) but need to better know how micro-organisms transfer to milk or cheese environment =Need to track at strain level
  - o but some bacterial species dominant on teat not dominant in milk. Will they then express cheese and become dominant?

❑ Is it the best strategy to give an advantage to certain species at milk production in regard with the reduction of diversity in the core and rind of cheese?

❑ Is Claude Bernard right **"The microbe is nothing, the "terrain " (surrounding) is everything"**
  - o Are sensorial cheese qualities mainly govern by cheese making and ripening process ?

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Happy cow!!!

Thank you

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