

Insights into the microbial biodiversity of cheese



Cornelia Bär^{1*}, Aline Moser¹, Daniel Wechsler¹, Stefan Irmeler¹

¹Agroscope, CH-3003 Bern; www.agroscope.ch

* cornelia.baer@agroscope.admin.ch

Introduction

Cheese quality is mainly influenced by microbes and their metabolic activities. In recent years, efforts have been undertaken to gain insights into the microbial complexity of cheese by classical culture-based methods. Since these techniques solely detect cultivable bacteria, only a part of the complexity and diversity of microbial communities within cheese could be investigated. Nowadays, due to progress in next-generation sequencing (NGS) technologies and computational methods for the analysis of high-throughput sequencing data, cultivable and non-cultivable bacteria of complex communities can be analyzed in a relatively short time and at low costs. We established a workflow based on 16S rRNA amplicon sequencing to study bacterial communities in two cheeses with quality defects.

Material

For the development of a workflow (Fig.2) in order to gain insights into the microbial community of cheese two cheeses with defects were used as starting material (Fig.1). The starter lactic acid bacteria were known and the presence of the problem causing bacteria (*) were additionally confirmed by other methods.

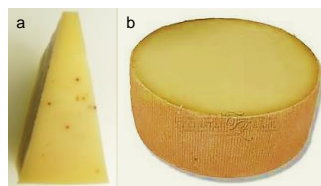


Fig.1: A pasteurized goat milk cheese with red spots (a) and a raw milk cheese (Tête de Moine) with a "burning" taste (b) were used as starting material.

Workflow

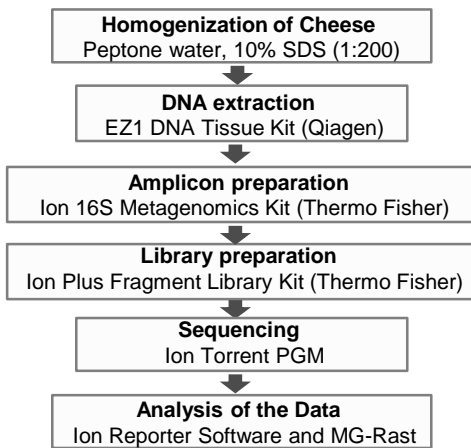


Fig.2: Workflow established for investigation of the bacterial community in cheese.



Fig.3: Primer set of the Ion Metagenomics Kit targets seven of nine hypervariable regions (green squares, shown for *E. coli* 16S rRNA gene).

Conclusions

- ❖ detection of starter bacteria (shown for genus level in Tab.1)
- ❖ loss of microbial diversity in cheese from pasteurized milk (Tab.1) compared to cheese made from raw milk
- ❖ identification of bacteria producing undesired metabolites (Tab.1, (*); Fig.4, species framed in red)
- ❖ detection of yet unclassified bacteria and identification of various bacteria not belonging to lactic acid bacteria (Tab.1 and Fig.4)

Metabolic activities of non-lactic acid bacteria are often not known and their influence on cheese ripening needs to be studied. Although there are still obstacles to be overcome before cheese metagenomics can be used as a routine diagnostic tool, the presented work shows that metagenomics provides new opportunities, perspectives and ways to explain food fermentation on the molecular level. Further research in this field will bring solutions to cheesemakers that are affected by cheese quality problems.

Bacteria	Raw Milk Cheese	Pasteurized Milk Cheese
Bacillus	+ 0.17 %	-
Brachy bacterium	+ 0.58 %	-
Brevibacterium	+ 11.9 %	+ 0.22 %
Carnobacterium	+ 0.32 %	-
Corynebacterium	+ 4 %	-
Jonesia	+ 0.17 %	-
Lactobacillus	+ 26.9 % (SLAB)	+ 0.77 %
Lactococcus	+ 0.22 %	+ 70.3 % (SLAB)
Leifsonia	+ 1.56 %	-
Leuconostoc	+ 0.36 %	+ 0.23 %
Microbacterium	+ 3 %	-
Pediococcus	+ 1 %	-
Rhodococcus	+ 0.8 %	-
Staphylococcus	+ 11.3 %	-
Streptococcus	+ 6.6 % (SLAB)	+ 2 %
Tetrasphaera	+ 0.21 %	-
Weissella	+ 0.075 %	-
Enterococcus	+ 0.1 %	+ 4 %
Nocardiopsis	-	+ 0.3 %
Propionibacterium*	-	+ 4.9 %
Unclassified bacteria	+ 31.6 %	+ 16.9 %

Tab.1: Comparison of the most abundant bacterial genus found in a raw milk cheese (Tête de Moine) and a cheese made of pasteurized goat milk illustrates the loss of diversity due to pasteurization (% of reads annotated to the rRNA genes on genus level; SLAB: starter lactic acid bacteria). Species of the genus *Propionibacterium** were found to be responsible for formation of the red spots in the pasteurized goat milk cheese.

Metagenome of a raw milk cheese (Tête de Moine) with a "burning" taste

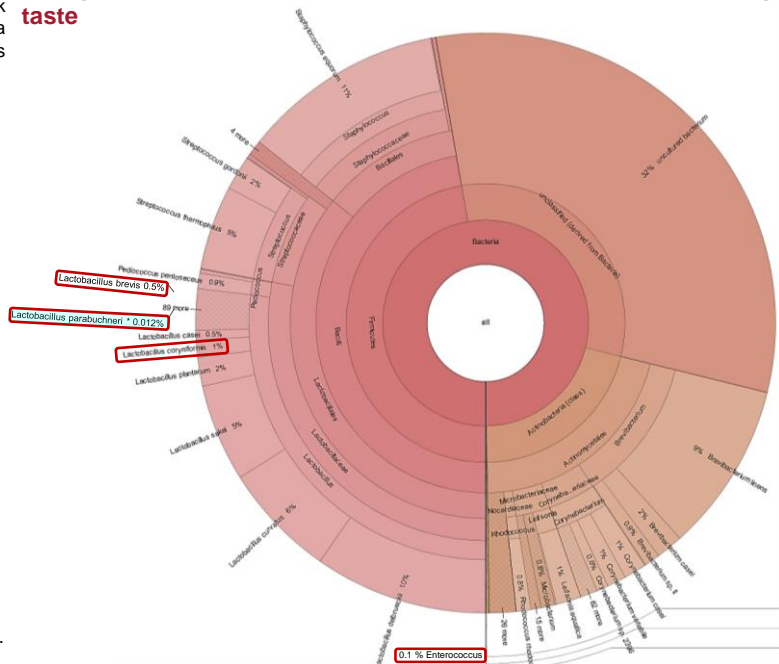


Fig.4: Bacterial community in a Tête de Moine sample (consensus view at the species level) annotated and taxonomical assigned by MG-RAST. A total number of 159 bacteria were found. Bacteria with the potential to form biogenic amines are marked in red. *Lb. parabuchneri** was found to be responsible for the "burning" taste by production of histamine (colored turquoise).