

# Analysis of milk microbial profiles using 16S rRNA gene sequencing in milk somatic cells and fat

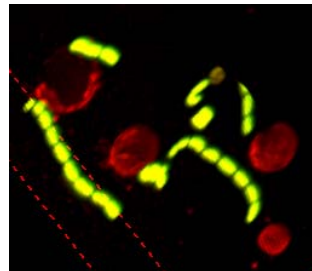
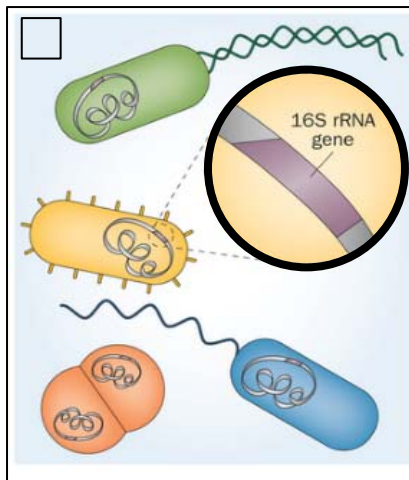
Juan F. Medrano

Anna Cuzco\*

Alma Islas-Trejo

Armand Sanchez\*

Olga Francino\*

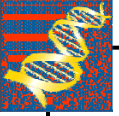


*Dept. of Animal Science  
University of California, Davis, USA*

*\* Molecular Genetics Veterinary  
Service (SVG), Veterinary School,  
Universitat Autònoma de  
Barcelona, Spain*

WCGALP, Auckland, NZ Feb 13, 2018

Whiteside et al., 2015. Nat Rev.  
Urol.



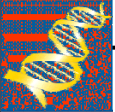
# Approach used to examined the Microbiota of milk

- Examined bacterial diversity in two milk tissues: somatic cells and fat.
- Collected milk samples with cannula and no-cannula to harvest endogenous milk vs milk exposed to the external environment.



Exp 1. Examined longitudinal composition of bacterial populations by sampling the same cow at 15, 90 and 120 dim. (7 cows)

Exp 2. Compared healthy vs mastitic quarters from the same cow. (3 cows)



# Material and methods:



2nd lactation  
Holstein cows  
UCDavis



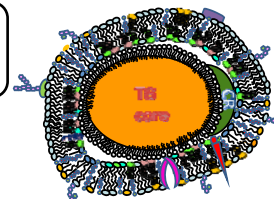
50 ml of fresh milk  
3hr after milking  
With and w/o  
cannula.

Centrifugation  
1000g,  
10 min, 4°C



Fat layer

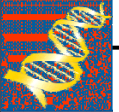
Stored at  
-70C



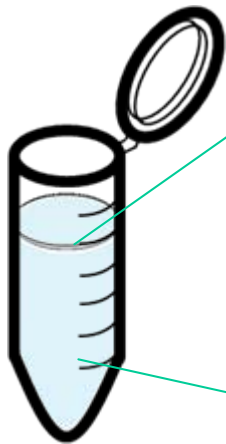
Pellet  
Somatic cells  
(epithelial cells,  
leukocytes)

SC

Lysis  
buffer 55C



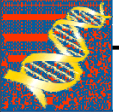
## Material and methods:



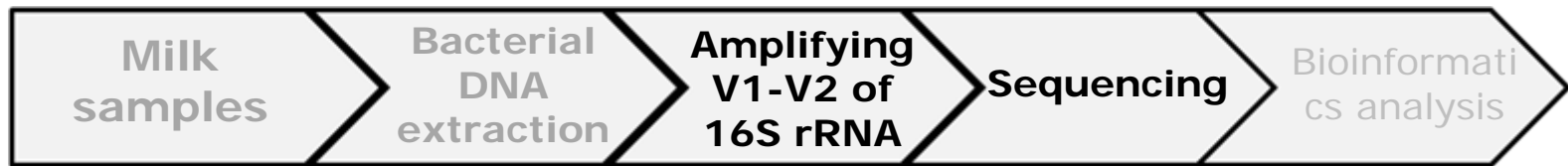
Bacterial DNA from the **fat fraction** was extracted using **Phenol/Chloroform**

Bacterial DNA from **somatic cells** was extracted using the **PowerFood® Microbial DNA isolation kit**.



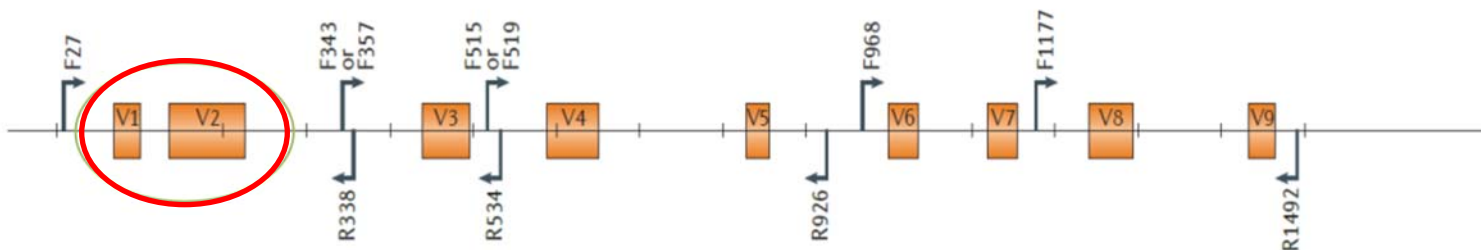


## Material and methods:



### Amplification of **V1-V2 regions of 16S rRNA gene**

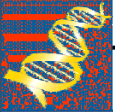
- Highly conserved gene with 9 hypervariable regions for taxonomic classification.



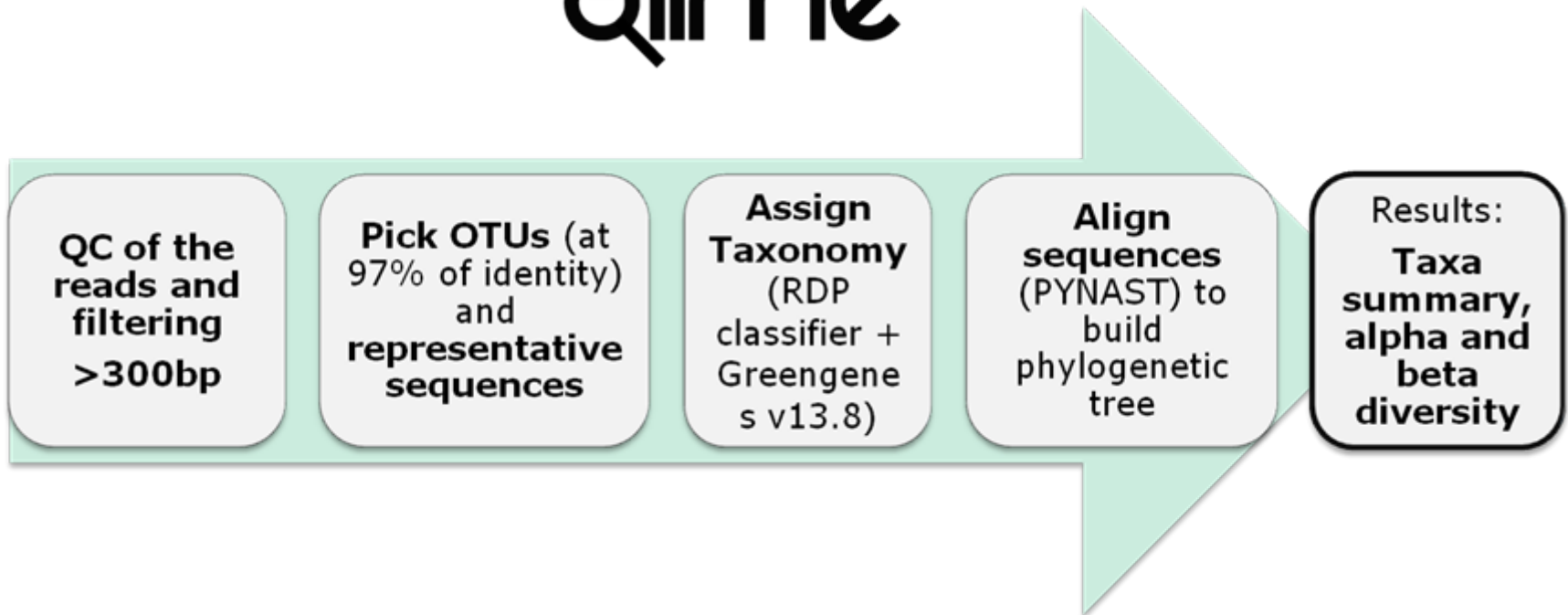
- 40-barcoded samples were pooled to run two 318™ Chips in an **Ion Torrent PGM** sequencing platform.

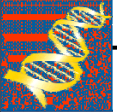






## Material and methods:





# Material and methods:

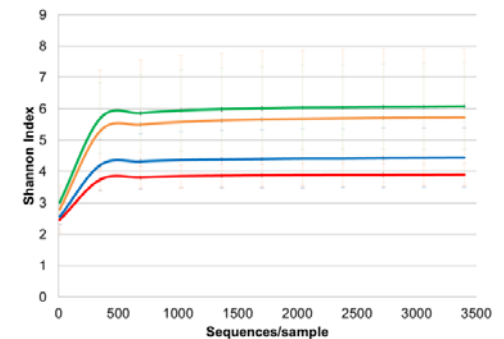


## Alpha diversity:

*“How many kinds of microbes are in a community?”*

- **Richness:**  $n^0$  of species per sample
- **Evenness (Shannon index):**  $n^0$  of species per sample considering their relative abundances

## Rarefaction plots

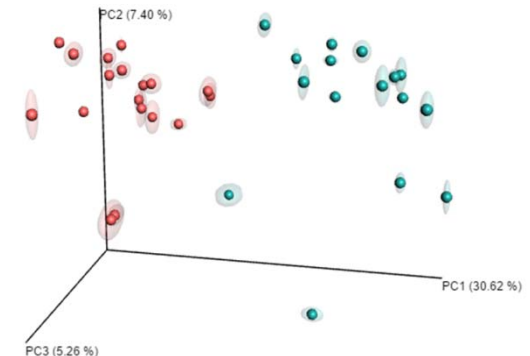


## Beta diversity:

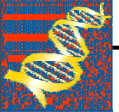
*“How do the communities differ from each other?”*

- **Unweighted UniFrac:** phylogeny and  $n^0$  of species.
- **Weighted UniFrac:** phylogeny,  $n^0$  of species and relative abundances.

## PCoA plots

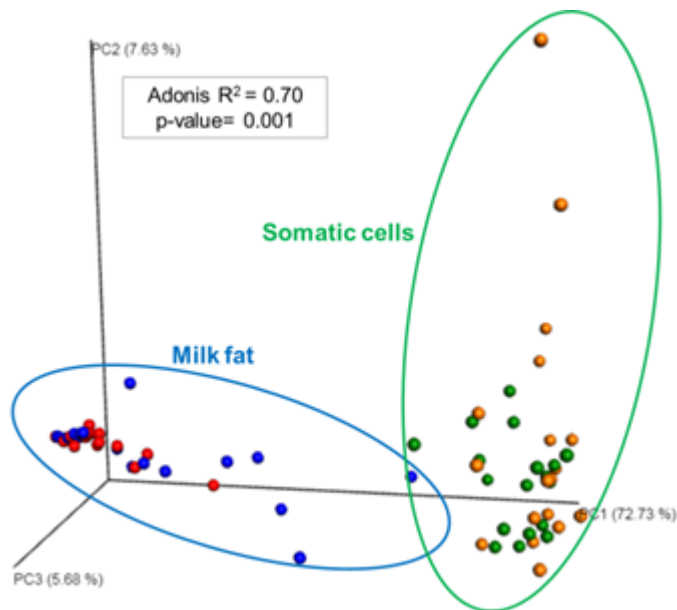




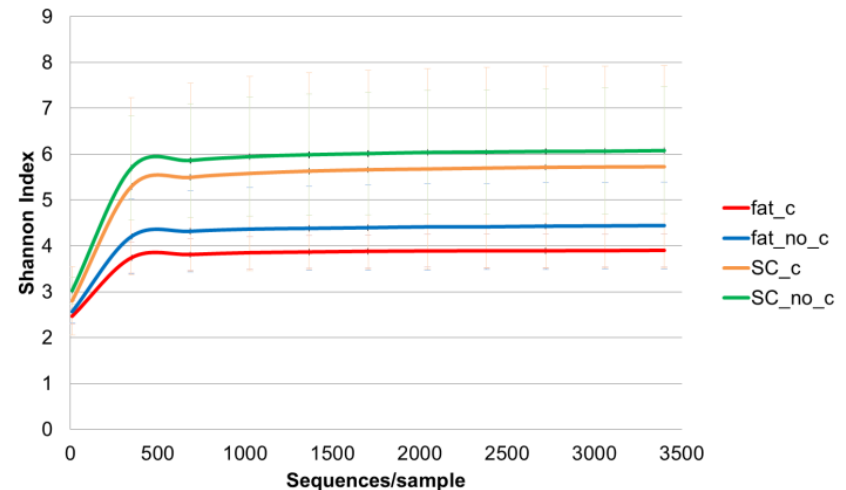


# Results: Microbiota in somatic cells vs fat

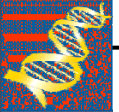
Somatic cells and fat fractions presented a very different microbiota structure.



Somatic cells microbiota was more diverse than milk fat microbiota



Samples obtained with no cannula presented higher diversity values, despite no statistically significant differences



# Results: Microbiota in somatic cells vs fat (cannula)

## Fat

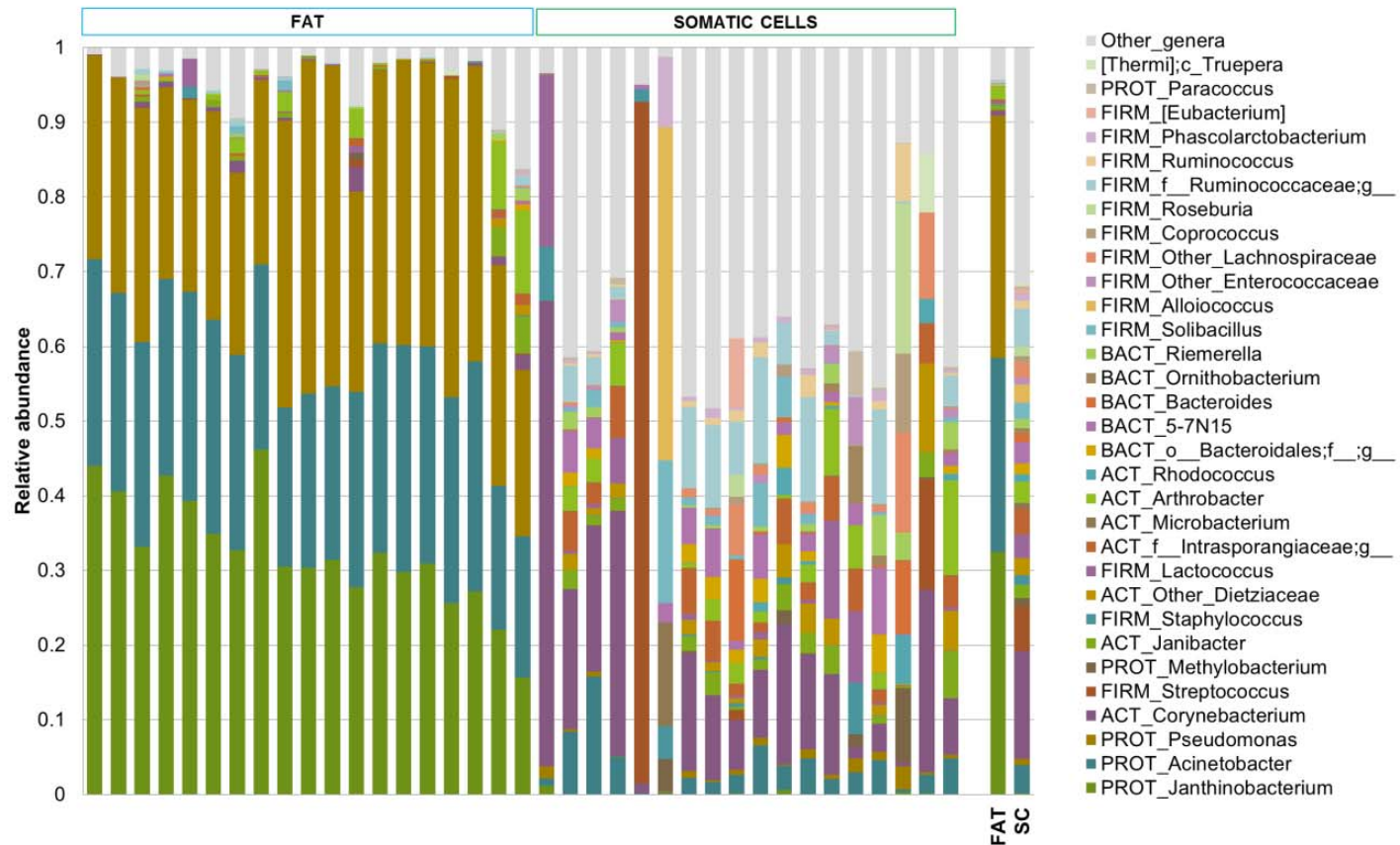
**Homogeneous composition**

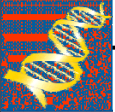
*Janthinobacterium,*  
*Acinetobacter* and  
*Pseudomonas*

## Somatic cells

**Higher diversity and variability**

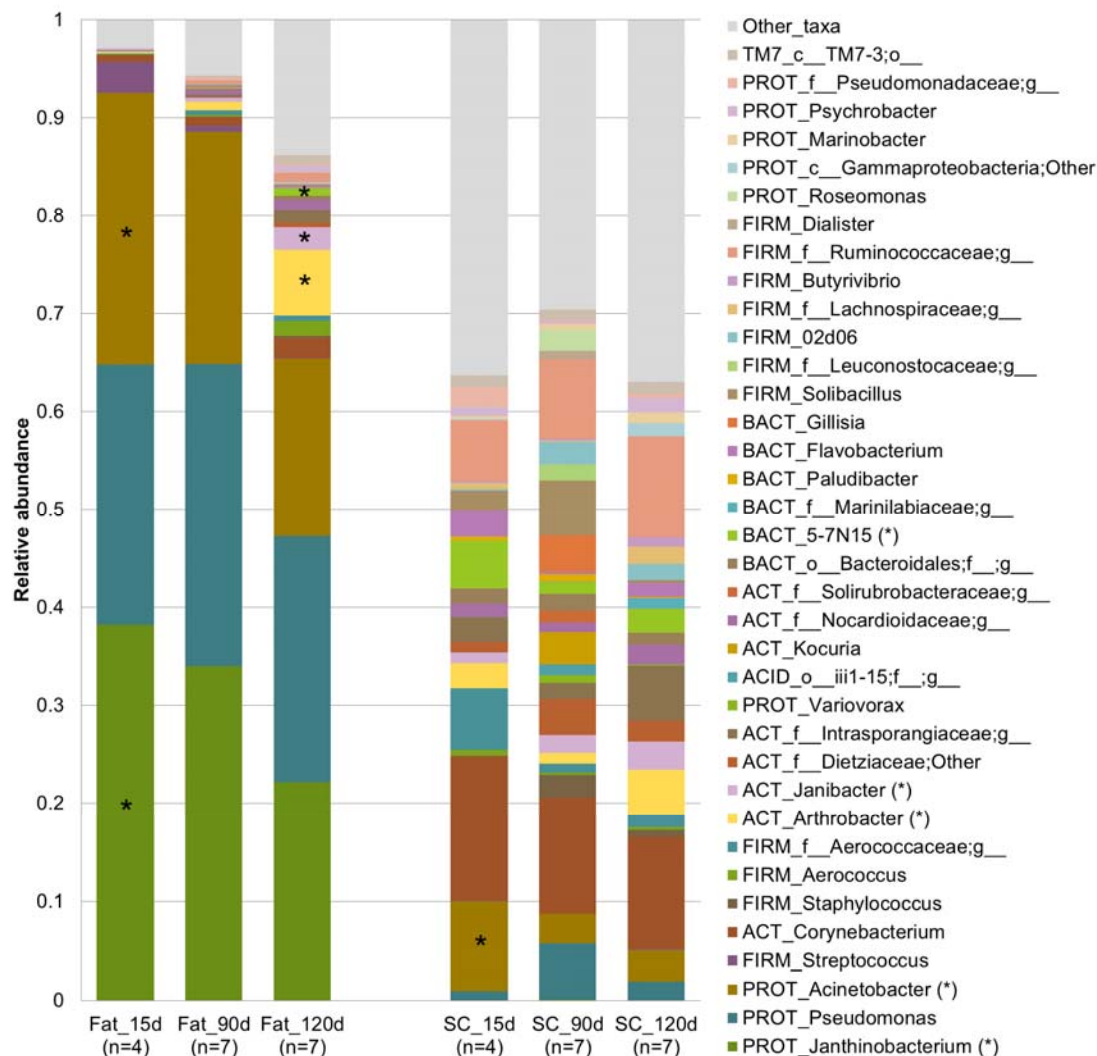
*Corynebacterium, Ruminococcaceae,*  
*Intrasporangiaceae, Acinetobacter,*  
*Lactococcus, Arthobacter, 5-7N15, etc.*





# Results: Microbiota through days in lactation (no cannula)

Bovine milk microbiota through days in lactation

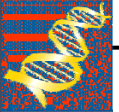


Taxonomic differences through lactation were more abundant in Fat cells:

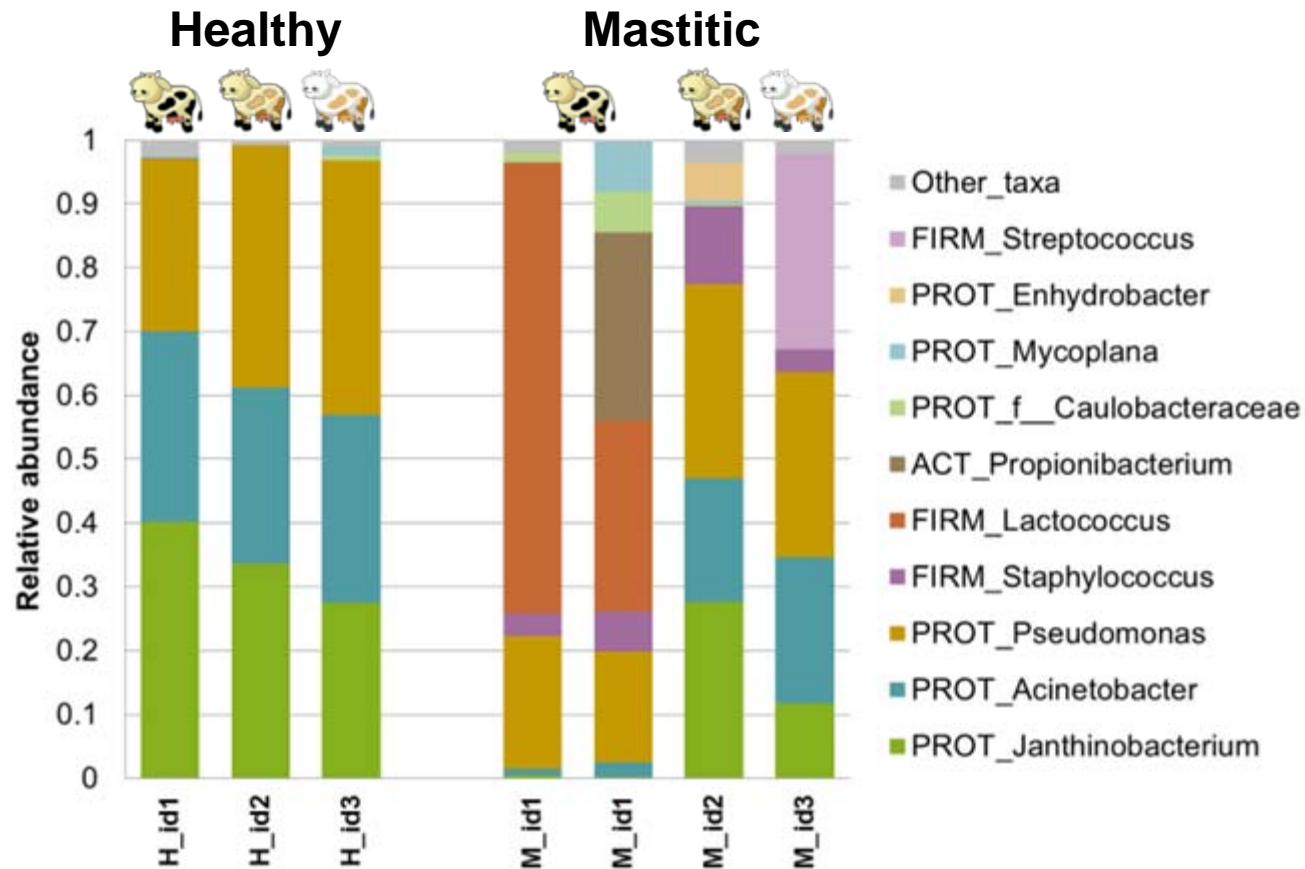
**-15 DIM cows:**  
overrepresentation of  
*Janthinobacterium*,  
*Pseudomonas*, *Acinetobacter*

**-120 DIM cows:**  
overrepresentation of  
*Arthrobacter*, *Janibacter*, 5-7N15

In Somatic cells,  
*Acinetobacter* was also overrepresented in 15 DIM cows.



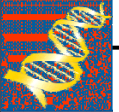
# Results: Microbiota in healthy and mastitic fat samples



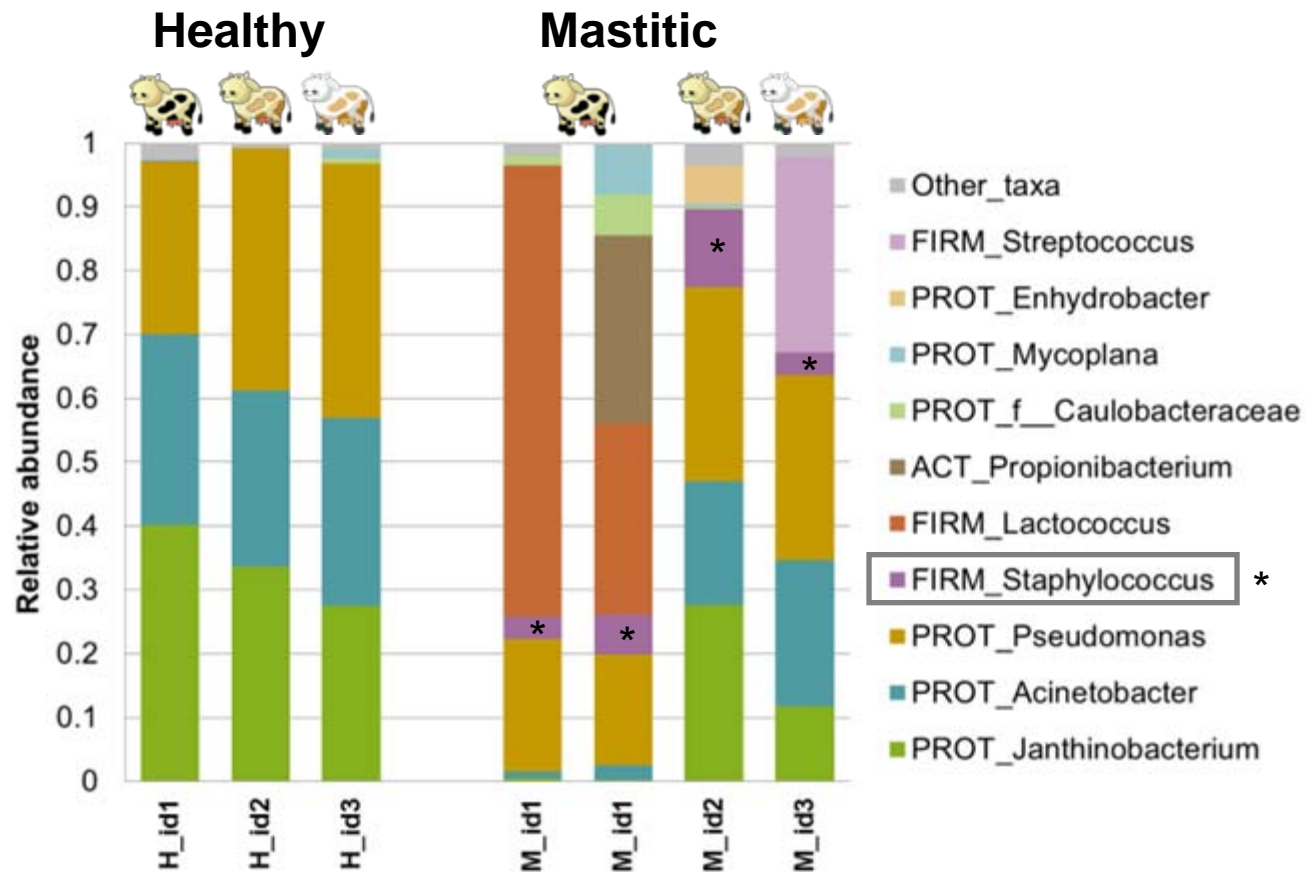
AKP

Healthy quarters - homogeneous taxonomy similar to healthy dim samples  
(*Janthinobacterium*, *Acinetobacter* and *Pseudomonas*)

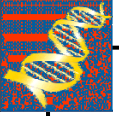
Genera detected on mastitic quarters:  
*Lactococcus*, *Propionibacterium*, *Streptococcus*, *Staphylococcus*



# Results: Microbiota in healthy and mastitic fat samples



Significant differences between healthy and mastitis were detected on fat:  
***Staphylococcus* were overrepresented in all mastitic quarters**

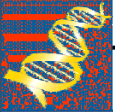


# Conclusions

---

- We developed a sampling and analytical methodology to assess milk microbiota in somatic cells and fat tissues.
- Milk somatic cells and fat are very distinct tissues in microbial affinity and are essential tissues to study milk microbial diversity.
- Milk collected from the endogenous environment of the udder, using a cannula, demonstrates a large microbial diversity in the gland and it is not sterile.
- Somatic cells presented a more diverse microbiota, fat samples were more homogeneous, but gave more insights in detecting significant differences between biological groups (cannula vs no cannula, mastitis vs healthy).
- Milk fat from bovine mastitis presented a diverse bacterial profile, with high abundances of some previously linked or isolated taxa to mastitis.





Thank you !!!

