

# Active but undetected, detected but inactive; DNA- and RNA-based amplicon sequencing analyses reveal divergent pictures of the bacterial communities in cheese

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## Introduction

Each ecosystem displays specificities making the study of its microbiome particular. In cheese, bacteria are immobilized in a solid matrix, and DNA stays intact for a relatively long period of time, either free in the matrix, or in metabolically active, dormant, or dead cells. It therefore reflects the history of the succession of populations. On the other hand, results obtained by the means of RNA-based analyses would rather reflect the metabolically active members of the bacterial community at a given time-point, as rRNA is much less stable over time.

## Objectives

Determine strengths and limitations of 16S amplicon-based sequencing analyses using **DNA** and **RNA** for **3 issues** in cheese research (temporal evolution during maturation, spatial variability, manufacturing process).

## Material and Methods

- Extraction of DNA and RNA
- PCR amplification (primers 8f / 355r) of 16S rRNA gene
- NGS sequencing: IonTorrent PGM
- Compare **present** and **metabolically active** members of the bacterial communities
- 3 Swiss cheese types** analyzed (Gruyère, Emmentaler, Raclette)

## Global picture

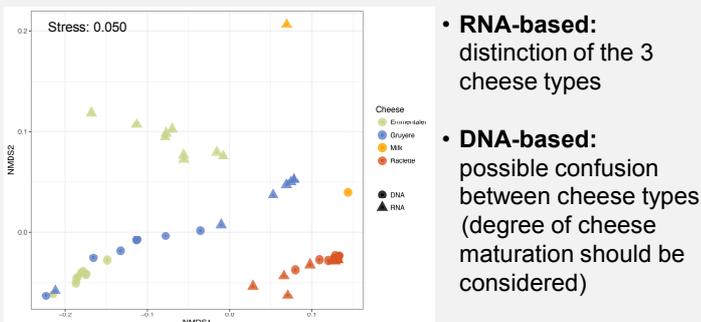


Fig.1. NMDS of all analyzed samples and milk used for the manufacture of the Gruyère cheese.

- RNA-based:** distinction of the 3 cheese types
- DNA-based:** possible confusion between cheese types (degree of cheese maturation should be considered)

## Spatial variability

- Radically **different pictures** provided by present or active bacterial communities.
- Zonation not reflected** in the bacterial community structures.

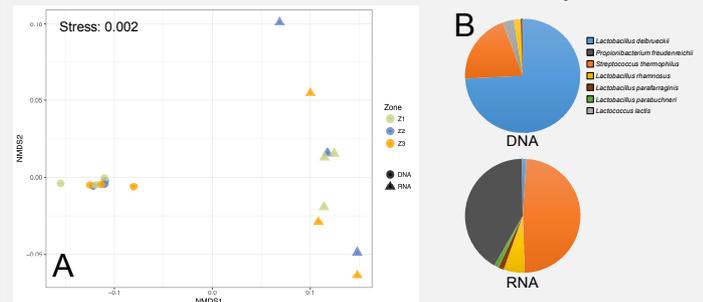


Fig.3. A. NMDS of samples extracted from 3 different zones of an Emmentaler cheese produced 3 consecutive days with identical conditions. B. Differential composition of present and metabolically active bacterial community analyzed in the same sample (zone 2, 1<sup>st</sup> sampling day).

## Temporal evolution during maturation

- Present and active bacterial communities display **different structures**, except after 1 day of maturation.
- Present bacterial communities display similar but **delayed trend** as compared to the metabolically active bacterial community.

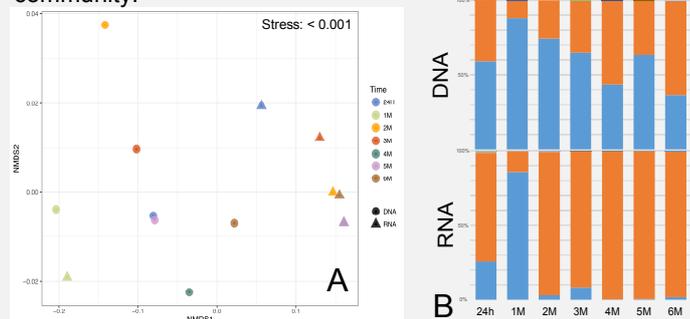


Fig.2. A. NMDS of samples extracted from Gruyère cheese during maturation (1 day to 6 months). B. Composition of the present and metabolically active bacterial community.

## Manufacturing process

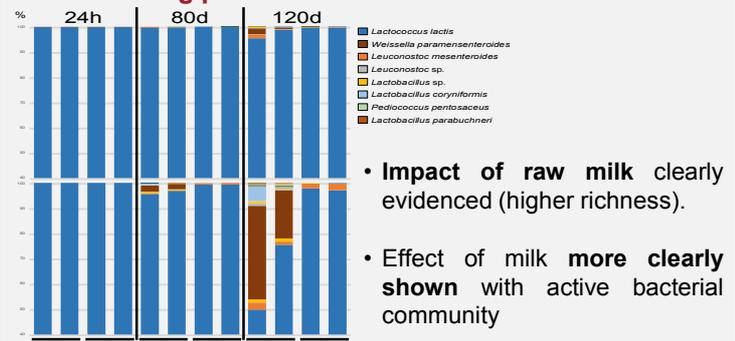


Fig.4. Bacterial community structures at 1, 80, and 120 days of maturation in 2 Raclette cheeses produced with raw (-) or pasteurized (+) milk, respectively.

## Conclusions

- RNA-based 16S amplicon sequencing analyses better suited to point out variations in bacterial communities.
- RNA-based analyses provide a snapshot of metabolically active members of bacterial communities
- Dominant species originating from the starter cultures may bias the bacterial community depicted using DNA-based analyses.

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