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Characterization of the microbial diversity of Belgian artisanal cheese using metagenetics

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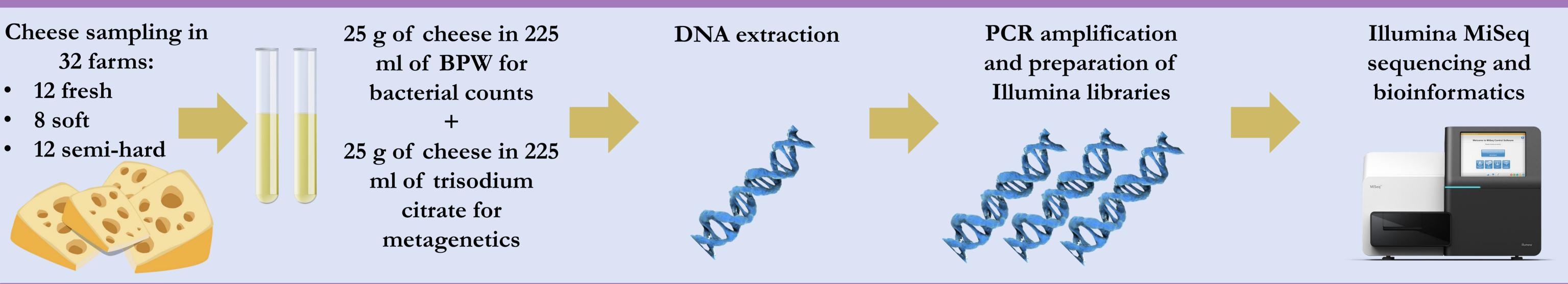
INTRODUCTION

More than 450 artisanal cheeses have been identified in Belgium during a survey, including typical products like Maquée, Herve or Abbaye. Cheese microflora originates from raw milk, from starters, and from environment. Microbial communities of cheese are thus highly complexe and diverse.

OBJECTIVES

- To identify bacteria found in artisanal cheese
- To study the ecological diversity of cheese microbiota
- To compare microbiota between major cheese families

MATERIALS AND METHOD

Cheese sampling in 32 farms:
- 12 fresh
- 8 soft
- 12 semi-hard

25 g of cheese in 225 ml of BPW for bacterial counts
+
25 g of cheese in 225 ml of trisodium citrate for metagenetics

DNA extraction

PCR amplification and preparation of Illumina libraries

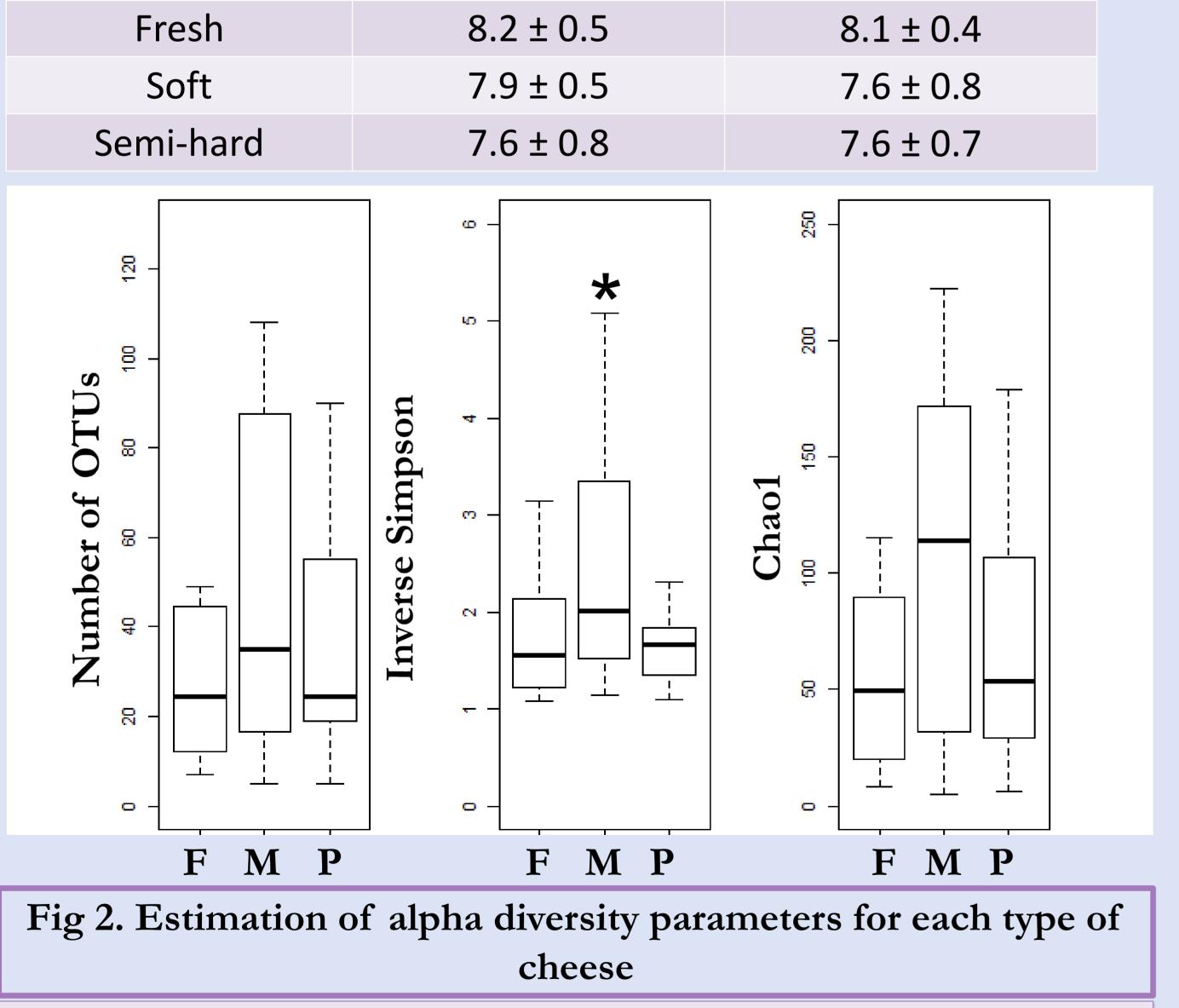Illumina MiSeq sequencing and bioinformatics

RESULTS

Table 1 shows that bacterial levels found in artisanal cheeses are around 8 $\log_{10}$ cfu/g., mainly corresponding to lactic acid bacteria. As a consequence, from 16S rRNA gene amplicon sequencing, after rarefaction to 10,000 sequences, genera *Lactococcus* and *Streptococcus* represented between 85 and 98 % of the reads in all cheese types (**Fig 1**). However, 3,202 OTUs were identified, belonging to 14 phyla and to 246 genera. Only 11 genera represented more than 0.1 % of the reads in at least one cheese type. *Brevibacterium* and *Brachybacterium* were much more frequent in soft cheeses. Surprisingly, strict anaerobes were identified, including *Bacteroides*, *Bifidobacterium* and *Preveotalla*.
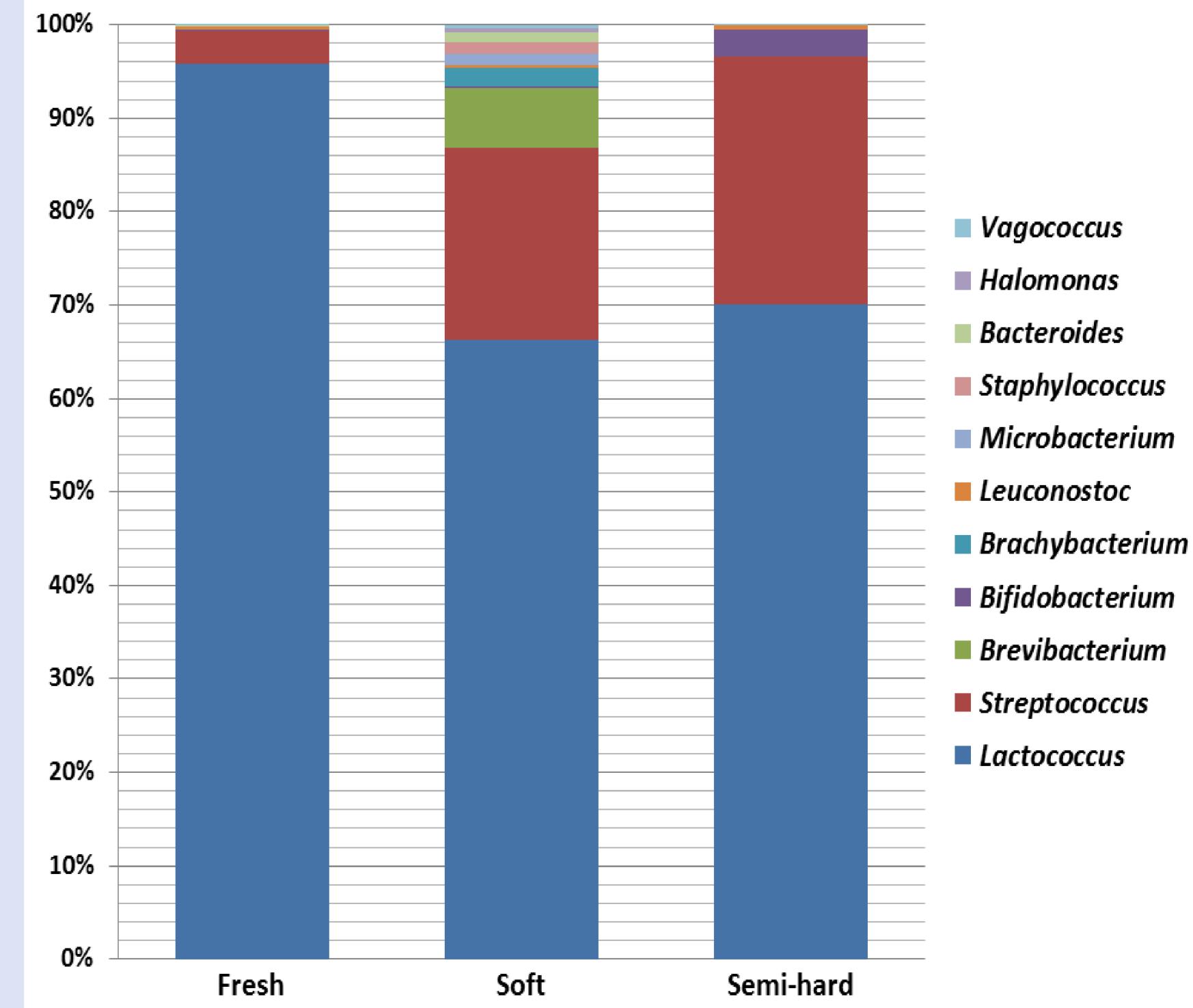
Type of cheese	TVC ($\log_{10}$ cfu/g)	LAB ($\log_{10}$ cfu/g)
Fresh	8.2 ± 0.5	8.1 ± 0.4
Soft	7.9 ± 0.5	7.6 ± 0.8
Semi-hard	7.6 ± 0.8	7.6 ± 0.7

Table 1. Total viable counts (TVC) and lactic acid bacteria (LAB) counts by cheese type

Fig 2. Estimation of alpha diversity parameters for each type of cheese

Species evenness (Inverse Simpson index) was significantly higher in soft cheese than in other cheese types, with FDR p-values adjustments. Although huge variability was observed, no statistical differences were observed regarding richness (Number of OTUs and Chao1 index ; **Fig 2**). Given the predominance of two genera, AMOVA and HOMOVA tests were performed on a Jaccard distance matrix, instead than on a traditional Bray-Curtis matrix. Significant differences in beta diversity between semi-hard cheeses and other samples were observed.

Legend:
- *Vagococcus*
- *Halomonas*
- *Bacteroides*
- *Staphylococcus*
- *Microbacterium*
- *Leuconostoc*
- *Brachybacterium*
- *Bifidobacterium*
- *Brevibacterium*
- *Streptococcus*
- *Lactococcus*

Fig 1. Bacterial genera with at least 0.1% of relative abundance identified in each type of cheese

CONCLUSION

These results show that the dominant microflora of all types of cheese is composed of two genera: *Lactococcus* and *Streptococcus*. However, subdominant and minor populations vary between samples and should require more attention, especially while looking for bacteria or bacterial consortia able to act on the growth of pathogens such as *Listeria monocytogenes*. Further steps would be to identify a core microbiome for each type of cheese and to study the evolution of this microbiome during cheese storage. Investigating on the presence of anaerobic species is another alternative.