



# Milk microbiota: physiological and health implication.

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# Development of NGS: Inventory of many microbiota



## Gut Microbiota

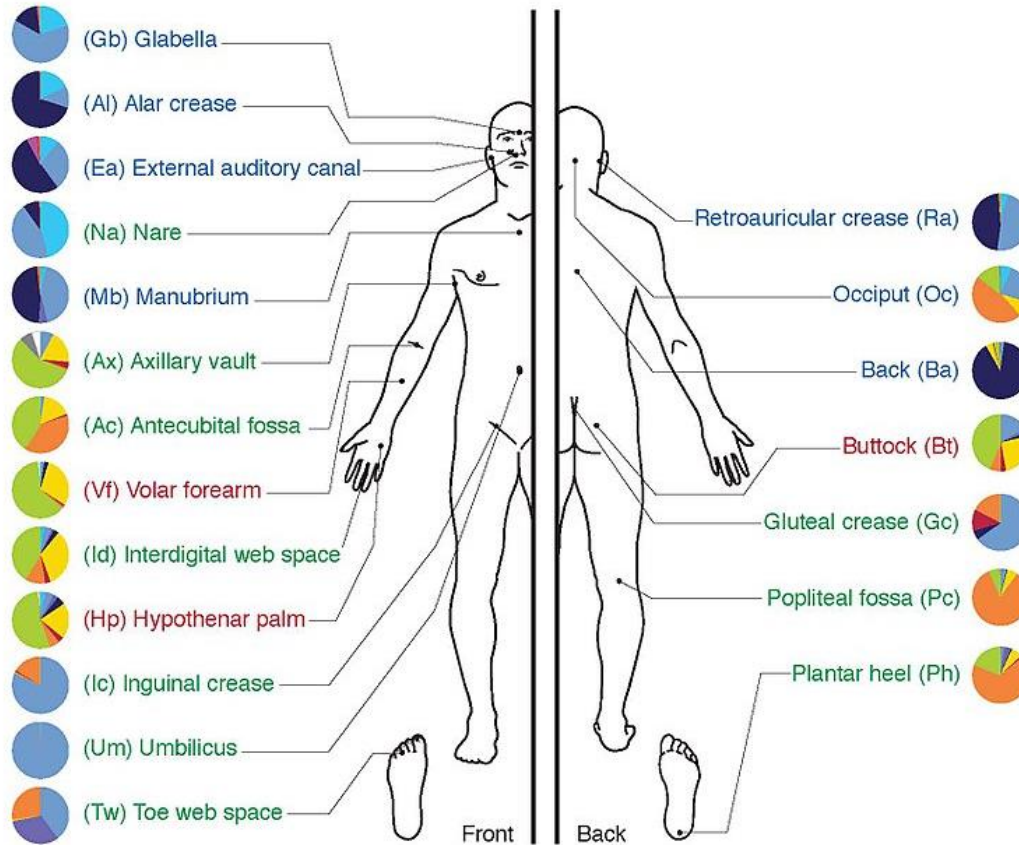


Pioneering studies  
Physiological functions, health benefits (when balanced)...  
Probiotics, Fecal transplantation...

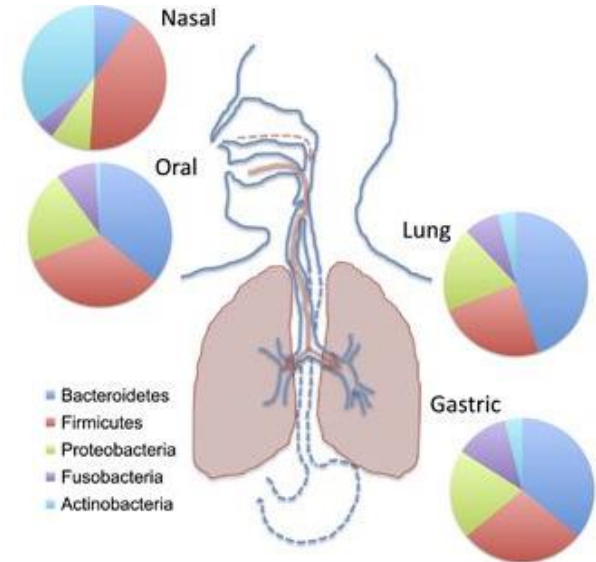
... Cf Talks by Jean-Marc, Philippe and Gwenaël.

# Microbes are everywhere!

## Skin microbiota



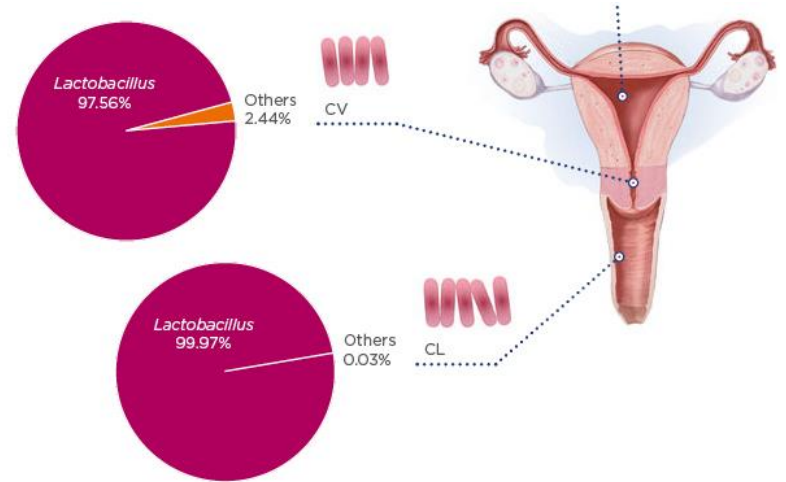
# Lung Microbiota



After Huffnagle & Lukacs, 2017. Mucosal Immunology Vol.10, p.299–306. doi:10.1038/mi.2016.108

Orally delivered probiotics are able to regulate immune responses in the respiratory system

# Vaginal Microbiota



The microbiota continuum along the female reproductive tract.

CL: lower third of vagina; CU: posterior fornix; CV: cervical mucus drawn from cervix; ET: endometrium/uterus; FL: fallopian tubes; PF: peritoneal fluid from the Pouch of Douglas.

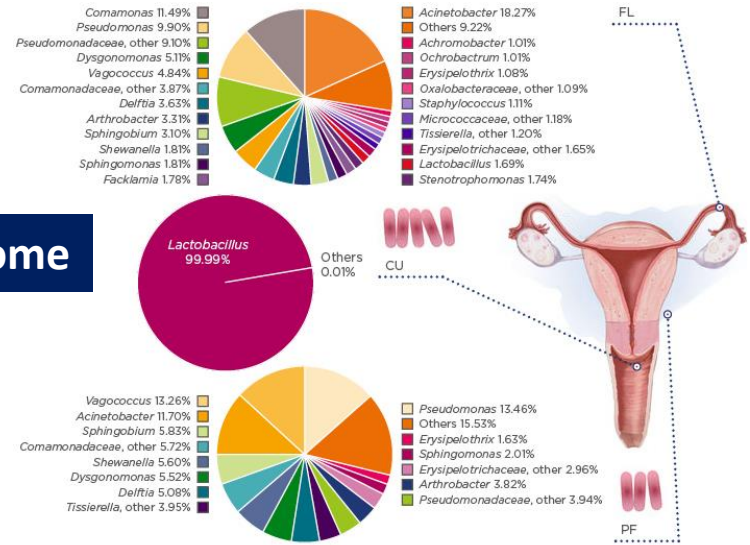
Adapted with permission from Chen et al.<sup>10</sup> The figure is reproduced with permission from Nature Publishing Group.

Dysbiosis in vaginal microbiota: open gate to microbial infections. Fungal (candidosis), bacterial (Neisseria, Staphylococcus...) and viral (HPV, HIV...)



# « Unexpected » Microbiota

## Uterine microbiome



## Placental microbiome

## Umbilic cord microbiome

## Meconium microbiome

... end of the « sterile womb paradigm »?



# Milk Microbiome: what are we talking about?





# Milk sterility: the paradigm is seriously questioned...



*Ralstonia*  
*Roseburia*  
*Clostridium*  
*Corynebacterium*  
*Faecalibacterium*  
*Lactobacillus*  
*Bifidobacterium*  
*Propionibacterium*  
*Pseudomonas*  
**Staphylococcus**  
**Streptococcus**  
*Bacteroides*  
*Acinetobacter*  
*Veillonella*  
*Lachnospiraceae*  
*Ruminococcaceae*  
*Enterococcus*  
*Prevotella*  
*Weissella*  
*Leuconostoc*  
*Lactococcus*  
*Citrobacter*  
*Serratia*



*Microbacterium*  
*Pediococcus*  
*Fusobacterium*  
*Propionibacterium*  
*Acinetobacter*  
*Bifidobacterium*  
*Pseudomonas*  
**Staphylococcus**  
**Streptococcus**  
*Lachnospiraceae*  
*Corynebacterium*  
*Bacteroides*  
*Enterococcus*  
*Ruminococcaceae*  
*Aerococcus*  
*Jeotgalicoccus*  
*Psychrobacter*  
*Enterobacter*



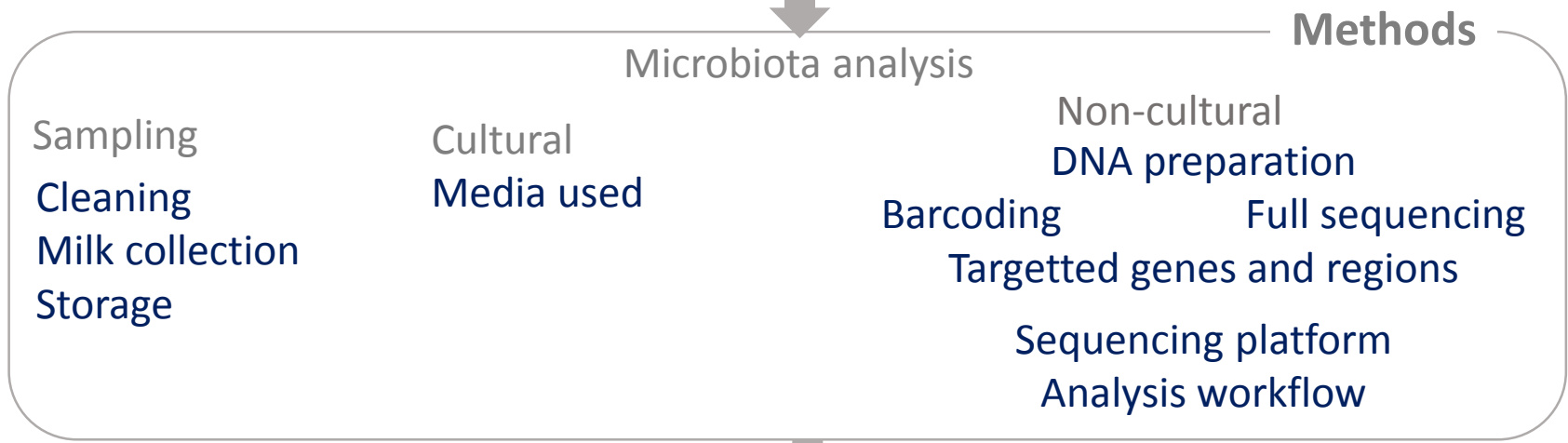
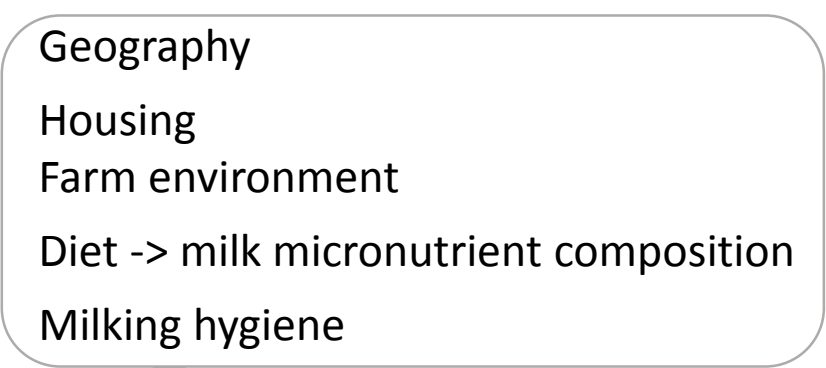
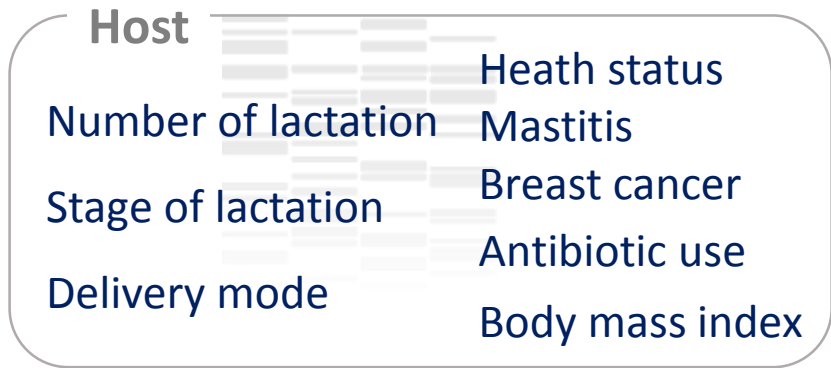
*Micrococcus*  
 5-7N15  
*Solibacillus*  
*Propionibacterium*  
*Pseudomonas*  
**Staphylococcus**  
*Aerococcus*  
*Clostridium*  
*Facklamia*  
*Trichococcus*  
*Turicibacter*  
*Acinetobacter*  
*Psychrobacter*



*Micrococcus*  
*Rhodococcus*  
*Arthrobacter*  
*Stenotrophomonas*  
*Pseudomonas*  
**Staphylococcus**  
*Streptococcus*  
*Phyllobacterium*  
*Rhizobium*  
*Agrobacterium*  
*Bacillus*

Oikonomou et al., submitted

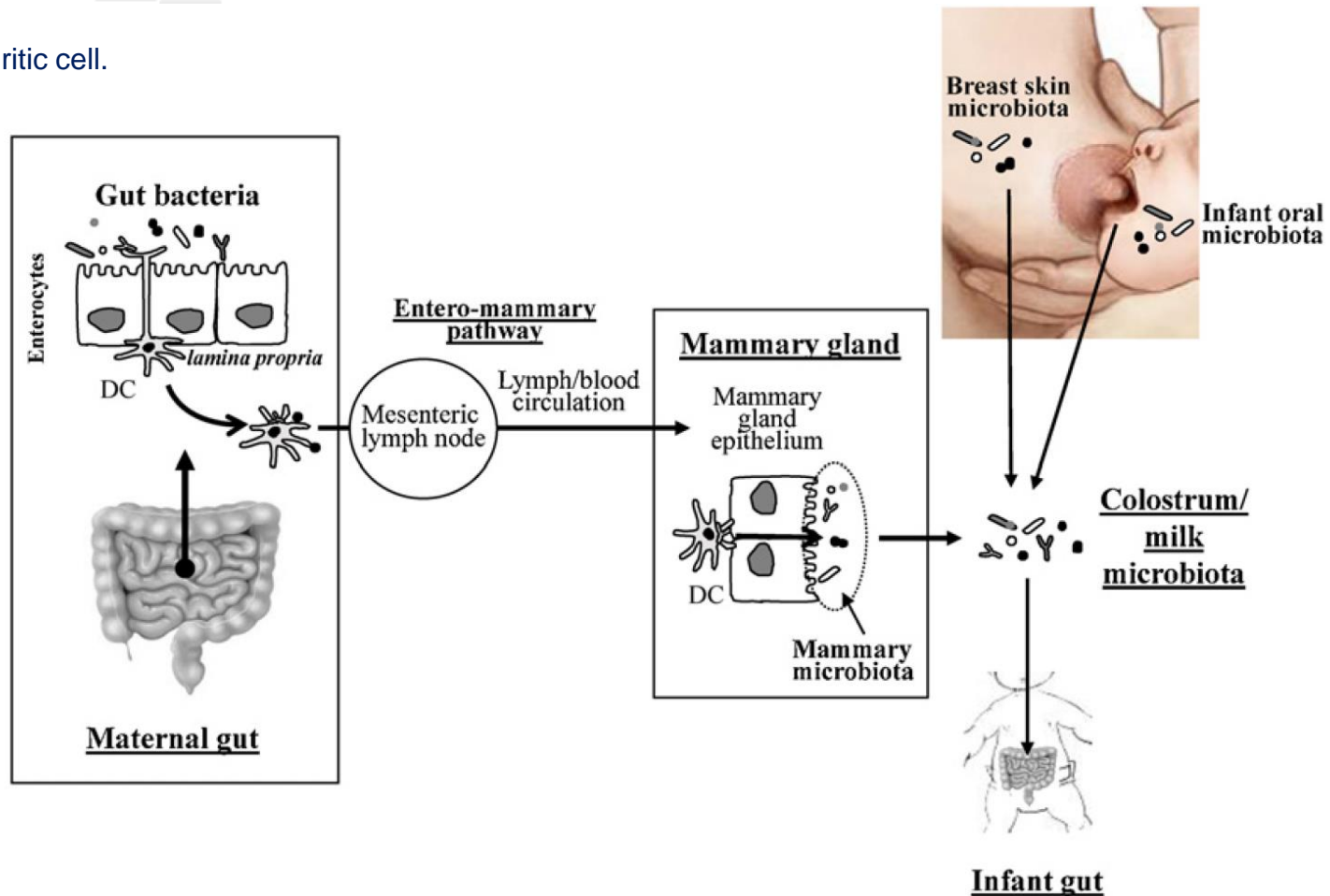
Red and orange: taxa shared between all human and animal species or present in 3 species out of 4 respectively. For human and bovine, taxa size reveals citation frequency



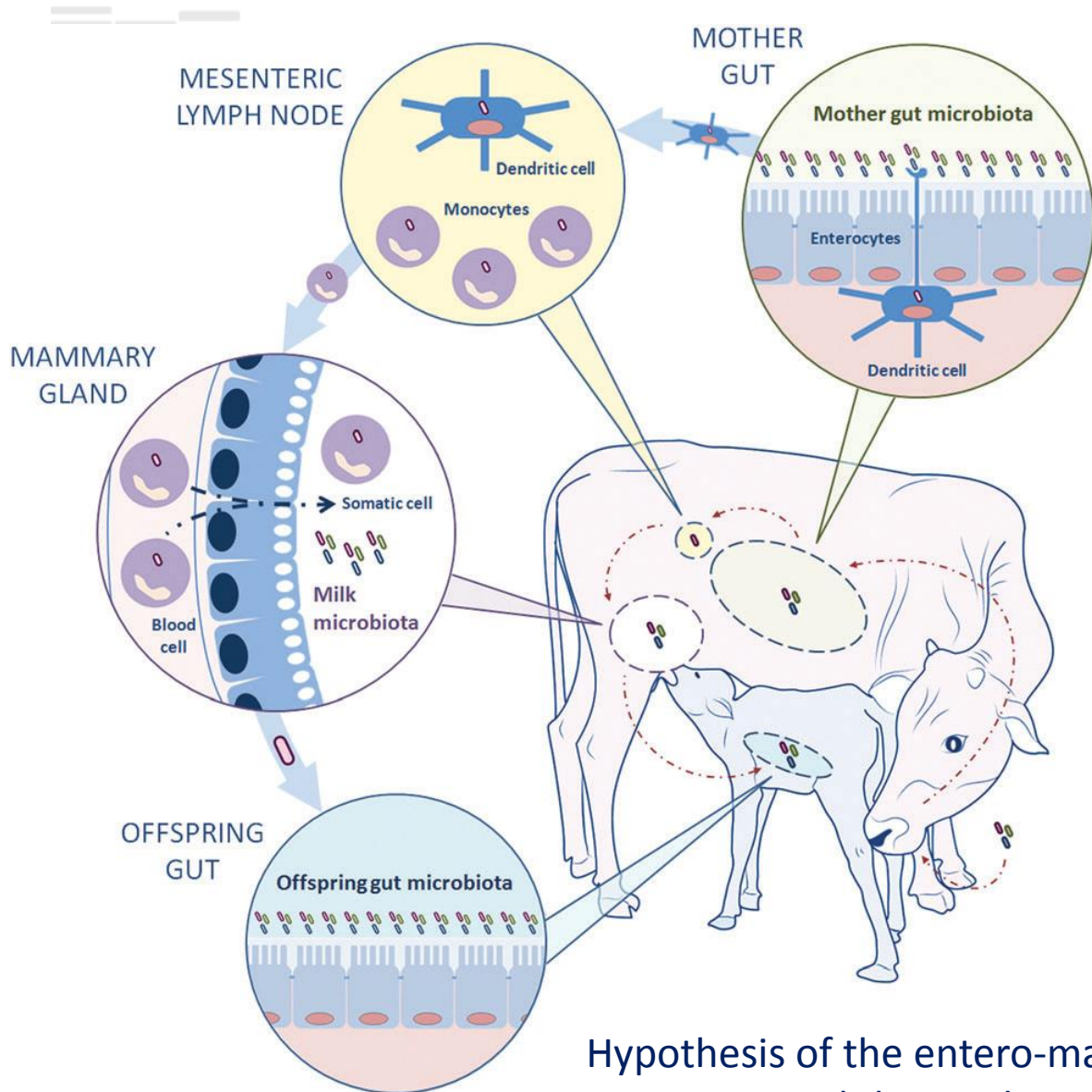
**Analysed microbiota or microbiome**

# Potential sources of the bacteria present in human colostrum and milk.

DC: dendritic cell.



After Fernandez et al., Pharmacological Research 69 (2013) 1– 102015



Hypothesis of the entero-mammary pathway in ruminants and the mother-offspring microbial flow

After Addis et al., 2016. Molecular Biosystems  
 DOI: 10.1039/c6mb00217j



# Roles of milk microbiota in the mother and offspring physiology and health





# Milk is more than a mere source of nutrients.

## Milk microbiota and maternal health

- link between milk microbiota dysbiosis and mother health status
- barrier effect against infections (mastitis)

## Milk microbiota and off-spring health

- maturation of the digestive tract, and of the digestive immune system.
- milk bacteria may serve as inoculum and play a role in the colonization and in the homeostasis of the gastrointestinal microbiota.



## There are still many questions...

Does a living milk microbiota exist?

Do they result from contamination?

What about non-bacterial microbiota and microbiota-associated functions?

How does host genetics influence the milk microbiota?

Does the milk microbiota have an influence on the calf gastrointestinal microbiota?

Can we manipulate the milk microbiota in order to improve mammary gland (and off-spring) health?





# Ecosystemic management of mammary gland health in dairy cows



# Mastitis



## Animal Health Mastitis

4896€/year for a herd of 100 dairy cows<sup>(1)</sup>  
(~200 M€/year in France)



**Huge economic loss**



## Lower « cheesability »<sup>(2)</sup>

**Food Safety**  
SFP outbreaks

(1) Halasa et al., 2009. Livest. Sc.  
(2) Le Maréchal et al., 2011. DST

# Mastitis



***S. aureus* mastitis difficult to cure and prone to recurrence**

**Mastitis is the first cause of antibiotic use in dairy herds**

**« Once a Staph cow, always a Staph cow »**



**Poorly efficient in eradicating *S. aureus* in the udder context**

(cure rate for *S. aureus* mastitis often <15% in cows)

**MRSA and other resistant strains are not a problem in mastitis treatment and milk production (until now)**

# Mastitis: prevention and treatment

## Prevention

## Treatment

### hygienic procedures



### Vaccines



Not effective

### Antibiotics



- Not always effective
- Dissemination of antibiotic resistance

## Alternative strategies

Genetic selection

New antibacterial molecules

Phage therapy

(lysostaphine)

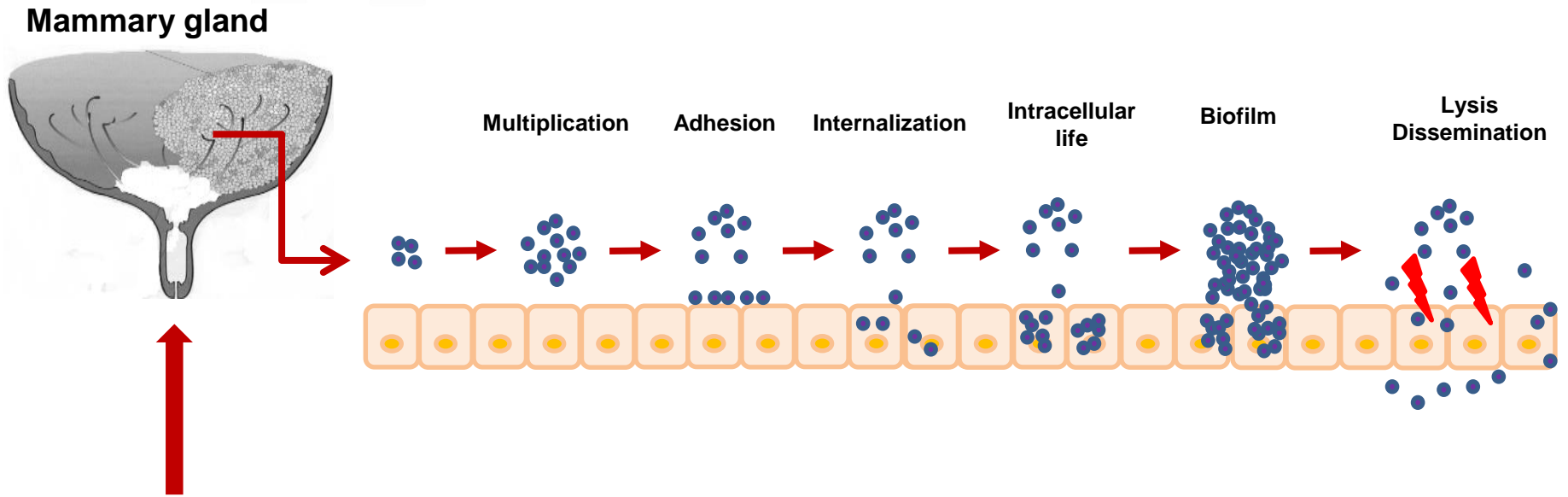
Essential oils

**Biological control**

Homeopathy

**Use of beneficial microbes**

# Infectious cycle

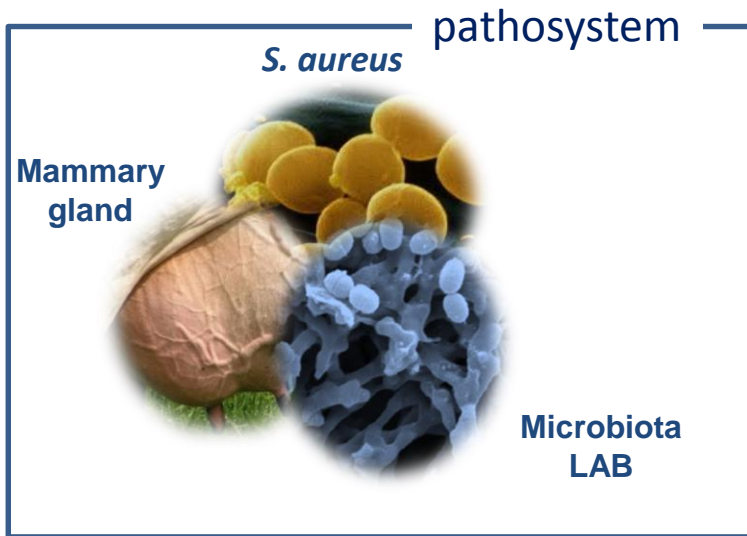


ascending colonization



Pathogen

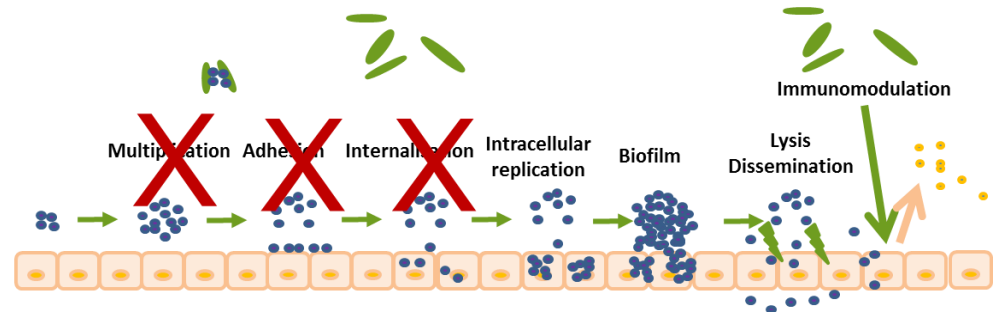
# Microbiological control



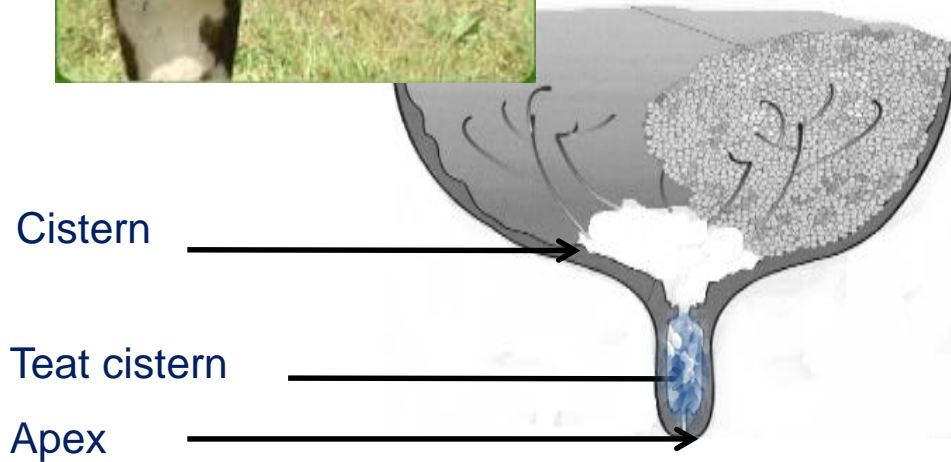
## Barrier effect of udder microbiota?



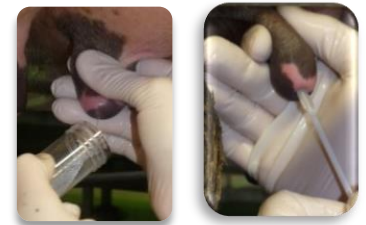
## Beneficial properties of LAB isolated from the mammary gland microbiota



# Main results

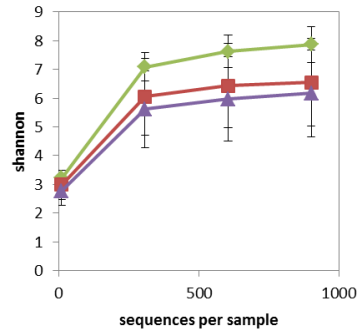


## Internal teat microbiota



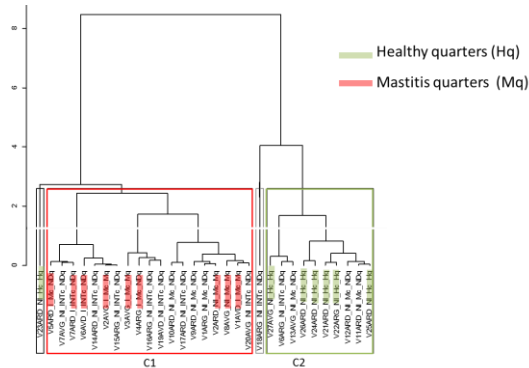
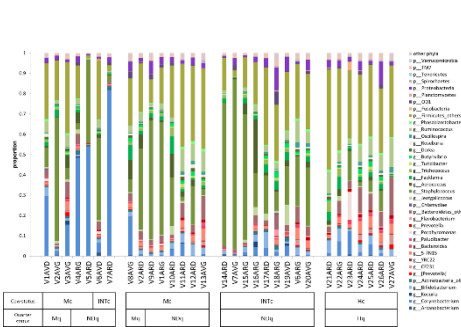
**Relation between microbiome composition and history of mastitis (apart from infectious episode)**

# Main results / Metataxonomics on bovine quarters

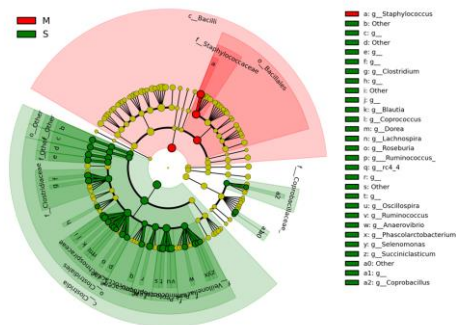


Healthy Quarters  
Mastitis quarters  
Non determined quarters

Higher diversity in healthy quarters



Separation between healthy quarters and quarters which had previously developed mastitis



## Taxonomic markers in relation to health status

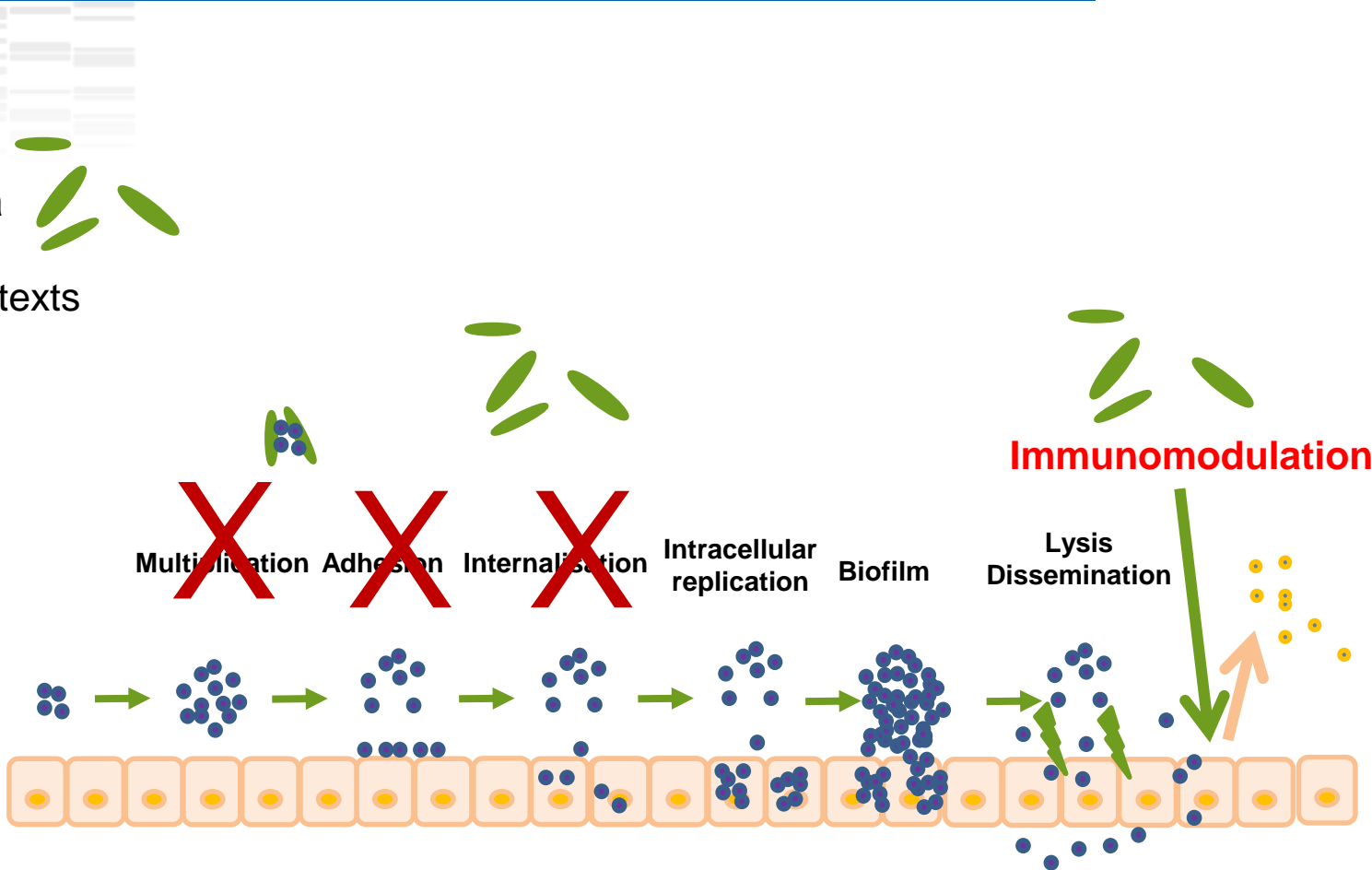
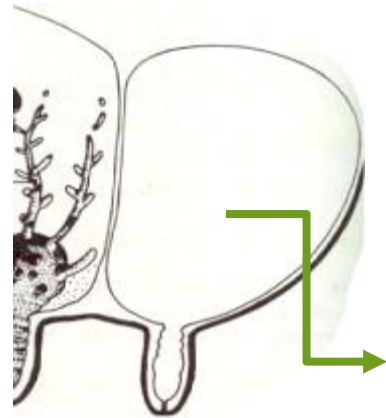
**Quarters with a history of mastitis (no ongoing mastitis)**  
higher proportion of C\_Bacilli (Staphylococcus)

**Healthy quarters**  
higher proportion of C\_Clostridia (Ruminococcus, Oscillospira, Roseburia, Dorea... ); P\_Bacteroidetes (Prevotella, Bacteroides, Paludibacter...) O\_Bifidobacteriales (Bifidobacterium)



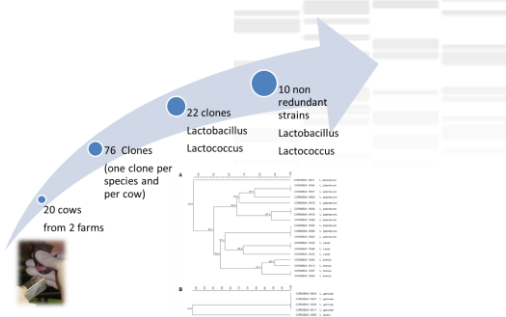
# Main results / Towards mammary probiotics?

**Lactic Acid Bacteria**  
-GRAS  
-Efficient in other contexts



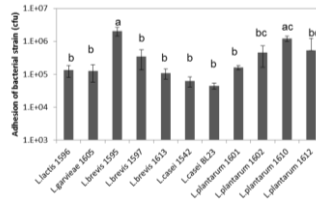
Isolated from the mammary gland microbiota...or not

# Main results / Towards mammary probiotics?

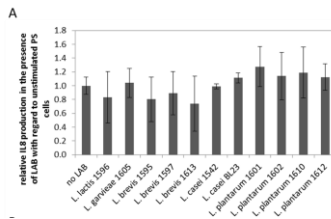


## Isolation of LAB candidate strains

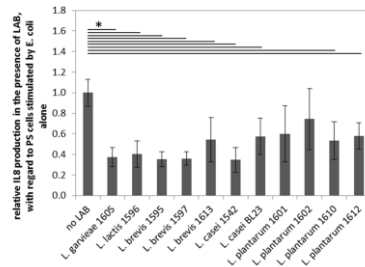
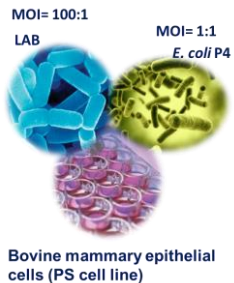
Bouchard et al., 2015. PLOS ONE. doi: 10.1371/journal.pone.0144831.  
 Seridan et al., 2015. Beneficial Microbes doi: 10.3920/BM2015.0019



## Colonization capacities (*in vitro*). Adhesion and internalization of LAB strains

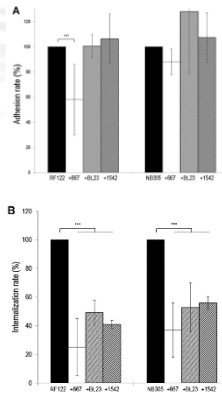
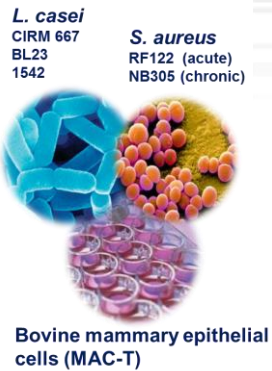


## Immunomodulation properties (*in vitro*) Some LAB modulate IL8 production in bMEC



## Immunomodulation properties (*in vitro*) LAB modulate IL8 production in infected bMEC

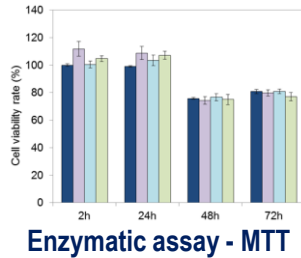
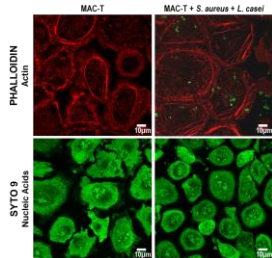
# Main results / Towards mammary probiotics?



***L. casei* inhibits *S. aureus* invasion in bMEC**

Strain-dependant inhibition of *S. aureus* adhesion by *L. casei*

Inhibition of *S. aureus* internalization by *L. casei*

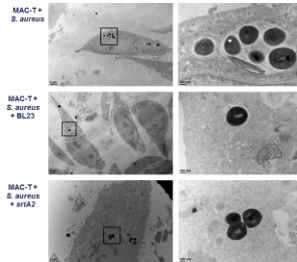
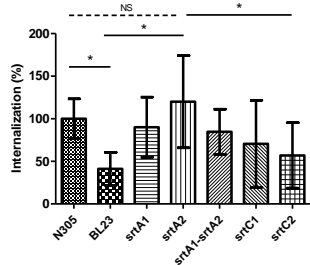


**No adverse effect on bMEC**

No impact on cell structure

No cytotoxicity in the conditions tested

Bouchard et al. 2013. Appl. Envir. Microbiol. doi: 10.1128/AEM.03323-12



**Role of *L. casei* sortases**

SrtA2 is involved in the inhibition of *S. aureus* internalization in bMEC and in the modulation of bMEC innate immune response

# Take home messages

**Mastitis : an old problem with new perspectives**

**Mammary gland microbiota plays a role in the udder health status**

Identification of marker species (eubiosis/dysbiosis)

Intervention to restore a balanced microbiota?

**Probiotic solutions as a promising strategy against mastitis**

Capacity to interfere with pathogen infectious cycle: competition for tissue colonization

Capacity to modulate the innate immune system

Promising results obtained *in vitro*...

...need further investigations on in vivo validation, on the molecular mechanisms involved.

# Towards mammary probiotics?

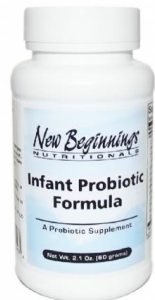


**Composition?**  
**Administration route?**  
**Regulation?**  
**Cost?**

## Probiotics for breastfeeding mothers...



## Probiotics in infant formulas...



What about a more complex microbiota in infant formulas?

# Thanks to...



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Patrice François

Jacques Schrenzel



**Thank you for your attention**  
**Muito obrigado pela sua atenção**

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