



Milk microbiota: physiological and health implication.

Yves LE LOIR
UMR 1253 STLO
INRA Agrocampus Ouest, Rennes, France





Development of NGS: Inventory of many microbiota



Gut Microbiota



Pioneering studies

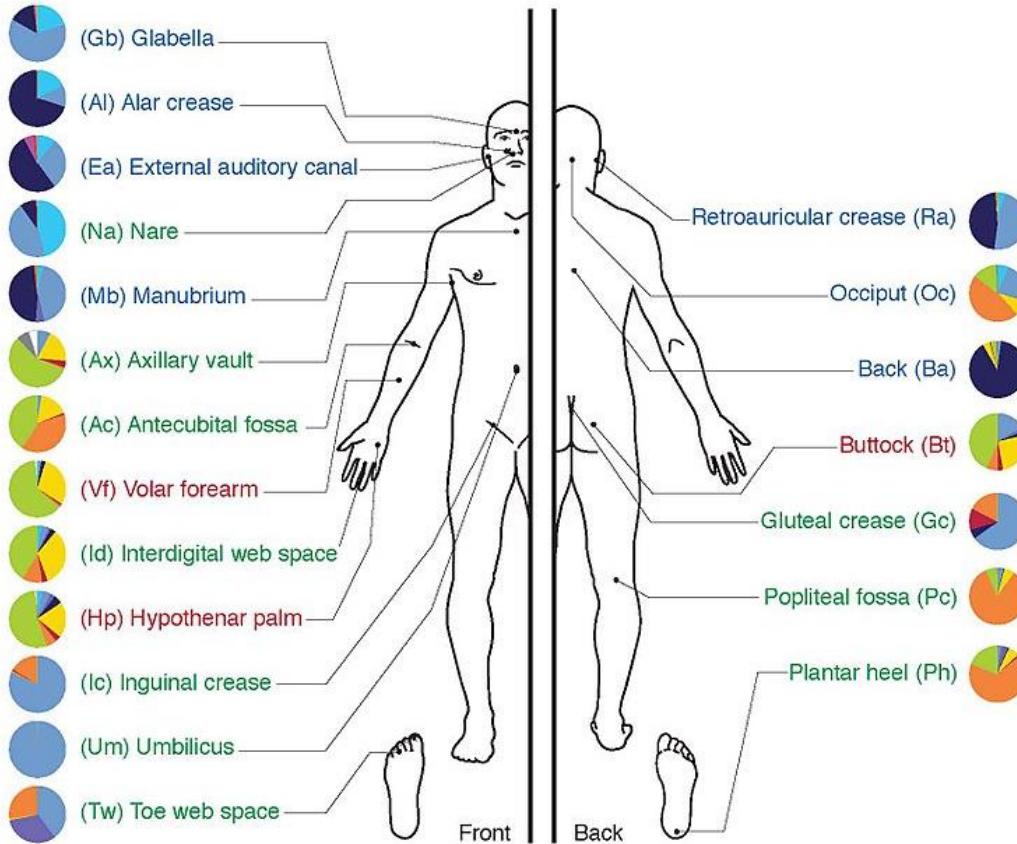
Physiological functions, health benefits (when balanced)...

Probiotics, Fecal transplantation...

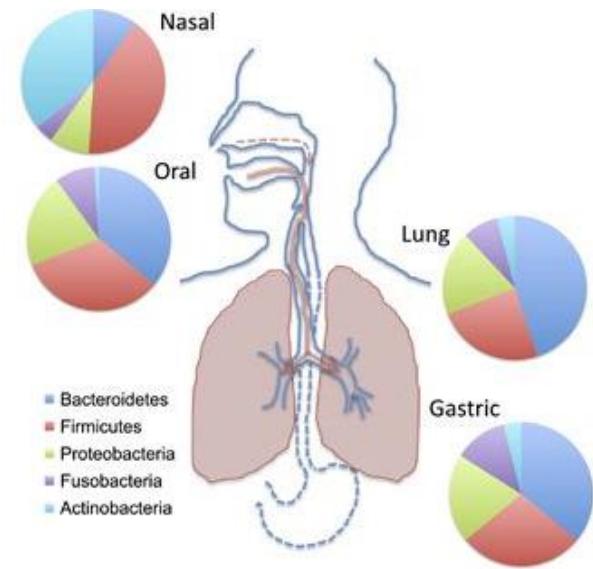
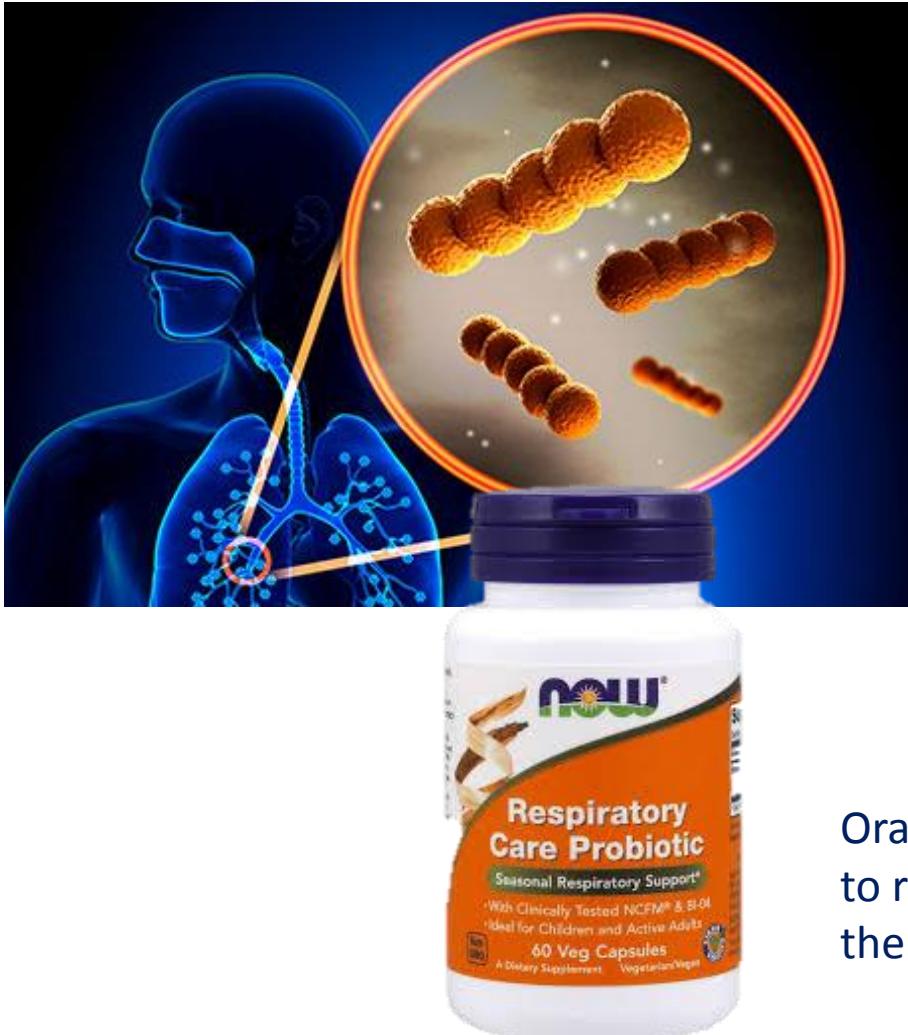
... Cf Talks by Jean-Marc, Philippe and Gwenaël.

Microbes are everywhere!

Skin microbiota



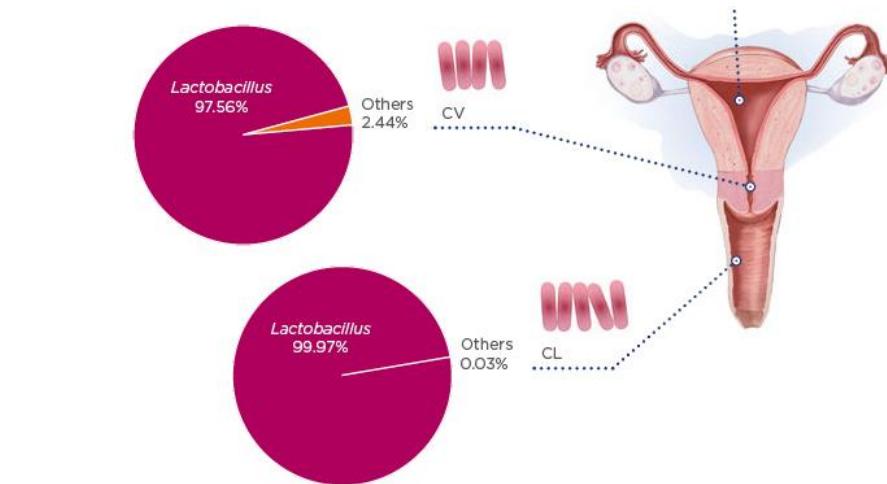
Lung Microbiota



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.10 The figure is reproduced with permission from Nature Publishing Group.

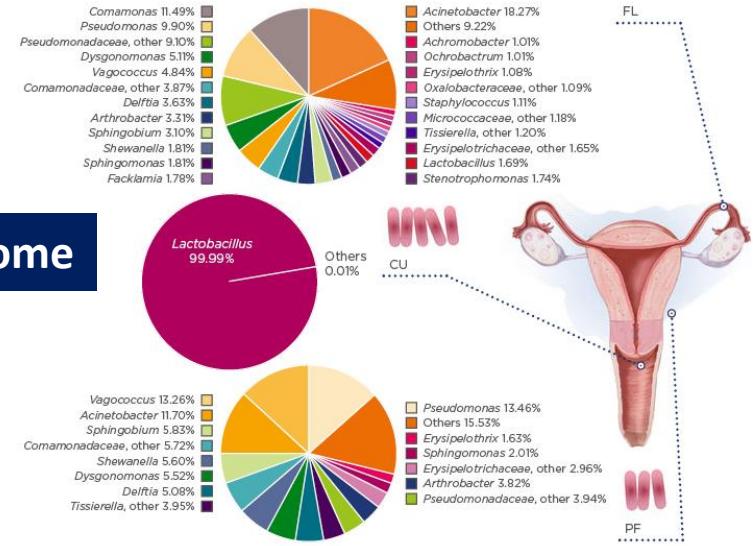


Dysbiosis in vaginal microbiota: open gate to microbial infections. Fungal (candidosis), bacterial (Neisseria, Staphylococcus...) and viral (HPV, HIV...)

« 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 questionned...



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



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



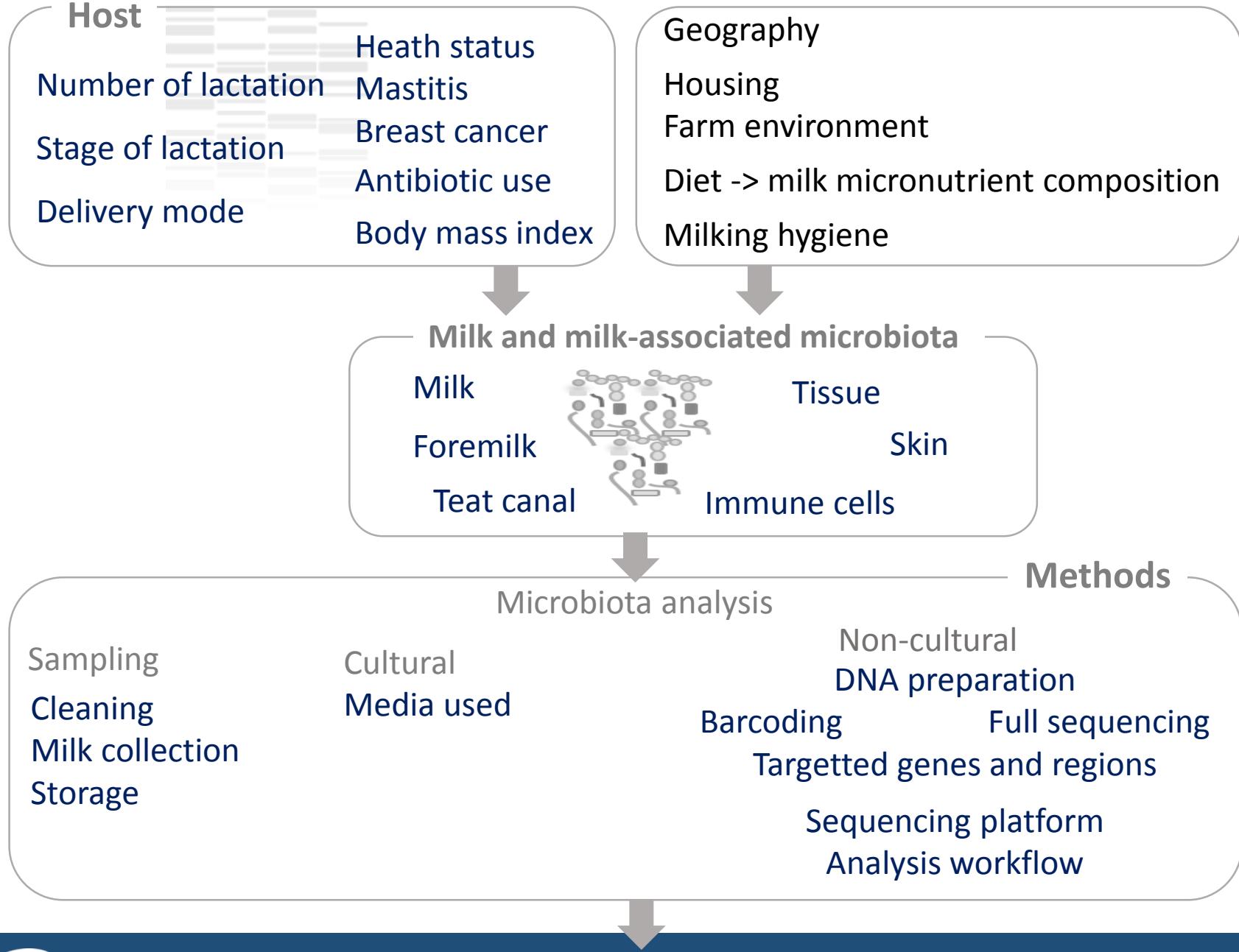
Micrococcus
5-7N15
Solibacillus
Propionibacterium
Pseudomonas
Staphylococcus
Aerococcus
Clostridium
Facklamia
Trichococcus
Turicibacter
Acinetobacter
Psychrobacter



Micrococcus
Rhodococcus
Arthrobacter
Stenotrophomonas
Pseudomonas
Staphylococcus
Streptococcus
Phyllobacterium
Rhizobium
Agrobacterium
Bacillus

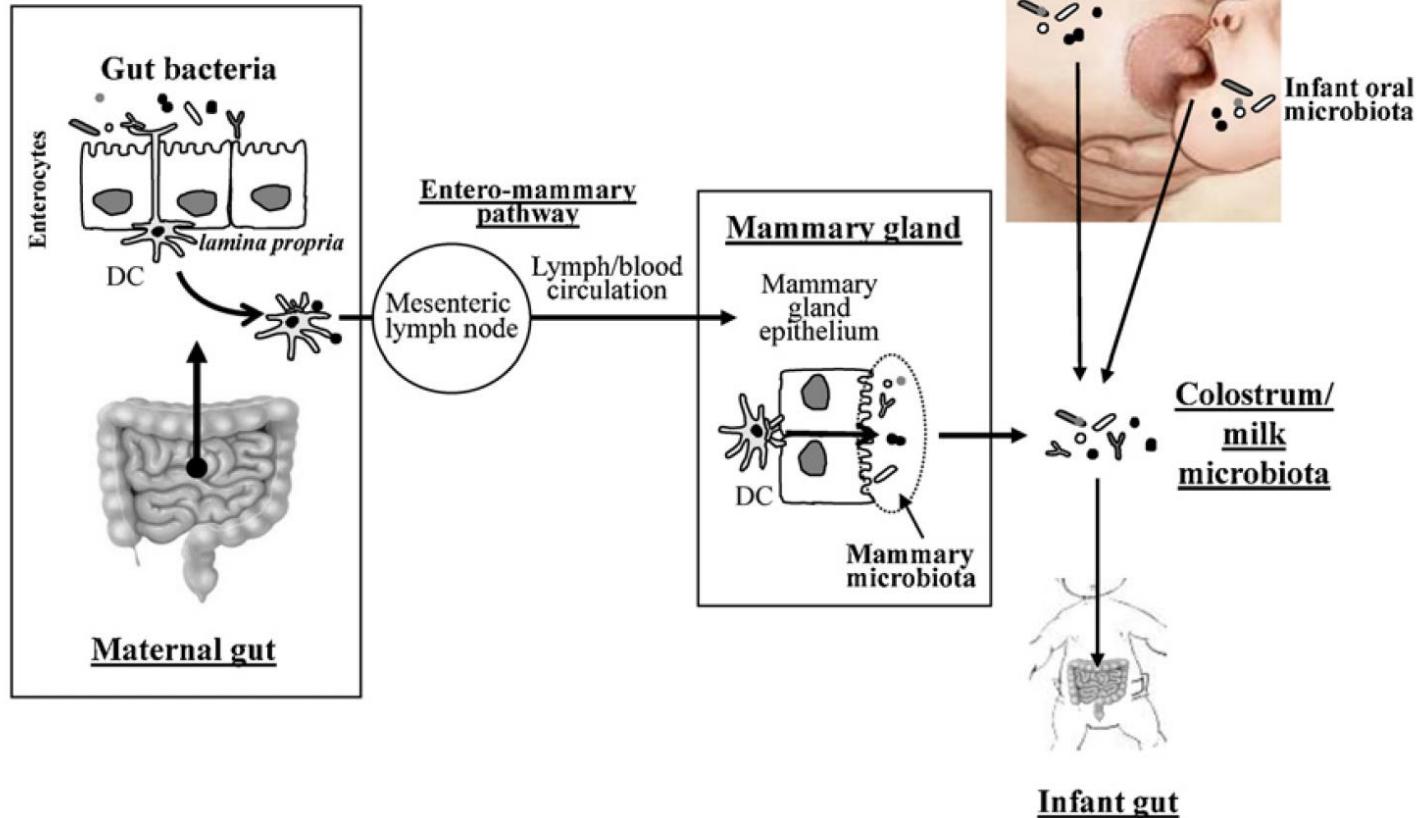
Oikonomou et al., submitted

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

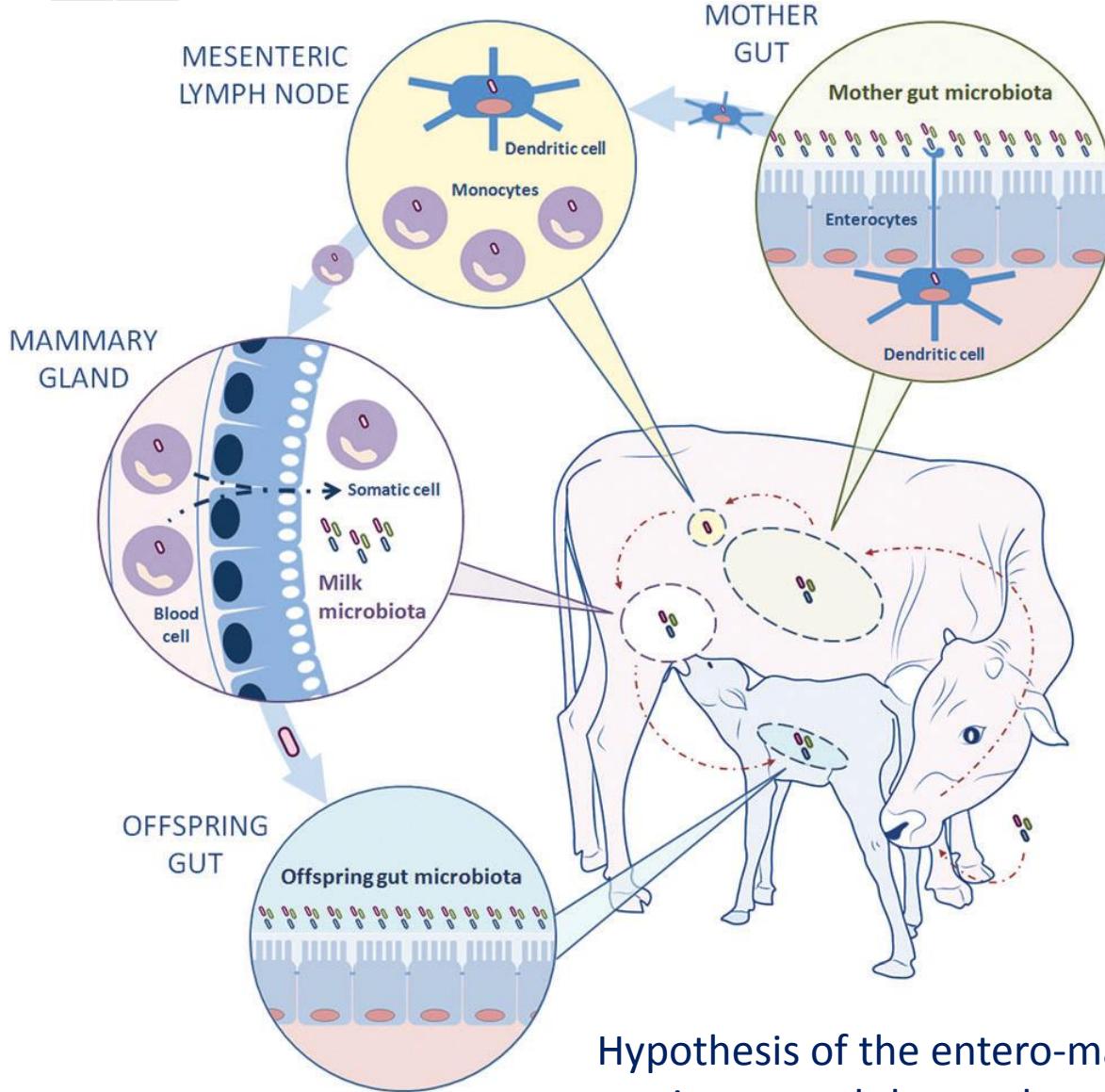


Potential sources of the bacteria present in human colostrum and milk.

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



Huge economic loss

1

Animal Health
Mastitis

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



Lower « cheesability »⁽²⁾

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

hygienic procedures



Vaccines



Treatment

Antibiotics



- Not always effective
- Dissemination of antibiotic resistance

Alternative strategies

Genetic selection

New antibacterial molecules

(lysostaphine)

Essential oils

Homeopathy

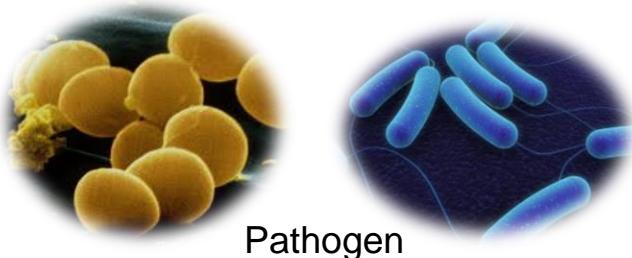
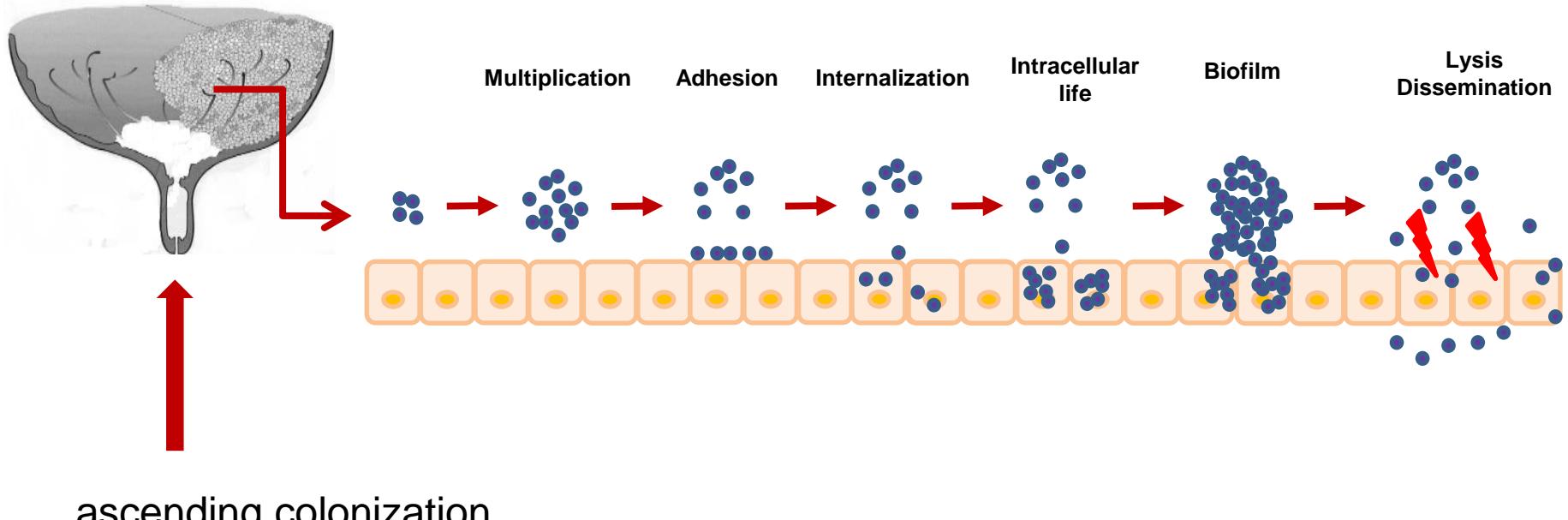
Phage therapy

Biological control

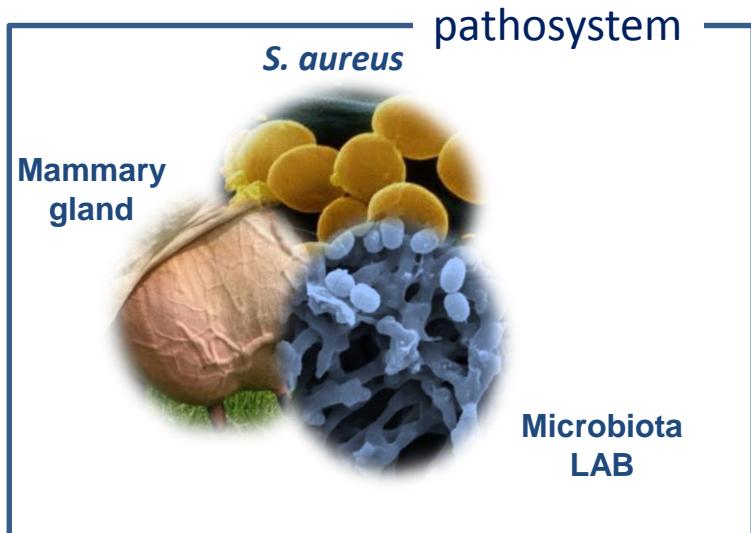
Use of beneficial microbes

Infectious cycle

Mammary gland



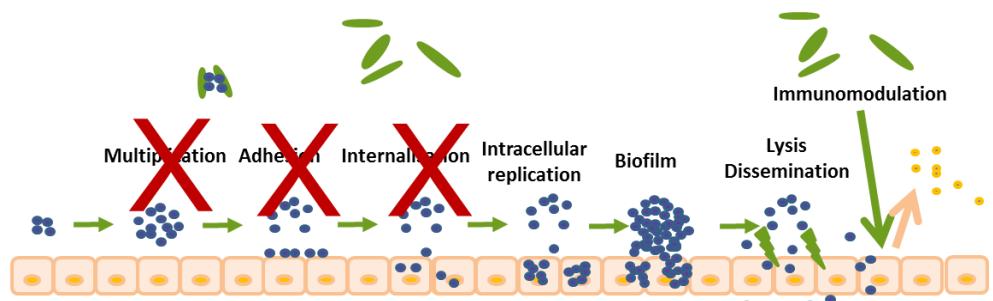
Microbiological control



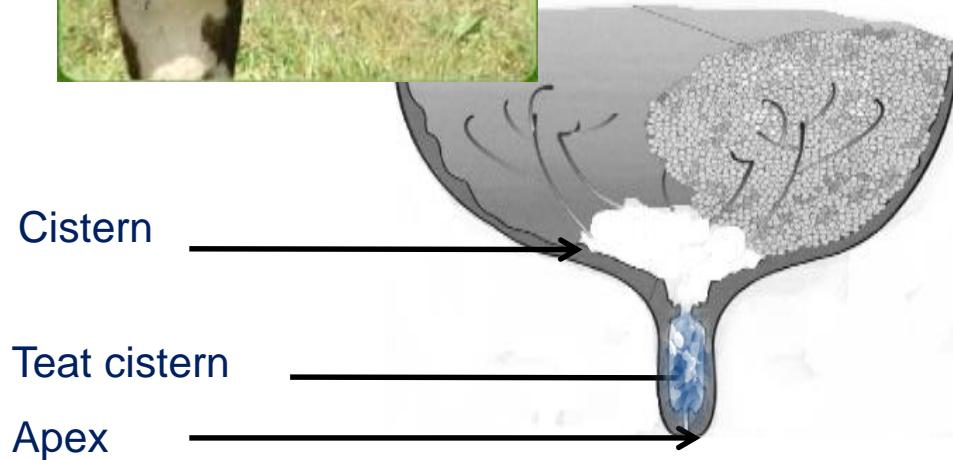
Barrier effect of udder microbiota?



Beneficial properties of LAB isolated from the mammary gland microbiota



Main results

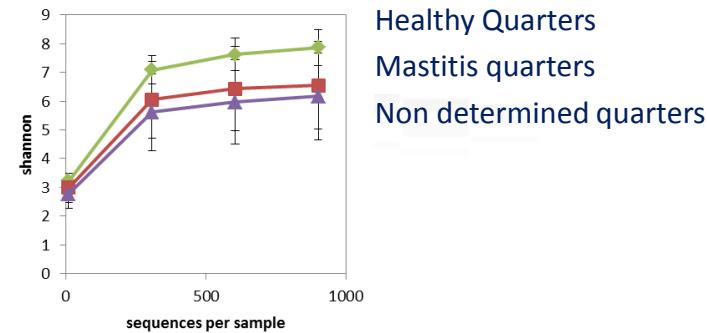


Internal teat microbiota

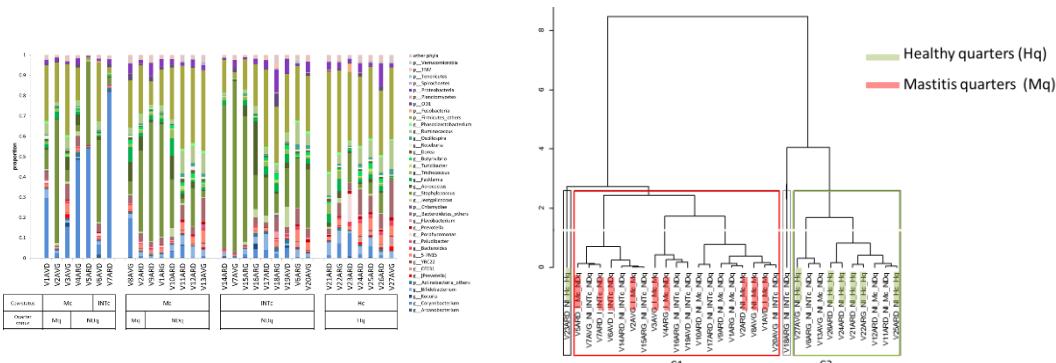


Relation between microbiome composition and history of mastitis (apart from infectious episode)

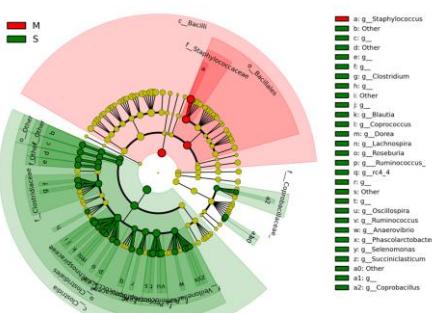
Main results / Metataxonomics on bovine quarters



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

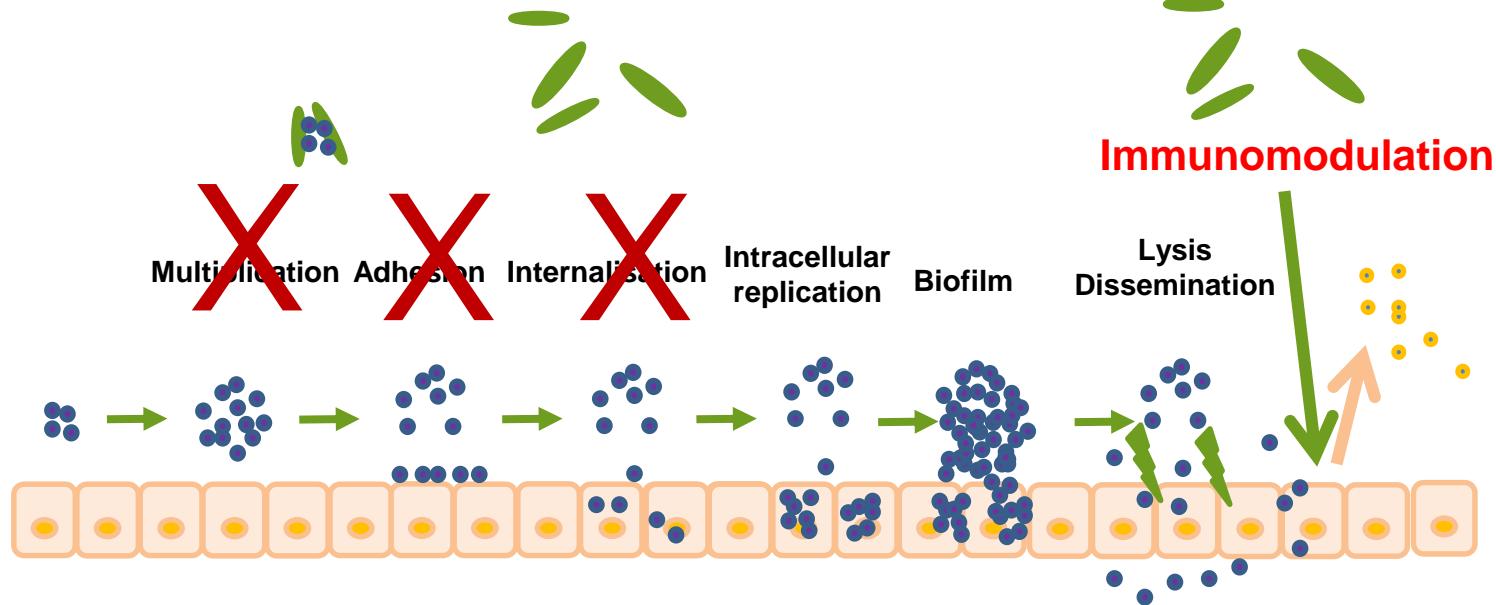
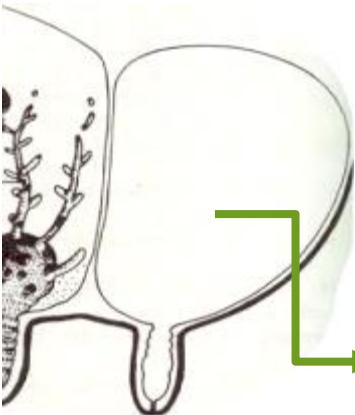
higher proportion of C_Clostridia (Ruminococcus, Oscillospira, Roseburia, Dorea...);
P_Bacteroidetes (Prevotella, Bacteroides, Paludibacter...) O_Bifidobacteriales
(Bifidobacterium)

Main results / Towards mammary probiotics?

Lactic Acid Bacteria

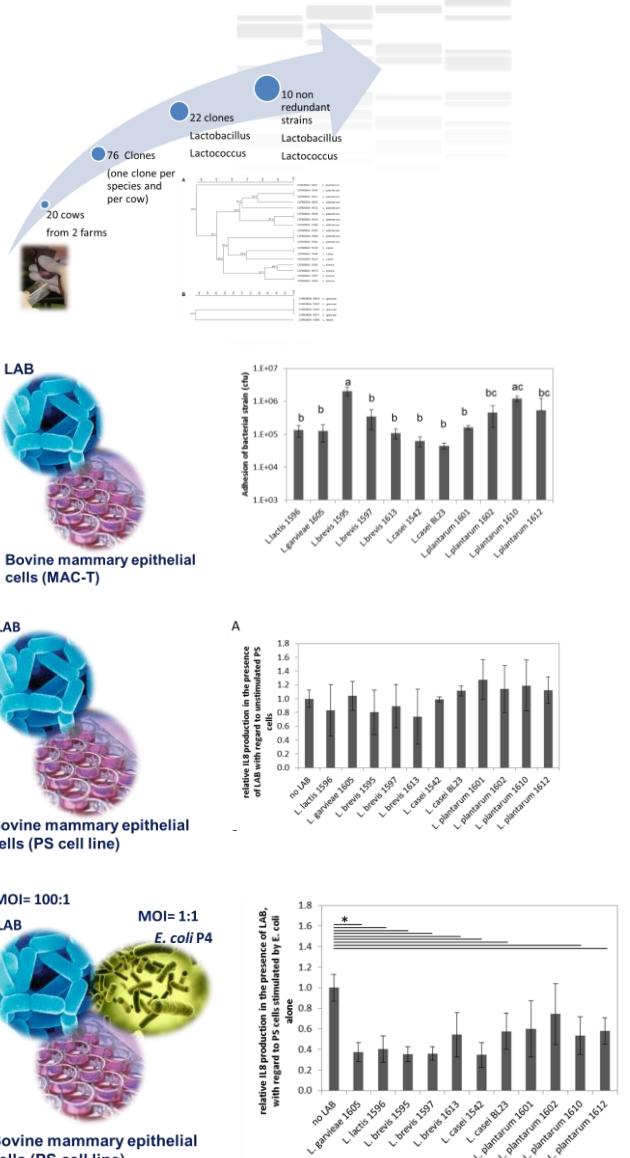
-GRAS

-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

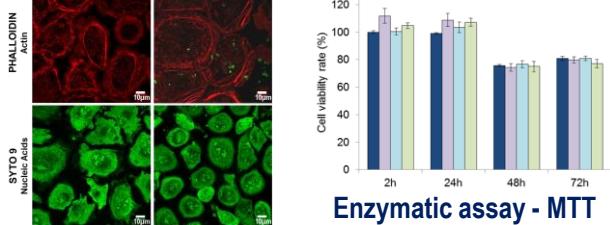
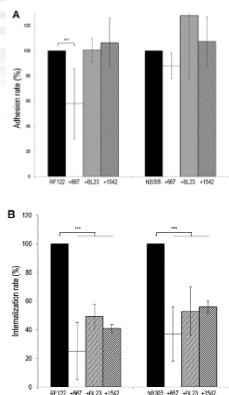
Main results / Towards mammary probiotics?

L. casei
CIRM 667
BL23
1542

S. aureus
RF122 (acute)
NB305 (chronic)



Bovine mammary epithelial cells (MAC-T)



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

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

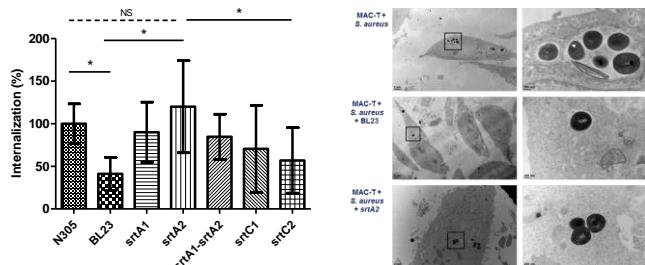
Inhibition of *S. aureus* internalization by *L. casei*

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?

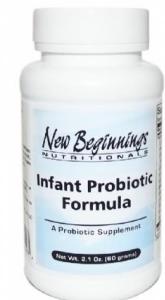
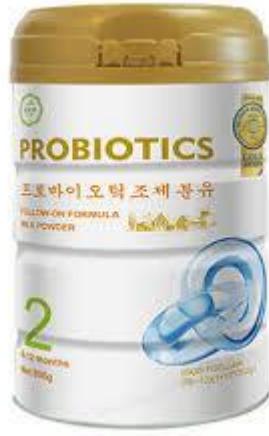


**Composition?
Administration route?
Regulation?
Cost?**

Probiotics for breastfeeding mothers...



Probiotics in infant formulas...



What about a more complex microbiota in infant formulas?

Thanks to...



Sergine Even

Yves Le Loir

Lucie Rault

Damien Bouchard

Renata de Faria Silva Souza

Bianca Seridan

Nadia Berkova

Hélène Falentin



ISP

P. Germon

J. Lassalas

P. Lamberton

J.-M. Aubry

P.-G. Marnet

PEGASE

P. Langella

L. Bermudez



Universidade Federal
de Minas Gerais
Vasco Azevedo
Jacques Nicoli



Fatima Nader-Macias
María Silvina Juárez Tomás
María Cecilia Leccese Terraf
Candelaria Gonzales-Moreno



Patrice François
Jacques Schrenzel





Thank you for your attention
Muito obrigado pela sua atenção

Contact:
yves.le-loir@inra.fr