

# Potential resident bacterial microbiota in udder tissues of culled cows sampled in abattoir



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## INTRODUCTION

Aseptic milking samples and microbiological analyses are used in routine for bovine mastitis diagnosis. In vivo practice, it is really difficult to sample mammary gland tissues (MGT) without risks for the cows' health or the milk production. This first study is thus designed and based on samples harvested at time of culling.

## OBJECTIVES

- Identify
  - Quantify
  - Compare
- Microbiota of macroscopically healthy MGT
  - Microbiota of cow milk of the paired quarter MGT
- Mainly by 16S rRNA amplicon sequencing and additional classical microbiological counts

## MATERIALS AND METHODS

- 13 milk and MGT samples, originated from the same quarter
- 7 reformed cows: 1-2 quarter per cow

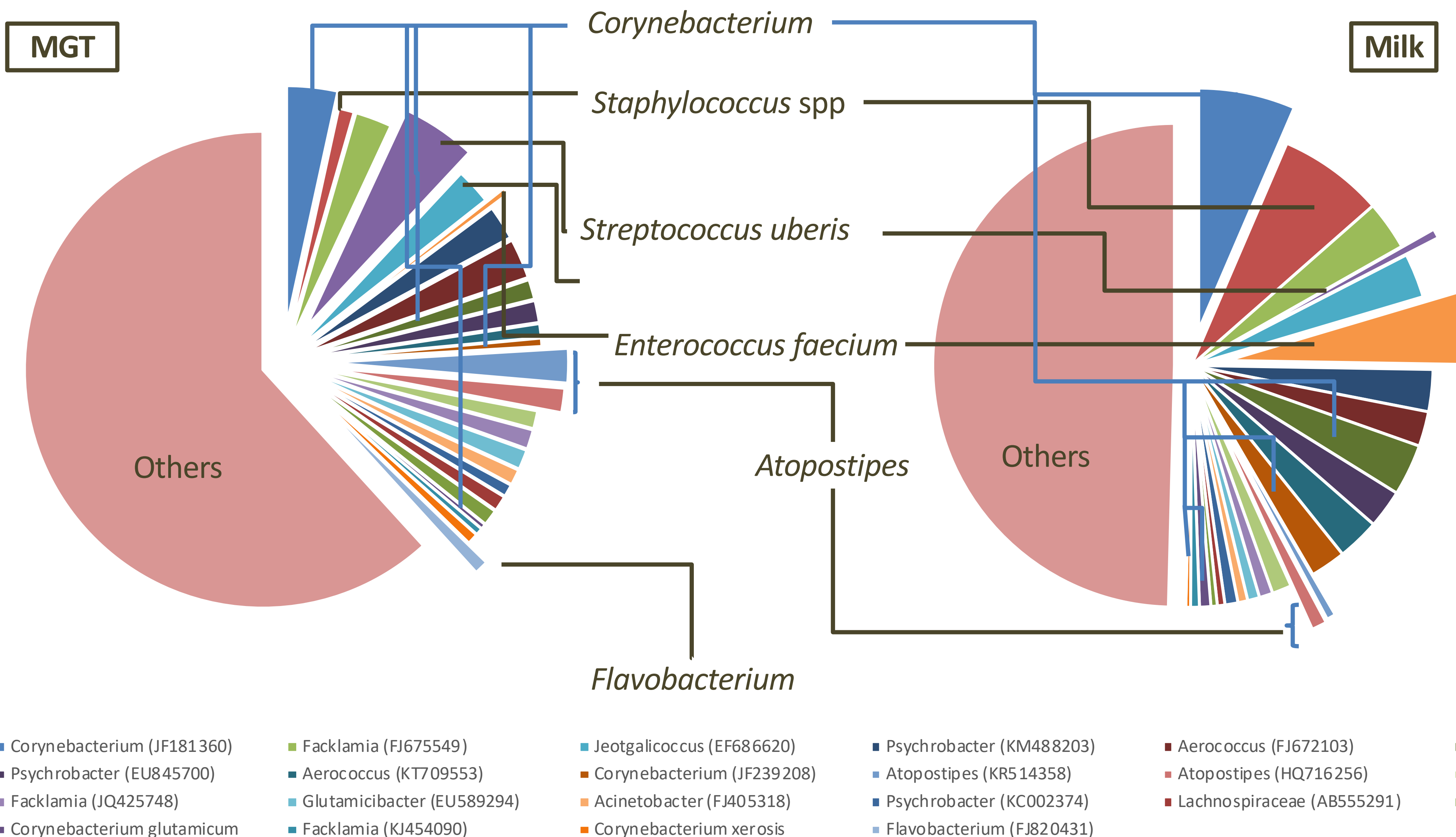
- 1 Aseptic milking
- 2 Culling
- 3 Aseptic mammary gland tissues sampling => 13 paired samples of milk secretions and MGT
- 4 Within 24h: total microflora counts
- 5 Metagenetic analysis
  - DNA extraction (Qiagen Blood & Tissue kit®)
  - PCR amplification & sequencing of the V1-V3 region of the 16S rRNA gene (Illumina MiSeq®)
  - Analysis by mothur tool (v1.39.5) & using SILVA database (v.128)
- 6 Statical analysis

## RESULTS AND DISCUSSION

**Microbiological analysis (Table 1):** Total microflora was generally higher in the milk than in the MGT for the plate inoculation on surface. On the contrary, deep plate seedings showed almost each time, more growth for the tissue compared to surface inoculation.

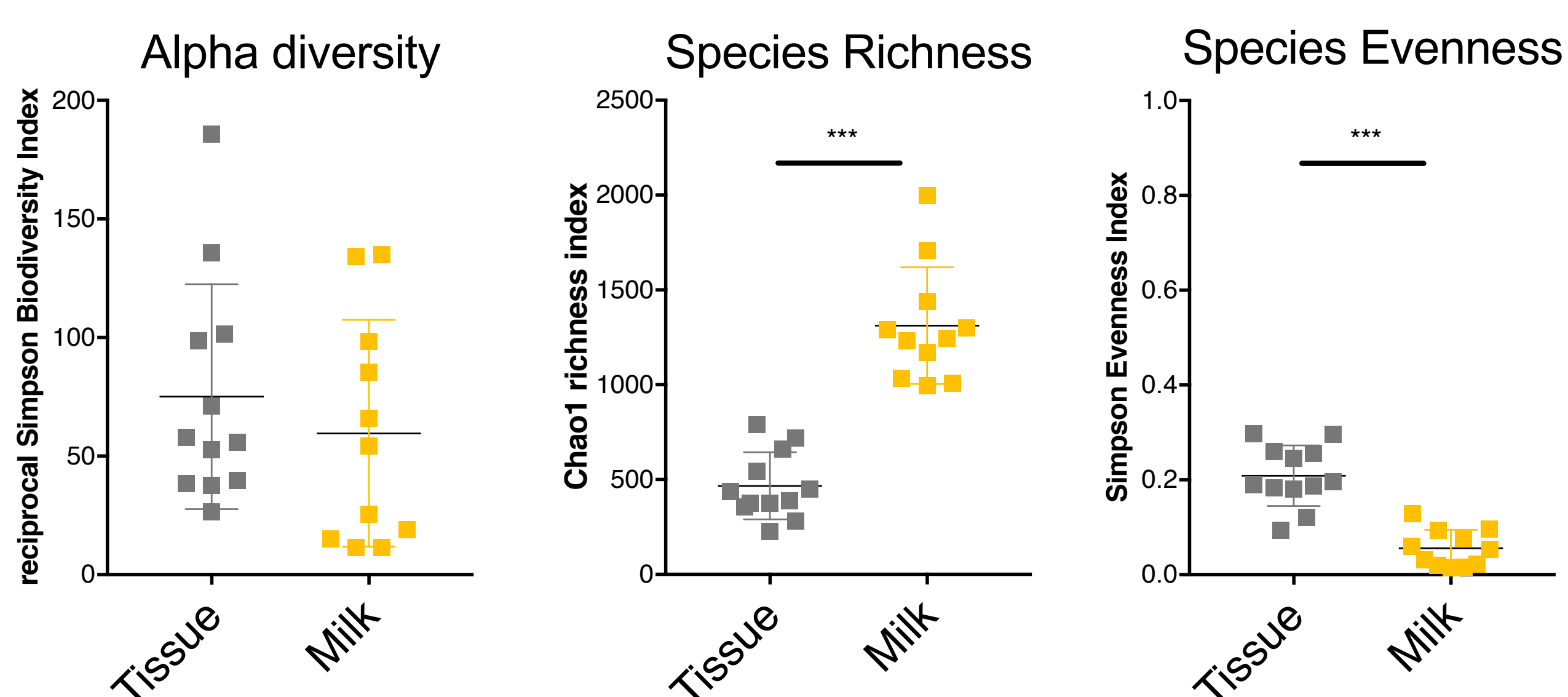
**Table 1:** Average total microbiological counts for both matrix

Media	MGT	Milk
PCA – surface plates	10 <sup>1</sup> CFU/g	10 <sup>2</sup> CFU/g

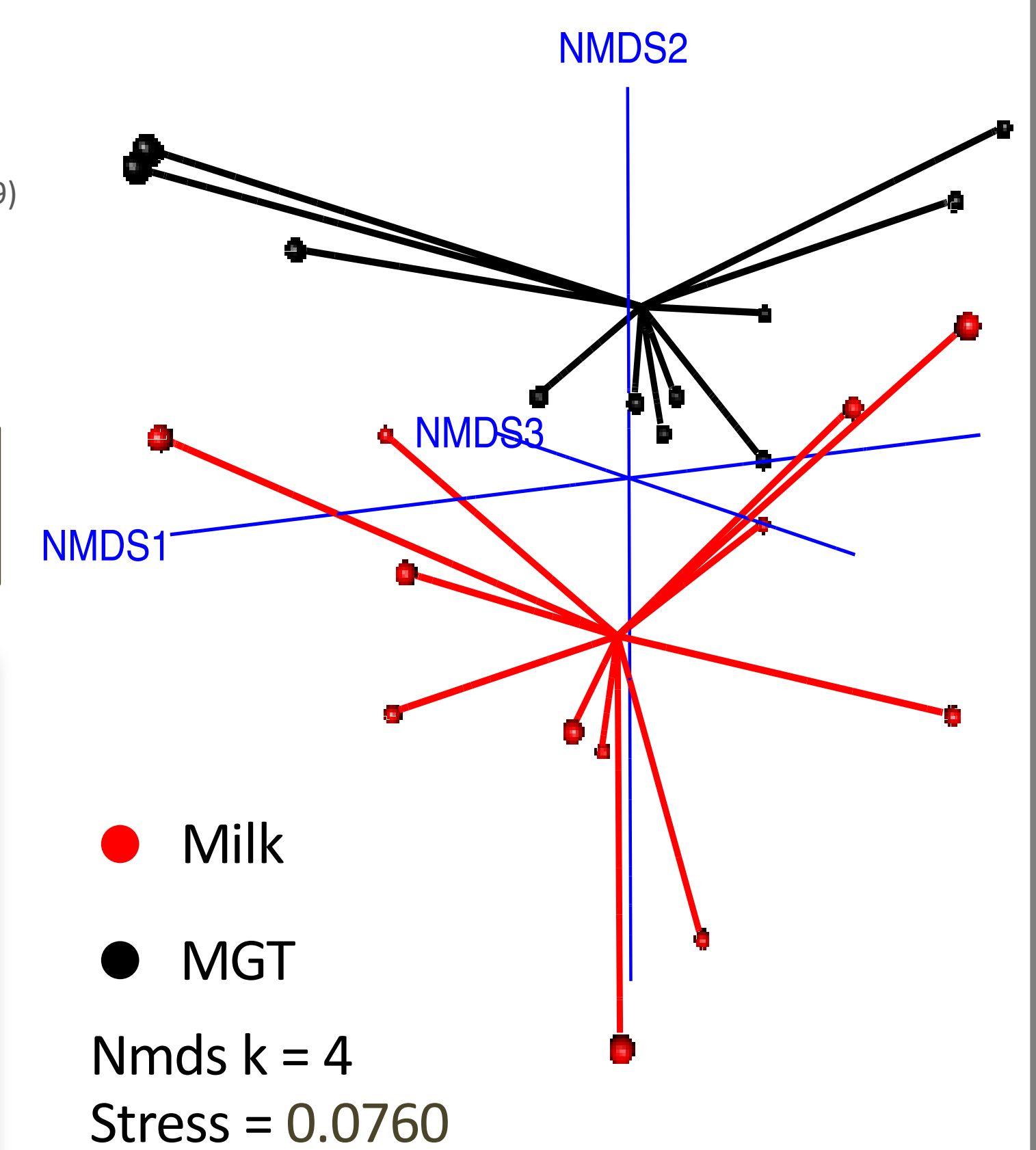


**16S rRNA gene amplicon sequencing (Fig 2):** 4679 species were identified from a total of 21007 OTUs in milk and MGT. *Corynebacterium* was the main genus generally found in the milk in higher proportions than in MGT. When it clearly dominated other populations in milk secretions, it could be found in the same quarter tissues. In case of identification of pathogenic bacteria in a milk sample, the same pathogen were detected in MGT but in very different proportions than in milk: higher for *Streptococcus uberis*, lower for *Staphylococcus spp* or *Enterococcus faecium*.

**Fig 2:** 25 first abundant species identified in both matrix. They represent a bit less than 50% in MGT and near from 50% in milk. Other species are taxa representing less than 0,5% in abundance among the total sequenced OTUs of our study.



**Statistical analysis (Fig 3 & 4):** We could not put in evidence a significant difference for alpha diversity in both matrix. However, data showed that species evenness and beta diversity were greater in mammary glands than in milk secretions. On the contrary, species richness is higher in milk samples. We also found that *Flavobacterium* and *Atopostipes* genera were statistically more abundant in MGT than in milk.



**Fig 4:** Non-metric Multi-Dimensional Scaling showing the clustering of the two bacterial populations.

**Fig 3:** Alpha biodiversity estimation through the species richness and evenness in both MGT and milk samples

## CONCLUSIONS

These results show a potential resident microflora in mammary glands of culled cows in abattoirs with notably, the genus *Corynebacterium* which is considered until now, belonging to the minor pathogen class of intra-mammary pathogen germs. 16S rRNA gene amplicon sequencing of milk samples could be a good indicator of the udder microbiota and health in the future but our first results must be completed and confirmed on a larger number of samples. Hypothesis about nature of such a resident flora will have to be confirmed on producing cows before studying bacterial-host interactions.