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# Microbial Communities of Raw Milk Cheeses, A Review

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**ABSTRACT**

Microbial communities play a fundamental role in shaping the taste, aroma, and texture of cheeses. They consist of starter and secondary microorganisms. Starters contribute to acid development during cheesemaking, while secondary microbiota play a crucial role in the ripening process. Their diversity is a subject of significant importance, shaped by various factors such as the cheesemaking environment, employed starters, physicochemical conditions, and manufacturing procedures. In this review, we attempted to provide an accurate picture of the microbial communities commonly found in raw milk cheeses and tried to list their origins, factors influencing their existence, and the approaches used for their screening. The research employed information retrieval methods, mainly focusing on specific keywords. We systematically searched various databases for relevant articles and reviews, prioritizing the retrieval of the most recent publications and those deemed most relevant to the objectives of this review. This review disclosed the frequently identified bacterial genera in cheeses, such as *Lactococcus* and *Lactobacillus*. In terms of fungi, regularly isolated species included *Candida*, *Kluyveromyces*, *Saccharomyces*, *Yarrowia*, *Goetrichum*, among others. Our investigation enabled the unveiling of both the core microbiota shared across diverse cheeses, crucial for cheese fermentation and ripening, and the variable microbiota contributing to the diversity in cheese characteristics.

## 1- Introduction

The story says that curd formation was accidentally discovered when milk was kept in bags made from animal stomachs. Hence, the idea of milk preservation through curdling emerged [1]. What initially began as milk conservation has transformed into an impressive variety of cheeses, each possessing its unique cheesemaking process, texture, flavor, and shape. Consequently, the classification systems developed for categorizing cheeses are diverse and sometimes confusing. To date, no classification scheme has been universally recognized as complete and satisfactory [2].

Today, there are over 4,000 varieties of cheese produced worldwide [3], with the majority manufactured in accordance with strict guidelines to ensure the quality of the final product. In 2015, global cheese production reached approximately 20 million tonnes, marking a 23% increase from 2005 to 2015 [4].

Obtaining various types of cheese, even when similar ingredients and manufacturing procedures are employed, raises questions about this enigmatic product. Scientific evidence supports the notion that cheese is a living system, and its variability stems from fermentation and ripening steps guided by a multitude of cheese microorganisms [5]. Understanding these primary actors in the cheese production process is crucial for a more precise appraisal and the enhancement of manufacturing conditions. The primary objective of this study was to compile the most recent, albeit not exhaustive, findings on the microbiota of raw milk cheese.

This review is divided into five main sections. We started by giving an overview of raw milk cheeses. Then, we listed the main sources of their indigenous microorganisms; we discussed some of the factors that influence their presence as well as the approaches that researchers use to screen them, including culture-dependent and culture-independent tools. Finally, we used research examples to summarize recent insights into cheese microbial populations. This review could provide valuable insights for novice researchers interested in cheese microbiology.

The information retrieval methods utilized in this research predominantly involved the use of specific keywords, including "raw milk," "artisanal cheese," "raw milk cheese," "metagenomics," "high throughput sequencing," and "cheese biodiversity." Various databases, such as the Web of Science, Science Direct, Pubmed and ResearchGate were systematically searched for relevant articles and reviews. The selection process prioritized the retrieval of the most recent publications and those deemed most relevant to the objectives of this review. The extensive reading of numerous articles and reviews aimed to extract the most pertinent information for a comprehensive understanding of the subject matter.

## 2- LITERATURE

### 2.1. Raw versus pasteurized milk cheeses

Raw milk, as defined by the code of hygienic practice for milk and milk products (CAC/RCP 57-2004), is "*milk which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect*." In contrast, pasteurized milk is defined as milk subjected to a heat treatment to reduce the number of any potential pathogenic microorganisms to a level at which they do not pose a significant health hazard. Pasteurization is commonly conducted at 72°C for 15 seconds [6].

Recently, cheeses crafted from raw milk have garnered renewed interest, primarily owing to their authentic taste, aromas, and pronounced flavors. The distinctiveness of raw milk cheeses arises from the rich and diverse microbial populations naturally present in the raw milk, including primary and secondary cultures [5].





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Nevertheless, the consumption of raw milk products is not without consequences and may pose serious impacts on public health. This is why public health authorities mandate a ripening period of at least 60 days for raw milk or raw milk cheese that has not undergone pasteurization [7]. It is widely acknowledged that pasteurization eliminates bacteria from raw milk, but it is also recognized that it eradicates microbial entities responsible for flavor and health benefits. For this reason, cheese connoisseurs often refer to pasteurized cheeses as "*dead cheeses*" [8].

Ensuring the production of a cheese that is both safe and flavorful is among the primary challenges faced by cheesemakers. To address this, some producers use selected starters isolated from high-quality raw milk cheeses to "*restitute*" the natural taste.

### 2.2 Microbial origin of raw milk cheeses

While a growing body of research explores the intricacies of cheese microbiota, few studies have delved into its origin. Cheese harbors several types of microbiota, including bacteria, yeasts, and molds, coexisting at different stages of production—both during manufacturing and ripening—forming a dynamic and complex ecosystem. These microorganisms predominantly originate from milk (a key ingredient in cheesemaking) and the cheese-making environment.

#### 2.2.1. Origins of milk microbiota

Milk provides an ideal environment for a diverse microbiota, owing to its rich composition of fats, proteins, sugars, vitamins, and minerals. Several factors contribute to this microbial diversity, including the microbiota found on animals' teats, the overall farm environment, interactions with dairy workers, and the dairy equipment. For example, the teat skin microbiota demonstrates significant biodiversity, consisting of 66 different species from genera such as *Enterococcus*, *Pediococcus*, *Enterobacter*, *Pantoea*, *Aerococcus* and *Staphylococcus*—commonly found in raw milk. Many of these taxa have been identified as key contributors to flavor development during the cheese ripening process [9]. Stable air is also recognized as a significant source of contamination. Vacheyrou et al. [10] demonstrated this by examining the microbial communities in stable air alongside those in raw milk, revealing a notable transfer of microbiota. Additionally, various environmental factors, including soil and silage, can influence the microbial composition of milk. This was demonstrated in a study focusing on the mesophilic lactic acid bacteria (LAB) of milk. Indeed, *Staphylococcus warneri* and *Staphylococcus* sp. were identified in soil, silage, and milk suggesting a potential transfer of these strains from the farm ecosystem to the milk [11]. Moreover, research has shown that hygienic practices wield significant influence over the microbial diversity of raw milk. Under stringent hygiene conditions, milk predominantly features Corynebacteriaceae and Micrococcaceae. Conversely, when produced under less stringent hygienic conditions, milk reveals the presence of diverse taxa, including gram-negative bacteria, *Lactococcus lactis*, *Brevibacterium linens*, and *Leuconostoc mesenteroides* species. This illustrates the direct impact of farming practices on the microbial communities present in raw milk [12]. Milk equipment also plays a significant role. In a study conducted by Didienne et al. [13], it was found that wooden vats significantly enriched the microbial populations in stored milk. Interestingly, deliberate inoculation of milk with pathogens such as *Listeria monocytogenes*, *Salmonella*, or *Staphylococcus aureus* within the same research had no apparent effect on the biodiversity of the vats. The vats remained uncontaminated and uncolonized.

#### 2.2.3. Origins of cheese microbiota

The microbial composition of cheese is primarily influenced by the indigenous microbiota of milk. However, the cheesemaking environment, including surfaces, curd tanks, molding machines, and ripening



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rooms, also exerts a significant impact. Furthermore, the inclusion of starter cultures, such as whey, plays a crucial role in shaping the microbiota of cheese. Kamimura et al. [14] investigated microbial diversity in samples from the cheesemaking environment, cheese ingredients, and ripened Serra da Canastra cheese (21 days) across three different farms in Minas Gerais, Brazil. Among its key findings, the study indicates a strong link between the microbiota found in 'pingo' (a natural fermented whey) used as a starter culture and the ripened cheese. In a separate study, Quijada et al. [15] examined the bacterial communities of the Vorarlberger Bergkäse (VB) cheese rind and different processing surfaces in the ripening cellars. Certain bacterial groups, such as coryneforms, *Staphylococcus equorum*, and *Halomonas*, were found both in the cheese and on most environmental surfaces, suggesting their transfer to the cheese surface during ripening. Additionally, **Calasso et al.** [16] conducted another study revealing the presence of specific house microbiota, such as *Lactobacillus plantarum*, in the rind of Caciocavallo Pugliese cheese, the curd tank, and molding machines. Furthermore, other bacteria were detected in both the ripening rooms and the rinds of Caciotta and Caciocavallo Pugliese cheeses. In a comparative study exploring different production methods, primarily distinguished by the type of equipment used during cheese-making (stainless steel and wooden equipment), it was revealed that particular strains within the *Enterococcus* spp. group including *Enterococcus faecalis*, *Enterococcus casseliflavus*, and *Enterococcus gallinarum* originated from wooden vats and persisted during the ripening of Caciocavallo Palermitano cheese. In contrast, these strains were not present in the cheese made by the standard method [17].

### 2.3. Factors influencing cheese microbiota

The growth of microbial communities can be either positively or negatively influenced throughout the cheesemaking process. Several factors come into play, including the pasteurization and refrigeration of milk, the temperature of curd cooking, pH levels, salt content, the season of cheesemaking, and various other parameters. While investigating the impact of milk refrigeration on cheese microbiota, it was found that towards the end of the ripening process for Serra de Estrela cheese, non-refrigerated milk cheeses exhibited elevated counts of Enterobacteriaceae and diminished counts of LAB in comparison to refrigerated milk cheeses [18]. In a separate study examining the effect of salt on starter viability, strains of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar diacetylactis, and *L. lactis* subsp. *lactis* isolated from DL-starter commercial cultures underwent viability tests using varying NaCl concentrations. The results revealed strain-dependent outcomes but indicated a negative correlation between viability and elevated NaCl concentrations for all strains [19]. The growth of certain strains can also be influenced by the cooking temperature. In a study involving a semi-hard cheese with *Streptococcus thermophilus* as a starter, the levels of this strain were observed to be impacted by the cooking temperature during production. Notably, elevating the cooking temperature from 47°C or 50°C to 53°C resulted in a reduction in the mean viable cell numbers of *S. thermophilus* strains during various manufacturing stages, particularly during the transition from maximum scald temperature to pre-press and brining steps. Conversely, it was observed that the cooking temperature had no discernible effect on the viability of *Lactobacillus helveticus* and other non-starter lactic acid bacteria (NSLAB) strains [20]. The pH of whey during drainage also plays a pivotal role in influencing the microbial composition of cheese. In a specific study, the reduction in whey pH during the drainage process of mozzarella cheese was linked to higher counts of mesophilic and thermophilic lactobacilli during the subsequent refrigeration phase of the cheese [21]. The season of cheese manufacturing may also exert an influence on cheese microbiota. Artisanal cheeses collected





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during the dry and rainy seasons exhibited distinct microbial patterns. In the dry season, cheeses were predominantly characterized by the presence of *Enterococcus* and *Lactobacillus*, while in the rainy season, there was a prevalence of *Lactococcus* and *Weissella* genera [22]. The composition of the starter culture also plays a crucial role in shaping the bacterial growth of naturally occurring bacteria in cheese. The investigation into the microbiology of a 90-day ripened Graviera cheese delved into the impact of natural starter culture (NSC) and commercial starter culture (CSC). The study revealed that nonstarter bacteria, specifically *Lactobacillus paracasei* and *Lactobacillus plantarum*, prevailed in cheeses produced with commercial starters. In contrast, cheeses crafted with natural starters predominantly featured indigenous strains of *Enterococcus faecium* and *Enterococcus durans* [23].

## 2.4. Investigation of the cheese microbiota by Culture-dependent and culture-independent techniques

With the advent of molecular methods, researchers have attained a more comprehensive understanding of microbial communities in cheese. Numerous molecular tools are currently at their disposal, and these can be categorized into two types: culture-dependent and culture-independent methods. The former necessitates an isolation and culturing step, while the latter omits this requirement, enabling the analysis of extracted DNA/RNA directly from the matrix of interest.

### 2.4.1 Culture-dependent methods

**Randomly Amplified Polymorphic DNA (RAPD)** is a PCR-based method that employs single small primers and low annealing temperatures. This generates multiple bands, usually 3–12 bands of varying sizes, providing distinct profiles for each strain. The method is quick and easy to operate, but its major drawbacks include poor reproducibility and a lack of standardization among laboratories [24].

**Repetitive Sequence-Based PCR (rep-PCR)** technique amplifies genomic regions between repetitive sequences using primers like REP, ERIC, or BOX. Each species produces unique patterns with bands of varying sizes, enabling differentiation from others [25].

**16S rRNA gene sequencing**, a widely used technique, allows species identification by providing information about the partial or complete nucleotide sequence of the 16S rRNA gene [26]. The obtained sequence is compared with a database, such as NCBI, containing numerous available sequences to determine closely related species.

### 2.4.2. Culture-Independent methods

Among PCR-based culture-independent methods, examples include **Temporal Temperature Gel Electrophoresis (TTGE)** and **Denaturant Gradient Gel Electrophoresis (DGGE)**. TTGE employs a constant temperature and denaturant, while DGGE utilizes an increasing gradient of chemical denaturant (urea and formamide) along with a constant temperature. This allows the separation of amplicons of similar length but with dissimilarities in nucleotide sequence. For species identification, the bands obtained are either compared with co-migrated reference strain amplicons or excised from the gel for sequencing [27].

Culture-independent approaches also include the single-strand conformation polymorphism (SSCP) method. In this technique, the sequence signature governs the single-stranded DNA conformation [28].

High-throughput sequencing (HTS) is increasingly utilized and gaining popularity for analyzing microbes in food samples. This technique can be used in three ways: by targeting a specific sequence, mainly the 16S rRNA gene (amplicon sequencing), by analyzing non-targeted sequences (shotgun


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metagenomics), or by investigating the total expressed genes in a sample (metatranscriptomics, RNASeq) [29].

### 2.5. Current state-of-knowledge on cheese microbial communities

#### 2.5.1. Fresh unripened cheeses

Table 1

The microbial composition of fresh unripened cheeses is detailed in Table 1. In the variety of freshly made unripened cheeses, Ranchero cheese underwent a thorough examination of its LAB, revealing a total of 172 isolated species. Within this diverse spectrum, 42.5% were identified as *Lactococcus* spp., 41.8% as *Enterococcus* spp., and 8.1% as *Lactobacillus* spp. When categorizing *Lactococcus* spp. at the species level, *L. lactis* subsp. *lactis*, succeeded by *L. garvieae*, emerged as the most prevalent species [30]**.** El Galiou et al. [31] explored the microbiota of Jben cheese made with goat milk by using different cheesemaking procedures. In the cheese produced using the traditional cheesemaking method involving calf rennet and uncooled milk, the following genera were identified: *Enterococcus*, *Lactococcus*, *Lactobacillus* and *Listeria*. An investigation into yeast communities in artisanal cheeses from various regions in Morocco revealed the presence of 18 species distributed in a cheese-dependent manner. The most common species included *Kluyveromyces lactis, Saccharomyces cerevisiae, Yarrowia lipolytica, Candida parapsilosis, Kazachstania unispora, Kluyveromyces marxianus and Pichia fermentans* [32]**.** In another study, LAB were isolated from Oaxaca cheese at various stages of processing and sequenced using the 16S rDNA gene. The main species found in ready-made Oaxaca cheese were *E. faecalis*, followed by *L. lactis* subsp. *lactis*, *E. faecium*, *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei and* finally *Lactobacillus rhamnosus*. Additionally, Rep-PCR analysis showed high molecular diversity within *L. lactis* species [33]**.** In a comprehensive examination of Buryatian cottage cheese, PacBio sequencing identified seven distinct phyla in samples from various regions. Notably, Firmicutes (73.57%) and Proteobacteria (26.27%) emerged as the predominant phyla. Both culture-dependent and culture-independent methods revealed the presence of diverse genera, including *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Acetobacter, Klebsiella, Acinetobacter, Pseudomonas, Raoutella* among others. Remarkably, the most abundant genera observed were *Lactococcus* spp. and *Streptococcus* spp. [34]**.**

Mendez Romero et al. [35], investigated the bacterial composition of 36 samples of fresco cheese from Sonora, Mexico. According to HTS results, the most representative phylum was Firmicutes, followed by Proteobacteria. At the family level, Streptococcaceae dominated with 52.87%, followed by Lactobacillaceae (9.61%) and Leuconostocaceae (7.19%). The representative genera included *Lactococcus* with a relative abundance of 28.22%, followed by *Streptococcus* (16.08%), *Lactobacillus* (8.03%), and *Leuconostoc* with a percentage of 6.82%. The Venn diagram facilitated the identification of shared genera, namely *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., and *Leuconostoc* spp. PCA analysis indicated that the dominant genera are *Lactococcus* spp., *Streptococcus* spp., and *Leuconostoc* spp.

#### 2.5.2. Soft cheeses

**Table 2**

The microorganisms found in soft cheeses are presented in table 2. Minas cheese, sourced from distinct geographical regions (Serro, Canastra, Araxá, Serra do Salitre, and Campo das Vertentes), underwent analysis using both culture-dependent methods (sequencing of LAB isolates) and independent methods (LH-PCR, Rep-PCR). Ten genera were discerned, with *Lactobacillus* emerging as the most prevalent. Additional taxa included *Lactococcus, Enterococcus, Weissella,*




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*Pediococcus*, *Leuconostoc*, *Streptococcus*, *Escherichia*, *Kocuria*, and *Staphylococcus*. Distinct microbial patterns were observed in the cheeses, suggesting a dependence on the cheese farm and geographical region. Furthermore, four species were exclusively found in specific cheeses; *Lactobacillus delbrueckii* in Canastra cheese, *Kocuria kristinae* in Serra do Salitre cheese, and (*L. garvieae*, *Lactobacillus acidipiscis*) in Campo das Vertentes cheese [36]. Three white pickled cheeses collected at various ripening stages from two distinct locations (L1) and (L2), underwent yeast and mold content analysis. All of the cheeses were dominated by *Debaryomyces hansenni* and *Candida zeylanoides*. Only cheese from location two was found to have *Y. lipolytica* and *Galactomyces goetrichum. K. lactis* was detected in the three cheeses. No mold was observed in any of the cheese samples [37].Tomme d'Orchies cheese was evaluated for its surface and core microbiota. For ripened cheese (21 days), *Streptococcus* spp. and *Lactococcus* spp. were the most abundant genera in the cheese's core while *Corynebacterium* spp. and *Brevibacterium* spp. were the least abundant. Notably, bacterial diversity was more pronounced on the cheese's surface compared to the core. Major taxa identified in the ripened cheese included *Corynebacterium* spp. and *Psychrobacter* spp. [38].

Metagenomic analysis uncovered a notable fungal diversity, particularly yeast, in Tomme d'Orchies cheese. Similar to a prior study (84), the surface microbiota exhibited greater diversity compared to the core microbiota. The prevalent operational taxonomic units (OTUs) in the cheese were *Y. lipolytica* and *G. goetrichum.* Despite its use as a starter, *D. hansenii* exhibited low percentages in both the core (0.02%-0.08%) and on the surface (0.1%). Mold genera identified included *Aspergillus, Lewia, Nectria, Myrothecium, Cladosporium and Mucor.* The core was dominated by *A. niger* and *Lewia infectoria,* while the surface was dominated by *A. niger and C. cladosporioides* [39]. Exploration of the bacterial and fungal populations of Portuguese Queijo de Azeitao PDO cheese was conducted through 16S and 26S rRNA gene amplicon sequencing, respectively. Nine 20-day ripened samples were collected from three different producers. Results revealed that *L. mesenteroides* and *L. lactis* were the most representative bacteria, followed by other species such as *L. zeae*, *L. kefiri*. Regarding fungal population, the cheeses were characterized by the dominance of *Y. lipolytica* followed by *C. ethanolica*, *K. zeylanoides*, *G. candidum*, *G. goetrichum* and others [40].

### 2.5.3. Semi-hard cheeses

**Table 3**

The microbial composition of semi-hard cheeses is depicted in Table 3. A metagenomic analysis of Serra da Canastra cheese involved sequencing the V3-V4 region of the 16S rRNA gene. The microbial content of the cheese remained consistent across farms, despite variations in whey and milk. Major bacterial players included Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes. The relative abundance of Firmicutes exceeded 86% regardless of farm origin. It was also observed that the bacterial composition of the final product was influenced by the Non-Starter Cultures (NSC) used, as identical microorganisms (*Streptococcus* spp. and *Lactococcus* spp.) were found in both [14]. The bacterial composition of Paipa cheese from different producers was examined through 16S rRNA gene sequencing, revealing producer-dependent variations. The predominant phyla included Firmicutes with the highest percentages ranging from 59.2% to 82%, followed by Proteobacteria, Actinobacteria, and Bacteroidetes. Lactic Acid Bacteria constituted a significant component, primarily characterized by the *Lactococcus* genus. The *Enterococcus* genus was consistently identified in all samples. Apart from LAB, spoilage bacteria, pathogens, and potentially toxin-



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producing bacteria were also detected [41]. Higher bacterial differences were identified in a study comparing Serpa cheese from PDO and non-PDO industries. Microflora was investigated using both culture-dependent and culture-independent methods. *Lb. brevis* was prominently present in non-PDO cheeses, while *Lb. paracasei/casei* dominated in PDO cheeses. High-throughput sequencing confirmed the presence of LAB, with the dominant genera being *Lactococcus* spp., *Lactobacillus* spp., and *Leuconostoc* spp. Enterobacteria were detected at the end of the ripening process [42]. High-throughput sequencing was employed to examine the microbiota of Gouda cheese. The primary genera identified were *Lactococcus* spp. and *Leuconostoc* spp., maintaining prevalence throughout the ripening period from the 6th to the 24th week. In fact, these genera constituted the starter composition added to the milk. Species belonging to the coliform group exhibited a gradual decrease during ripening, reaching negligible levels in matured cheese [43].

The bacterial communities of 120-day Raclette du Valais PDO cheeses were examined through 16S rRNA gene amplicon sequencing. All 21 analyzed samples contained *L. paracasei*, *L. lactis*, *L. helveticus* and *S. thermophilus*. More than 80% of the samples exhibited the presence of ten distinct species, all belonging to the Streptococcaceae or Lactobacillaceae families [44]. Thirteen Van Herby cheese samples from diverse Turkish regions showed varied results based on enrichment (OP) or non-enrichment (KOP). In KOP samples, *Lactobacillus*, *Lactococcus*, and *Streptococcus* prevailed, while OP samples displayed dominance by *Enterococcus*, *Streptococcus*, and *Bacillus*. At the species level, KOP had *L. raffinolactis* and *Streptococcus salivarius* prevalence, while enrichment led to *E. faecalis* and *S. salivarius* dominance. Despite differences, Firmicutes was the most abundant phylum in both KOP and OP samples [45]. Table 3 lists the most prevalent genera.

### 2.5.4. Hard cheeses

**Table 4**

Table 4 provides a comprehensive list of microbiota in various hard cheese varieties. A study comparing Silter cheese with and without an autochthonous starter culture unveiled that predominant species in the 200-day cheese belonged to the *Streptococcus* spp. and *Lactococcus* spp. genera. Some strains from these genera were employed as autochthonous starter cultures. Additionally, other genera were identified, including *Lactobacillus* spp., *Leuconostoc* spp., and *Enterococcus* spp. [46].

De Pasquale et al. [47] investigated metabolically active bacteria in Pecorino Siciliano cheese using pyrosequencing. The most abundant genera were *Lactobacillus* spp. and *Streptococcus* spp. Additionally, sub-dominant genera, each constituting less than 0.4%, were identified, including *Planococcus, Alkalibacterium, Enterococcus, L. and Peptostreptococcus*. Culture-independent methods were employed to analyze Istrian cheese from two farms at various ripening stages. Microbial populations from both farms are summarized in Table 4. In the 90-day cheese from farm 1, *L. lactis* (86.47%) predominated, followed by the *Enterococcus* spp. (7.05%) genus. Conversely, in cheese from farm 2, *Enterococcus* spp. (42.65%) and *Streptococcus parauberis* (40.33%) were the most prevalent. Detection of *E. coli/S. flexneri* and *Salmonella* spp. exclusively occurred in farm 2 cheeses, with high percentages in fresh cheeses: 58.98% for *E. coli/S. flexneri* and 20.44% for *Salmonella* spp. [48]**.** The microbiota of Alberquilla cheese was investigated using both culture-dependent (RAPD-PCR, species-specific or 16S rRNA gene sequencing) and culture-independent methods (PCR-TTGE and 16S gene sequencing for TTGE bands). Various isolates were categorized into different species, with the majority belonging to the *Lactobacillus* genus, particularly *Lb. paracasei*. Gram-negative



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bacteria, primarily *H. alvei*, were also well-represented. Direct DNA analysis via the TTGE approach revealed *L. lactis* and *Enterococcus* spp. as the most common species. Additionally, culture-dependent approaches exclusively detected *L. pseudomesenteroides* and *P. urinaequi* [49]. A metagenomic study examined four samples of Bulgarian green cheese for their microbial content. The dominant phylum was Firmicutes (50.61%), followed by Actinobacteria (42.96%) and Proteobacteria (6.43%). Representative genera included *Streptococcus* spp., *Lactobacillus* spp., *Lactococcus* spp., *Staphylococcus* spp., *Brevibacterium* spp., and *Corynebacterium* spp. In the fungal population, species belonging to Ascomycota were prevalent, including *P. roqueforti*, *S. flava*, *D. hansenii*, *P. membranifaciens*, and others [50].

## 3- CONCLUSIONS

While not exhaustive, the data and examples presented in this review provide a comprehensive overview of the microbial communities present in raw milk cheeses, allowing for an assessment of their origins and the factors influencing their growth and presence. Based on the current knowledge, it can be inferred that raw milk cheeses serve as significant reservoirs of microbial populations, primarily originating from raw milk and Natural Whey Starters (NSW) used in the cheesemaking process. These ingredients themselves undergo inoculation from the environment, equipment, and farmers. Additionally, contamination can occur during various stages of cheesemaking and ripening. Procedures like pasteurization, milk refrigeration, curd cooking, salting, and ripening exert direct influences on microbial growth during cheesemaking.

It is crucial to note that the outcomes derived from microbial screening heavily rely on the methodology employed; culture-dependent methods identify viable and dominant cells, while culture-independent techniques such as HTS offer an overview of the existing microbiota, typically at the genus level and without distinguishing between dead and live cells. Therefore, the combination of both approaches is of utmost importance.

Based on the aforementioned findings, it is evident that microbial communities vary depending on the type of cheese under study. Nevertheless, the most commonly identified taxa in cheeses include Firmicutes, *Lactococcus* spp., and *Lactobacillus* spp. In terms of fungi, the frequently isolated species belong to *Candida*, *Kluyveromyces*, *Saccharomyces*, *Yarrowia*, *Goetrichum*, and others.

## 4-References

[1] Harbutt J., 2015. *World Cheese Book*. London: DK publishing.

[2] Almena-Aliste M. & Mietton B., 2014. Cheese Classification, Characterisation and categorization: A Global Perspective. Microbiology Spectrum 2, 1-29.

[3] Sekban H. and Taracki Z., 2020. Effects of different starter cultures on the ripening characteristics of Golot cheese. Nutrition and Food Science 51(4): 664-676.

[4] IDF, 2016. The World Dairy Situation, Brussels, Belgium, 1-6.

[5] Čanžek Majhenič A., Bogovič Matijašić B., Trmčić A. & Rogelj I., 2014. Tailor-Made Starter Cultures for Preserving the Uniqueness of Traditional Cheeses. *In*: V Ravishankar Rai & Jamuna A. Bai. Beneficial microbes in fermented and functional foods. USA: CRC press, 15-30.

[6] FAO, 2004. Code of Hygienic Practice for Milk and Milk Products CAC/RCP 57-2004.

[7] Donnelly C.W., 2004. Growth and Survival of Microbial Pathogens in Cheese. *In:* Fox P.F., Guinee T.P., Cogan T.M., & McSweeney P.L.H eds. Cheese, Chemistry, Physics &



[ DOI: 10.22034/FSCT.21.150.121 ]

Microbiology, Vo1, 3rd ed. USA: Elsevier, 541-560.

[8] Tunick M., 2014. The Science of Cheese. New York, NY, USA; OUP USA.

[9] Verdier-Metz I., Michel V., Delbès C., & Montel M-C, 2009. Do milking practices influence the bacterial diversity of raw milk? Food Microbiology 26: 305-310.

[10] Vacheyrou M. et al., 2011. Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms. International Journal of Food Microbiology 146: 253-262.

[11] Luiz L.M.P. et al. 2016. Mesophilic Lactic Acid Bacteria Diversity Encountered in Brazilian Farms Producing Milk with Particular Interest in *Lactococcus lactis* Strains. Current Microbiology 73: 503-511.

[12] Verdier-Metz I. et al., 2012. Cow Teat Skin, a Potential Source of Diverse Microbial Populations for Cheese Production. Applied Environmental Microbiology 78: 326-333.

[13] Didienne R. et al., 2012. Characteristics of microbial biofilm on wooden vats (‘gerles’) in PDO Salers cheese. International Journal of Food Microbiology 156: 91-10120.

[14] Kamimura B.A. et al. 2020. Amplicon sequencing reveals the bacterial diversity in milk, dairy premises and Serra da Canastra artisanal cheeses produced by three different farms. Food Microbiology 89: 103453.

[15] Quijada N.M et al.., 2018. Autochthonous facility-specific microbiota dominates washed-rind Austrian hard cheese surfaces and its production environment. International Journal of Food Microbiology 267: 54-61.

[16] Calasso M. et al. 2016. Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at the same dairy plant. Food Microbiology 54: 115-126.

[17] Di Grigoli A. et al. 2015. The influence of the wooden equipment employed for cheese manufacture on the characteristics of a traditional stretched cheese during ripening. Food Microbiology 46: 81-91.

[18] Tavaria F.K., Reis P.J.M. & Malcata F.X., 2006. Effect of dairy farm and milk refrigeration on microbiological and microstructural characteristics of matured Serra da Estrela cheese. International Dairy Journal 16: 895-902.

[19] Kristensen L.S., Siegumfeldt H., Larsen N., Jespersen L., 2020. Diversity in NaCl tolerance of Lactococcus lactis strains from DL-starter cultures for production of semi-hard cheeses. International Dairy Journal 105: 104673.

[20] Sheehana J.J., Fenelona M.A., Wilkinsonb M.G., McSweeney P.L.H., 2007. Effect of cook temperature on starter and non-starter lactic acid bacteria viability, cheese composition and ripening indices of a semi-hard cheese manufactured using thermophilic cultures. International Dairy Journal 17: 704-716.

[21] Yazici F., Dervisoglu M., Akgun A. & Aydemir O., 2010. Effect of whey pH at drainage on physicochemical, biochemical, microbiological, and sensory properties of Mozzarella cheese made from buffalo milk during refrigerated storage. International Journal of Dairy Science 93: 5010-5019.

[22] De Freitas Martins M.C. et al. 2018. Bacterial diversity of artisanal cheese from the Amazonian region of Brazil during the dry and rainy seasons. Food Research International 108: 295-300.

[23] Vanderaa E., Kakouria A., Koukkoub A-I., Samelis J., 2019. Major ecological shifts within the dominant nonstarter lactic acid bacteria in mature Greek Graviera cheese as affected by

the starter culture type. International Journal of Food Microbiology 290:15-26.

[24] Ivey M.L. and Phister T.G., 2011. Detection and identification of microorganisms in wine: a review of molecular techniques. Journal of Industrial Microbiology and Biotechnology 38: 1619-1634.

[25] Lupski J.R., and Weinstock G.M., 1992. Interspersed Repetitive DNA Sequences in Prokaryotic Genomes. Journal of Bacteriology 174: 4525-4529.

[26] Randazzo C.L., Caggia C., Neviani,E., 2009. Application of molecular approaches to study lactic acid bacteria in artisanal cheeses. Journal of Microbiological Methods 78: 1-9.

[27] Rosello-Soto E. et al. 2018. New Trends in Molecular Techniques to Identify Microorganisms in Dairy Products. *In*: El Sheikha AF., Levin R. & Xu J. eds. *Molecular Techniques in Food Microbiology: Safety, Biotechnology, Authenticity and Traceability*. UK*:* John Wiley and Sons Inc., 309-318.

[28] Rosello-Soto E., et al. 2018. Current and New Insights on Molecular Methods to Identify Microbial Growth in Fruit Juices. *In:* El Sheikha AF., Levin R. & Xu J. eds. *Molecular Techniques in Food Microbiology: Safety, Biotechnology, Authenticity and Traceability*. UK: John Wiley and Sons Inc., 145-154.

[29] Yeluri Jonnala BR. et al. 2018. Sequencing of the Cheese Microbiome and its relevance to industry. Frontiers in Microbiology 9: 1020.

[30] Gallegos-Acevedo MA. et al. 2018. Microbial characterization and diversity of artisanal Ranchero cheese with emphasis in *Lactococcus* strains. LWT-Food Science and Technology 39: 143-148.

[31] El Galiou O. et al. 2015. Chemical and microbiological characteristics of traditional homemade fresh goat cheeses from Northern Morocco. Small Ruminant Research 129: 108-113.

[32] Ouadghiri M. et al., 2014. Yeasts in Traditional Moroccan Goat Cheese. African Journal of Microbiology Research 8:3029-3037.

[33] Caro I. et al., 2020. Characterization of *Lactococcus* strains isolated from artisanal Oaxaca cheese. LWT-FOOD Science and Technology, 122: 109041.

[34] Jin H. et al., 2018. Using PacBio sequencing to investigate the bacterial microbiota of traditional Buryatian cottage cheese and comparison with Italian and Kazakhstan artisanal cheeses. International Journal of Dairy Science 101: 6885-6896.

[35] Mendez-Romero J. et al. 2021. Artisanal Fresco cheese from Sonora : Physicochemical composition, microbial quality, and bacterial characterization by high-throughput sequencing. International Journal of Dairy Technology 74: 359-370.

[36] Perin L.M. et al., 2017. Bacterial ecology of artisanal Minas cheeses assessed by culture dependent and -independent methods. Food Microbiology 65: 160-169

[37] Šuranská H. et al**.,** 2016. Characterisation of the yeast and mould biota in traditional white pickled cheeses by culture-dependent and independent molecular techniques. Folia Microbiology (Praha), 61: 455-463.

[38] Ceugniez A. et al. 2017. Use of a metagenetic approach to monitor the bacterial microbiota of “Tomme d’Orchies” cheese during the ripening process. International Journal of Food Microbiology 247: 65-69.

[39] Ceugniez A. et al. 2017. Fungal diversity of "Tomme d'Orchies" cheese during the ripening process as revealed by a metagenomic





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study. International Journal of Food Microbiology 258: 89-95.

[40] Cardinali F. et al. 2021. Microbial communities and volatile profile of Queijo de Azeitao PDO cheese, a traditional Mediterranean thistle-curdled cheese from Portugal. International Food Research Journal 147: 110537.

[41] Castellanos-Rozo J. et al. 2020. Analysis of the Bacterial Diversity of Paipa Cheese (a Traditional Raw Cow's Milk Cheese from Colombia) by High-Throughput Sequencing. Microorganisms 8: 218.

[42] Gonçalves MTP. et al**.** 2018. Bacterial Communities in Serpa Cheese by Culture Dependent Techniques, 16S rRNA Gene Sequencing and High-throughput Sequencing Analysis. International Journal of Food Science, 83: 1333-1341.

[43] Park W. et al. 2019. Microbiological Characteristics of Gouda Cheese Manufactured with Pasteurized and Raw Milk during Ripening Using Next Generation Sequencing. Food Science and Animal Resources 39:585-600.

[44] Dreier M. et al. 2022. High-throughput qPCR and 16S rRNA gene amplicon sequencing as complementary methods for the investigation of the cheese microbiota. *BMC* Microbiology 22: 48.

[45] Sudağidan M. et al. 2021. 16S Bacterial Metagenomic Analysis of Herby Cheese (Otlu Peynir) Microbiota. Acta Veterinatia Eurasia 47: 188-196.

[46] Silvetti T. et al. 2017. Microbial population profile during ripening of Protected Designation of Origin (PDO) Silter cheese, produced with and without autochthonous starter culture. *LWT*-Food Science and Technology, 84: 821-831.

[47] De Pasquale I. et al. 2016. Spatial Distribution of the Metabolically Active Microbiota within Italian PDO Ewes' Milk Cheeses. PLoS One, 11, e0153213.

[48] Mrkonjic Fuka M. et al. 2013**.** Dynamics of Bacterial Communities during the Ripening Process of Different Croatian Cheese Types Derived from Raw Ewe's Milk Cheeses. PLoS One, 8, e80734.

[49] Abriouel H. et al. 2008. Biodiversity of the microbial community in a Spanish farmhouse cheese as revealed by culture-dependent and culture-independent methods. International Journal of Food Microbiology 127: 200-208.

[50] Dimov S.G. et al. 2021. NGS-Based Metagenomic Study of Four Traditional Bulgarian Green Cheeses from Tcherni Vit. LWT-Food Science and Technology 152.

**Table 1.** Microbiota of fresh unripened cheeses

| Cheese | Type of milk | Starter culture | Used methods | Microbial communities | Ref |
|---|---|---|---|---|---|
| **Ranchero** | Cow's milk | NA* | 16S rDNA sequencing for LAB isolates | *Enterococcus* spp.<br>*Lactobacillus* spp.<br>*L. garvieae*<br>*L. lactis* subsp. *lactis*<br>*Leuconostoc* spp.<br>*Streptococcus* spp. | [30] |
| **Jben (North of Morocco)** | Goat milk | NA* | Genus or species level PCR | *Enterococcus* spp.<br>*L. lactis* subsp. *lactis*<br>*L. lactis* subsp. *lactis cit*$^+$<br>*Lb. plantarum*<br>*Lb. paracasei* | [31] |





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| **Moroccan goat cheese** | Goat milk | ND** | -M13 PCR fingerprinting<br>-Ribosomal and protein-coding gene sequencing analysis | *K. lactis*<br>*S. cerevisiae*<br>*Y. lipolytica*<br>*C. parapsilosis*<br>*K. unispora*<br>*K. marxianus*<br>*P. fermentans*<br>*Candida inconspicua*<br>*Clavispora lusitaniae*<br>*C. zeylanoides*<br>*Filobasidium uniguttulatum*<br>*Torulaspora delbrueckii*<br>*K. unispora*<br>*Naumovozyma castellani*<br>*Barnettozyma califronica*<br>*Candida anglica*<br>*Kazachstania servazii*<br>*Rhodotorula mucilaginosa* | [32] |
| **Oaxaca "Pasta filata unripened"** | cow's milk | NA* | -16S rRNA gene sequencing for LAB isolates<br>-RAPD and Rep-PCR for *L. lactis* | *E. faecalis*<br>*L. lactis* subsp. *lactis*<br>*E. faecium*<br>*Lb. plantarum*<br>*Lb. paracasei* subsp. *paracasei*<br>*Lb. rhamnosus* | [33] |
| **Buryatian cottage cheese** | Cow's milk | Natural starter : handmade butter or cream | -16S rRNA gene sequencing for LAB isolates<br>-PacBio sequencing for whole cheese DNA | -*E. casseliflavus*<br>-*E. durans*<br>-*E. faecalis*<br>-*E. faecium*<br>-*Lactobacillus brevis*<br>-*L. delbrueckii* subsp. *bulgaricus*<br>-*Lactobacillus fermentum*<br>-*Lactobacillus gasseri*<br>-*Lb. paracasei*<br>-*Lb. plantarum*<br>-*L. lactis*<br>-*Leuconostoc lactis*<br>-*Leuconostoc pseudomesenteroides*<br>-*Pediococcus pentosaceus*<br>-*S. thermophilus*<br>-*Lactococcus raffinolactis*<br>-*Acetobacter cibinongensis*<br>-*Lactobacillus helveticus*<br>-*Klebsiella pneumonia*<br>-*Acinetobacter johnsonii*<br>-*Klebisella oxytoca*<br>-*Pseudomonas*<br>-*Raoutella* | [34] |
| **Artisanal fresco cheese from Sonora** | Cow milk | NA* | 16S rRNA gene sequencing (V3 region) | Streptococcaceae<br>Lactobacillaceae<br>Leuconostocaceae<br>Enterococcaceae | [35] |

*: the starter culture was not added

**: The authors did not mention whether they used the starter or not.

**Table 2.** Microorganisms found in soft cheeses

| Cheese | Type of milk | Starter culture | Used method | Microbial communities | Ref |
|---|---|---|---|---|---|
| **Minas (5 to 10 days)** | Cow's milk | Pingo | -LAB isolates sequencing<br>-Culture independent methods :<br>Rep-PCR<br>LH-PCR | -*Lb. paracasei*<br>-*Lb. plantarum*<br>-*Lb. rhamnosus*<br>-*Lactobacillus brevis*<br>-*Lactobacillus parabuchneri* | [36] |





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|---|---|---|---|---|---|
| | | | | -*Lactobacillus buchneri*<br>-*Lb. acidipiscis*<br>-*L. delbrueckii*<br>-*Lactobacillus curvatus*<br>-*Lactobacillus fermentum*<br>-*L. garvieae*<br>-*L. lactis* subsp. *lactis*<br>-*E. faecalis*<br>-*Weissella paramesenteroides*<br>-*Pediococcus acidilactici*<br>-*L. mesenteroides*<br>- *S. thermophilus*<br>-*Staphylococcus spp.*<br>-*Kocuria kristinae*<br>-*Escherichia coli* | |
| **Traditional white pickled cheese (Soft/semi hard) (1 to 10 days)** | ewe's and ewe's/cow's milk | NA* | -ITS-RFLP ITS and D1/D2 LSU rDNA sequencing of isolates<br>-LSU-DGGE and sequencing of discrete bands<br>-CI ITS-clone library RFLP | *D. hansenii*<br>*C. zeylanoides*<br>*Trichosporon ovoïdes*<br>*Torulaspora delbrueckii*<br>*K. lactis*<br>*Candida pararugosa*<br>*Y. lipolytica*<br>*Galactomyces geotrichum*<br>*S. cerevisiae*<br>*Cryptococcus albidus* | [37] |
| **Tomme d'Orchies (21 days)** | Cow's milk | Commercial starter | Metagenomic study by HTS | -*S. thermophilus*<br>-*L. lactis* subsp. *lactis*<br>-*L. lactis* subsp. *cremoris*<br>-*B. linens*<br>-*Lb. rhamnosus*<br>-*Lactobacillus malefermentans*<br>-*Corynebacterium variabile*<br>-*Corynebacterium flavescens*<br>-*Corynebacterium casei*<br>-*Lactobacillus helveticus*<br>-*Lactobacillus capillatus*<br>-*Lactobacillus sakei*<br>-*Leucobacter chromiireducens*<br>-*Peptostreptococcus russelli*<br>-*Psychrobacter celer*<br>-*Brachybacterium tyrofermentans*<br>-*Hafnia alvei*<br>-*Staphylococcus* spp.<br>-*Serratia* spp. | [38] |
| **Tomme d'Orchies (21 days)** | Cow's milk | *D. hansenii* as yeast commercial starter | Metagenomic analysis with HTS | ***Yeasts:***<br>-*Y. lipolytica*<br>-*Galactomyces geotrichum*<br>-*S. cerevisiae*<br>-*Cryptococcus curvatus*<br>-*K. unispora*<br>-*Candida rugosa*<br>-*Candida intermedia*<br>-*C. parapsilosis*<br>-*C. zeylanoides*<br>-*D. hansenii*<br>-*K. marxianus*<br>-*Torulaspora delbrueckii .*<br>-*Pichia fermentans*<br>-*Clavispora lustinae*<br>-*K. lactis*<br>-*Trichosporon gracile* | [39] |


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|---|---|---|---|---|---|
| | | | | *-Trichosporon asahii*<br>*-Trichosporon guehoae*<br>*-Trichosporon ovoides*<br>*-Trichosporon montevideense*<br>*-Malassezia restricta*<br>*-Exophiliala phaeomuriformis*<br><br>***Molds :***<br>*Aspergillus niger*<br>*Lewia infectoria*<br>*Cladosporium cladosporioides*<br>*Myrothecium sp.*<br>*Mucor circinelloides* | |
| **Queijo de Azeitao PDO (20-d)** | Ovine milk | NA* | 16S and 26S rRNA gene amplicon sequencing for bacteria and fungi (V3-V4 region for bacteria and D1 domain for fungi) | **Bacteria :**<br>*L. mesenteroides*<br>*L. lactis*<br>*Lacticaseibacillus zeae*<br>*Serratia* spp.<br>*Latilactobacillus sakei*<br>*Lactiplantibacillus plantarum*<br>*Leuconostoc gelidum*<br>*Lentilactobacillus kefiri*<br>*Levilactobacillus brevis*<br>*Weissella confusa*<br>*Corynebacterium variabile*<br>*Fusobacterium*<br>*Enterococcus pseudoavium*<br>*Carnobacterium gallinarum*<br><br>**Fungi :**<br>*Yarrowia lypolitica*<br>*Kurtzmaniella zeylanoides*<br>*G. goetrichum*<br>*Goetrichum candidum*<br>*Candida ethanolica*<br>*K. lactis*<br>*Goetrichum sylvicola*<br>*C. parapsilosis*<br>*D. hansenii*<br>*Candida pararugosa* | [40] |

*: the starter culture was not added

**: culture independent

**Table 3.** The microbial composition of semi-hard cheeses

| Cheese | Type of milk | Starter culture | Used method | Microbial communities | Ref. |
|---|---|---|---|---|---|
| **Serra da Canastra (21-d)** | cow's milk | pingo | Amplicon sequencing 16S rRNA gene V3-V4 | *Bacilli*<br>*Gammaproteobacteria*<br>*Clostridia* spp.<br>*Streptococcus* spp.<br>*Lactococcus* spp.<br>Actinobacteria<br>Bacteroidetes | [14] |
| **Paipa (28-d)** | cow's milk | NA* | HTS<br>16S rRNA gene V3-V4 | *Lactobacillales*<br>Enterobacteriaceae<br>Aeromonadaceae<br>Moraxellaceae<br>*Lactococcus* spp.<br>*Enterococcus* spp.<br>*Leuconostoc* spp. | [41] |



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|---|---|---|---|---|---|
| | | | | *Streptococcus* spp.<br>*Staphylococcus* spp.<br>*Enterobacter* spp.<br>*Serratia* spp.<br>*Citrobacter* spp.<br>*Aeromonas* spp.<br>*Acinetobacter* spp.<br>*Marinomonas* spp.<br>*Corynebacterium* spp.<br>*Chryseobacterium* spp. | |
| **Serpa (30-d)** | ewe's milk | NA* | 16S rRNA gene for isolates and HTS for CI** methods (V3V4) | Staphylococcaceae<br>Enterobacteriaceae<br>*Lactobacillales*<br>*Lactococcus* spp.<br>*Lactobacillus* spp.<br>*Leuconostoc* spp.<br>*Enterococcus* spp.<br>*Staphylococcus* spp.<br>*Klebsiella oxytoca*<br>*Hafinia alvei*<br>*Escherichia Coli* | [42] |
| **Gouda-type cheese (24th week)** | cow's milk | Commercial starter | Metagenomics : sequencing of 16S rRNA gene | -*L. lactis*<br>-*L.pseudomesenteroides*<br>-*Chryseobacterium bernardetii*<br>-*Chryseobacterium zeae*<br>-*S. equorum*<br>-*Brevibacterium aurantiacum*<br>-*L. laudensis*<br>-*Lb. plantarum*<br>-*Raoutella ornithinolytica*<br>-*Brachybacterium sacelli*<br>-*Agrobacterium tumefaciens*<br>-*Leclercia adecarboxylata*<br>-*Pseudomonas* spp. | [43] |
| **Raclette du Valais (120-d)** | Cow milk | *L. lactis* subsp *lactis*<br>*L. lactis* subsp. *cremoris*<br>*L. lactis* subsp. *lactis* biovar *diacetylactis*<br>And sometimes : *S. thermophilus* and/or *Lb. helveticus* | 16S rRNA gene amplicon sequencing (V1-V2 region) | *S. thermophilus*<br>*L. lactis*<br>*Lacticaseibacillus paracasei*<br>*Lactiplantibacillus pentosus*<br>*Lactiplantibacillus plantarum*<br>*Lentilactibacillus parabuchneri*<br>*Lb. helveticus*<br>*L. delbrueckii* | Dreier et al. 2022 |
| **Van Herby cheese (3-7 months)** | Sheep, goat or cow | *L. lactis* subsp *lactis*<br>*L. lactis* subsp. *cremoris* | 16S rRNA gene amplicon sequencing (V3-V4 region) | *Enterococcus* spp.<br>*Streptococcus* spp.<br>*Bacillus* spp.<br>*Lactococcus* spp.<br>*Lactobacillus* spp. | Sudağidan et al. 2021 |

*: the starter culture was not added

**: Culture independent

**Table 4.** Detailed list of microbiota belonging to different varieties of hard cheeses

| Cheese | Type of milk | Starter culture | Used method | Microbial communities | Ref. |
|---|---|---|---|---|---|
| **Silter cheese (200-d)** | Cow's milk | Natural starter culture | -RAPD-PCR<br>-LH-PCR<br>-Culture-independent sequencing | *Streptococcus* spp.<br>*Lactococcus* spp.<br>*Lactobacillus* spp.<br>*Leuconostoc* spp.<br>*Enterococcus* spp. | [46] |



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|---|---|---|---|---|---|
| **Pecorino Siciliano (4 months)** | Ewe's milk | NA* | 16S rRNA gene Pyrosequencing from RNA data | *Lactobacillus* spp.<br>*Streptococcus* spp.<br>*Staphylococcus* spp.<br>*Enterococcus* spp.<br>*Lactococcus* spp.<br>*Tetragenococcus* spp.<br>*Alkalibacterium* spp.<br>*Propionibacterium* spp.<br>*Brevibacterium* spp.<br>*Corynebacterium* spp.<br>*Cytophaga* spp.<br>*Escherichia* spp.<br>*Idiomarina* spp.<br>*Peptostreptococcus* spp.<br>*Planococcus* spp.<br>*Pseudomonas* spp. | [47] |
| **Istrian (90-d)** | Ewe's milk | NA* | Culture-independent tools :<br>-T-RFLP<br>-Pyrosequencing | *L. lactis*<br>*Enterococcus* spp.<br>*Macrococcus caseolyticus*<br>*L. mesenteroides*<br>*Lactobacillus casei/paracasei*<br>*Lb. plantarum*<br>Lactobacillus amylovorus<br>*Streptococcus gallolyticus*<br>*Streptococcus parauberis*<br>*Bifidobacterium thermophilum*<br>*Aerococcus viridans*<br>*S. equorum*<br>*Staphylococcus chromogenes*<br>*Staphylococcus saprophyticus*<br>*Corynebacterium casei*<br>*Corynebacterium variabile*<br>*Weissella paramesenteroides*<br>*Escherichia Coli/Shigella flexneri*<br>*Salmonella* spp.<br>*Brevibacterium* spp.<br>*Kosmotoga* spp.<br>*Petrotoga* spp.<br>*Megasphera elsdenii*<br>*Thermoanaerobacterium* spp. | [48] |
| **Alberquilla** | Mixture of goat and sheep milk | NA* | -RAPD-PCR, 16S sequencing and species-specific PCR for different isolates<br>-PCR-TTGE and sequencing for DNA directly extracted from cheese | *Lb. paracasei*<br>*Lb. plantarum*<br>*Lactobacillus brevis*<br>*Lactobacillus acidophilus*<br>*L. lactis*<br>*L. pseudomesenteroides*<br>*L. mesenteroides*<br>*E. faecium*<br>*Enterococcus devriesei*<br>*Pediococcus urinaequi*<br>*H. alvei*<br>*Escherichia coli*<br>*Shigella flexneri*<br>*Obesumbacterium proteus*<br>Nitrogen fixing bacteria | [49] |
| **Bulgarian green cheese (5 to 8 months)** | Ewe's or goats milk | Yogurt (*L. delbrueckii* spp. *bulgaricus* and *S. salivarius* spp. *thermophilus*) | HTS-based metagenomics (16S for bacteria and ITS2 for fungi) | Bacteria :<br>*Streptococcus* spp.<br>*Lactobacillus* spp.<br>*Lactococcus* spp.<br>*Staphylococcus* spp.<br>*Brevibacterium* spp. | Dimov et al., 2021 |




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| | | | | *Corynebacterium* spp.<br>*Kocuria* spp.<br>*Cobetia* spp.<br>*Psychrobacter* spp.<br>*Halomonas* spp.<br><br>Fungi:<br>*Penicillium roqueforti, Scopulariopsis flava,*<br>*D. hansenii ,*<br>*Pichia membranifaciens,*<br>*C. zeylanoides, K. lactis ,*<br>*Torulaspora delbrueckii,*<br>*Fusarium oxysporum Trichothecium roseum,*<br>*Circinella muscae.* | |

*: the starter culture was not added



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