

Microbial community dynamics in a traditional Swiss mountain cheese over 142 years of cheesemaking

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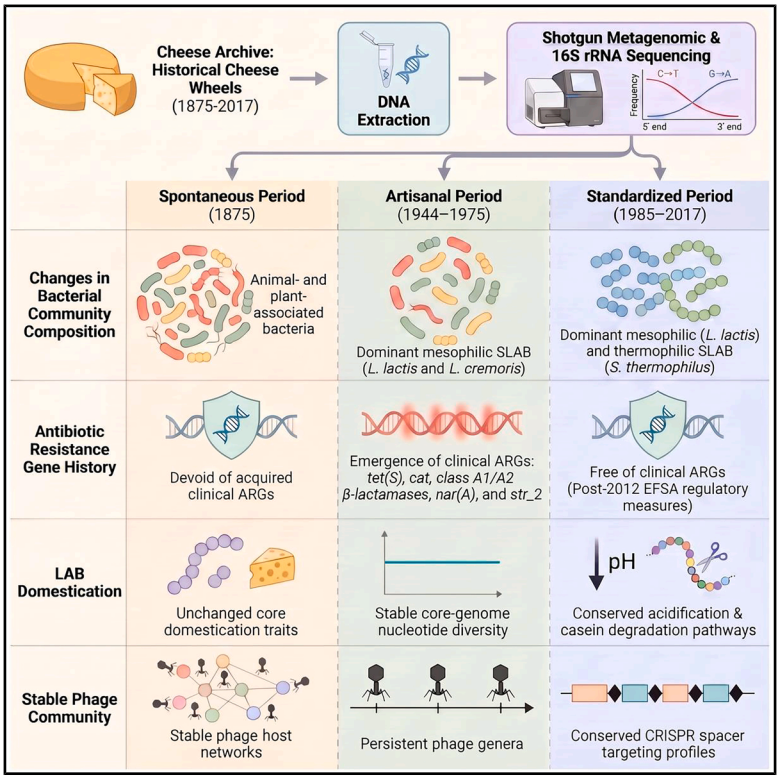
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Article

Current Biology

Microbial community dynamics in a traditional Swiss mountain cheese over 142 years of cheesemaking

Graphical abstract



Highlights

- A 142-year cheese archive traces how industrialization shaped cheese microbiomes
- Antibiotic-resistance genes in cheese mirrored historical veterinary drug use
- Starter bacteria and cheese phages were already established over a century ago
- The oldest cheese (1875) harbored a distinct, gut- and plant-taxa-rich microbiome

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In brief

How did modernizing cheesemaking change the microbes inside the cheese? By sequencing preserved traditional Alpine cheese wheels spanning 142 years from a single Alpine dairy, Somerville et al. trace shifts in bacterial composition, antibiotic resistance, and bacteriophages.

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Article

Microbial community dynamics in a traditional Swiss mountain cheese over 142 years of cheesemaking

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SUMMARY

The history of cheesemaking is deeply intertwined with the evolution of microbial communities, from spontaneous fermentation to modern, standardized practices. The rapid technological changes of the last century likely induced profound alterations in cheese microbial communities, yet this remains largely underexplored. Using shotgun metagenomics and 16S rRNA amplicon sequencing, we examined microbial community changes in Raclette du Valais, a traditional Swiss cheese, using preserved wheels from 1875 to 2017 from the same Alpine dairy. Our results reveal that significant differences in microbial community composition coincide with changes in production practices. The oldest cheese harbored a distinct bacterial community, dominated by *Lactiplantibacillus paraplantarum*, *Streptococcus thermophilus*, *Pseudolactococcus laudensis*, and gut-associated taxa. Antibiotic-resistance genes mirrored historical antibiotic use, lactic acid bacteria domestication predated the studied period, and bacteriophage genera from 1875 were already comparable to those of modern cheese factories. These findings highlight how human practices have shaped cheese microbiomes over time.

INTRODUCTION

Human practices have played a major role in shaping both the use and the evolutionary trajectories of microbial communities involved in food fermentation. Initially, the surplus of food from the shift to a herding lifestyle was serendipitously preserved by spontaneous fermentation,[1] before the deliberate reuse of fermented products to initiate new fermentations emerged.[2] In recent years, fermented foods have experienced renewed popularity, which drives an increased interest in the screening, selection, and optimization of microbial cultures for food applications.[3]

Hardly any other fermented food product has undergone such diversification as cheese. Since the earliest evidence of cheesemaking around 5200 BCE,[4] the over 1,000 cheese varieties produced today are a result of millennia of adaptation to climate, preservation needs, milk sources, cultural traditions, and trade, yielding distinct yet reproducible tastes and textures.[5,6] Despite these ancient origins, cheesemaking has likely undergone its most profound transformations in the past century, driven by advances in microbiology and technology that enabled major increases in production, standardization, and improvements in food safety (Figure 1).[7]

From a microbiological perspective, the last two centuries of cheesemaking can be divided into three partly overlapping periods. Until the turn of the 20th century (the "spontaneous" period), cheesemaking relied on spontaneous fermentation and/or early forms of back-slopping, the reuse of microbial

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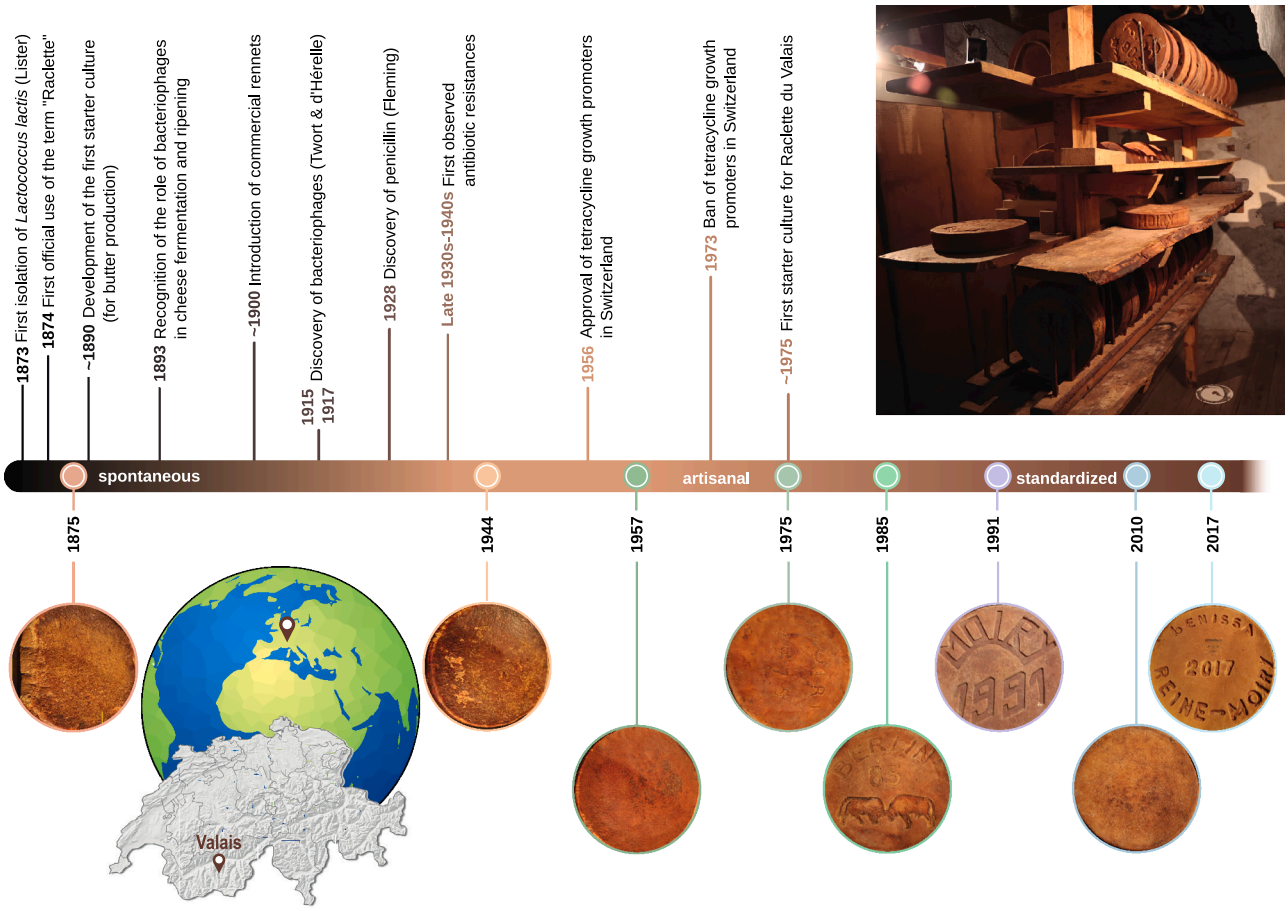


Figure 1. Timeline of the cheese wheels investigated in this study

The cheese wheels investigated are shown along a timeline, with key technical and scientific events that likely shaped cheese microbial communities. The globe (left) indicates the location of Grimentz, canton Valais, Switzerland, where the cheese wheels were produced.

communities from previous batches.[8,9] Nevertheless, cheeses were already recognized as standardized products with type-specific characteristics.[8] The following "artisanal" period saw a growing recognition of the microbial role and refinement of cheesemaking techniques,[1] most notably through natural starter cultures obtained by back-slopping or incubating raw milk at room temperature, and commercial rennet.[10] This period coincided with the discovery of antibiotics, which were widely and often excessively used in human healthcare and animal husbandry in the second half of the 20th century, including as growth promoters and for prophylaxis in livestock, which represented a major driver of antimicrobial resistance.[11] Finally, the adoption of commercial starter cultures marked the beginning of the "standardized" period, which enabled industrial-scale production through increased mechanization, enhanced hygiene, and technical improvements such as temperature control, alongside growing knowledge of cheese microbiology, chemistry, and biochemistry, and the advent of fermentation-produced chymosin replacing the traditional rennet extracted from calf stomachs and the microbial rennets used after the 1960s.[7,12]

The microbiological impact of these transitions was likely far-reaching. Improved hygiene was associated with declining bacterial diversity in cheese,[13] while antibiotics altered milk quality and microbial composition, at times even causing cheesemaking failures,[14–16] and contributed to the spread of antimicrobial resistance.[16,17] Successive changes in inoculation practices, from spontaneous fermentation involving microorganisms from milk, the dairy environment, and calf rennet, through back-slopping and artisanal starter cultures, to standardized commercial starter cultures, probably drove the most profound changes in bacterial community composition, with cascading effects on associated bacteriophage populations. Lytic bacteriophages pose a significant risk to consistent cheese production.[18] While phages were co-discovered in 1915 and 1917, respectively, by Frederick Twort and Félix d'Hérelle, lactic acid bacteria (LAB) phages were first reported in 1935, though these viruses were likely present since the earliest days of cheesemaking.[19,20] Phages found in modern cheese dairies, adapted to the narrow range of LAB used in starter cultures, likely differ substantially from those circulating during the spontaneous fermentation era.

Assessing the impact of these historical transitions on cheese microbial communities has long been hampered by the lack of samples spanning all production eras. While modern

genomes reveal population structure, only ancient samples can show how microbial communities actually evolved over time. Several ancient cheese remnants have been discovered,[21,22] but they are isolated snapshots of early dairy practices and cannot capture the key technological developments of the last century.

Our study was based on a rare historical series of samples rooted in an Alpine valley of the canton Valais, Switzerland, where a transhumance-related funerary tradition, the "meal of the dead," persisted into the early 20th century. In this custom, a cheese wheel acquired on a special occasion was kept until the owner's death and served at their funeral.[23,24] As a remnant of this tradition, a few cheese wheels were still produced and kept throughout the late 19th and 20th centuries for commemorative purposes.[25] We obtained eight well-preserved wheels of a traditional semi-hard, smear-ripened cheese in the Raclette du Valais tradition, made from raw cow's milk[26] and produced from the same Alpine pasture over 142 years (1875–2017), which provided a unique chronological archive to trace microbial dynamics across this critical phase of cheesemaking. Here, we show that ancient microbial DNA can be recovered from the paste of eight historical cheese wheels, and we use these data to investigate long-term changes in bacterial communities, antibiotic-resistance genes, LAB domestication, and bacteriophage diversity.

RESULTS AND DISCUSSION

DNA within the cheese matrix has remained well preserved for 142 years

To explore microbiological changes over more than a century, we sequenced DNA from the paste of eight cheese wheels produced between 1875 and 2017 (Figure 1). Shotgun metagenomic sequencing yielded between 4.7 and 122 gigabases (Gb) per sample. The majority of reads were of prokaryotic origin (45%–85%; Figure 2A), with the remainder largely attributed to eukaryotes (mean = 33%) and phages (mean = 5%). Most eukaryotic reads originated from cows, with traces of sheep and goat DNA that were absent in the 2010 and 2017 samples (Figure 2B), indicating that sheep and goat milk had still been used at this Alpine dairy until three decades ago.

Although milk is a known cryoprotectant,[27] little is known about DNA's long-term stability in processed milk stored at cellar temperature. To assess DNA quality and minimize artifacts, we examined nucleotide misincorporation patterns at the 3′ and 5′ ends of the DNA fragments.[28] We focused on four key bacterial species typically present in Raclette du Valais cheese, *Lactococcus lactis*, *Lactococcus cremoris*, *S. thermophilus*, and *Lacticaseibacillus paracasei*,[29] and considered damage only if mapping coverage exceeded 10. Damage frequencies at both the 3′ and the 5′ ends ranged from 0.3% to 5% and declined sharply within

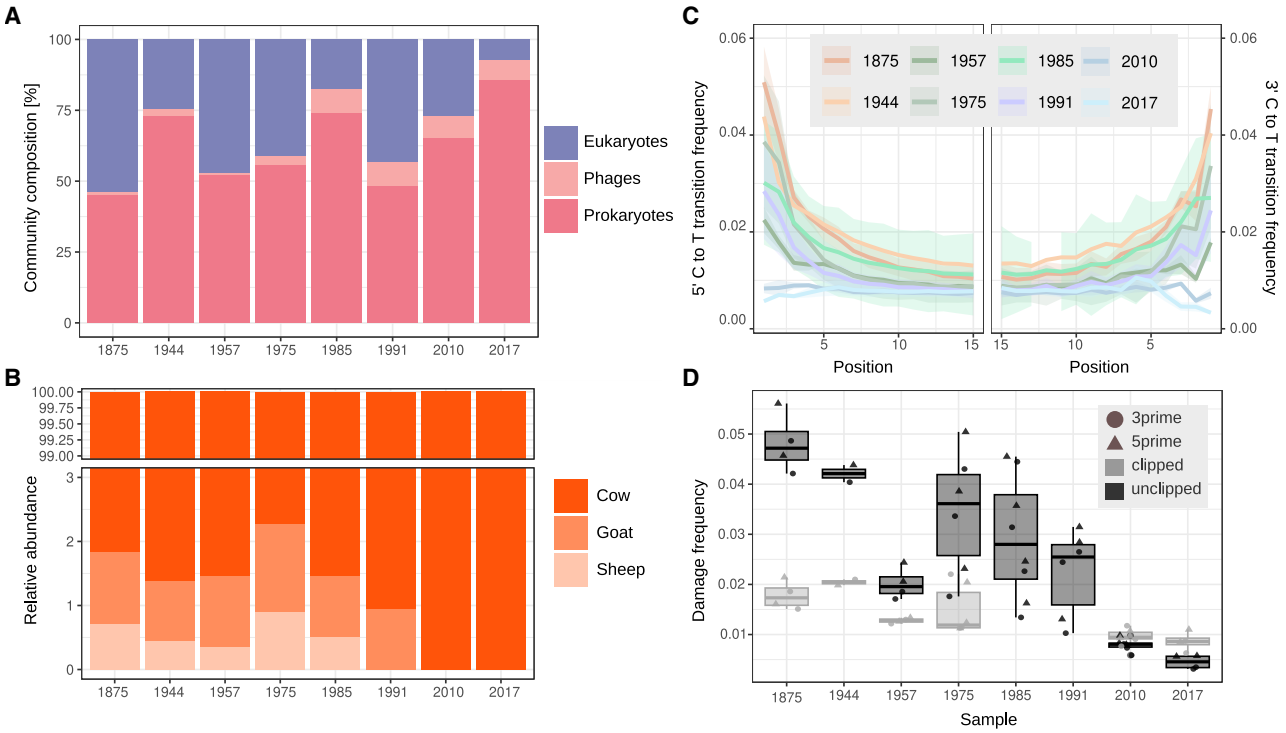


Figure 2. DNA sequencing and degradation over time

(A) Proportion of prokaryotic, eukaryotic, and phage reads.

(B) Origin of eukaryotic reads, categorized as cow, sheep, or goat.

(C) The damage frequency at the 5′ and 3′ ends of the reads.

(D) Changes in end damage frequencies over time.

See also Figure S10 and Data S1.

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the first 6 bp (Figure 2C). This degradation signal decreased significantly over time (0.028% per year; $R^2 = 0.51$, $p < 0.001$; Figure 2D). After clipping the first and last 6 bp, diagenetic degradation artifacts were effectively removed (Figure 2D).

The degradation pattern is consistent with ancient DNA and no modern contamination. The 1875 sample exhibited DNA nucleotide misincorporation frequencies of 4%–5% at the 3′ and 5′ ends, indicating modest degradation for 150-year-old material, which is comparable to oral microbiomes from the 16th–17th centuries (4%–8%) and lower than medieval samples (1,000- to 2,000-year-old oral microbiomes: 20%–30%) and a 5,700-year-old oral microbiome (12.7%–17.2%).[30–32] This suggests that cheese represents a stable matrix for long-term microbial DNA preservation, thus opening opportunities for high-resolution analyses of ancient food microbiomes, including functional-, species-, and strain-level profiling. Recently, Liu et al. confirmed this potential by characterizing the microbiome of a 3,500-year-old kefir cheese using shotgun sequencing and reconstructing the genome of *Lactobacillus kefiranofaciens* through targeted DNA capture and sequencing.[22]

Bacterial community changes reflect 20th century cheesemaking modernization

Cheesemaking relies on a specialized bacterial community responsible for milk fermentation. To observe changes in the bacterial community composition, 16S rRNA amplicon sequencing was performed. Between 105 k and 1.4 M reads were obtained, resulting in 1,326 amplicon sequence variants (ASVs) from 305 bacterial species. The species were categorized into four groups: "starter lactic acid bacteria" (SLAB), "dairy bacteria," "animal-associated bacteria," and "other bacteria" (Figure 3A). Thirty-two species exceeded 0.1% median relative abundance (Figure S1). The cumulative relative abundance of subdominant species (<0.1%) ranged from 0.6% to 7.5% of the community and declined over time in all four species groups (Figure S2; linear model: $R^2 = 0.49$, $p = 0.014$). However, neither species richness (Figure S3; linear regression, $R^2 = 0.0679$, $p = 0.2652$) nor Shannon diversity (Figure S3; linear regression, $R^2 = 0.1777$, $p = 0.164$) declined significantly over time. In contrast, richness at the family level, likely a better proxy for broader functional diversity in dairy products, declined over time (Figure 3B; linear regression, $R^2 = 0.3179$, $p = 0.0663$).

Further, we assessed temporal changes in microbiome composition at the species level (Figures 3A and 3C). The 1875 sample was notably divergent, despite the presence of typical starter species, which was mainly due to (1) a higher relative abundance (21.1%) of strict animal-associated bacteria and (2) high relative abundances of the dairy species *Pseudolactococcus laudensis* (11.1%) and the plant-associated *Lactiplantibacillus paraplantarum* (28.9%), all of which declined markedly in subsequent samples. The animal-associated species were not typical fecal contamination indicators (i.e., *Enterobacteriaceae* and *Enterococcaceae*) but strict anaerobes typical of calves' stomachs,[33,34] thus suggesting they originated from rennet extraction from a calf's stomach using fresh whey, a common practice at the time[35] that disappeared with the advent of commercial rennets by the late 19th century.[36] In contrast, *L. paraplantarum* may have been derived from traditional plant-based mastitis treatments,[37] the dairy environment and utensils, or both. Its high relative abundance suggests active proliferation during maturation rather than mere contamination, implying a possible significant functional role in this traditional cheese's fermentation and ripening.

Three SLABs, *L. lactis*, *L. cremoris*, and *S. thermophilus*, were the most prevalent species, with their relative abundance increasing over time, particularly from 1944 onward (Figure 3A). The dissimilarity between 1875 and 1944 likely reflects a transformation in cheesemaking technology, from the spontaneous

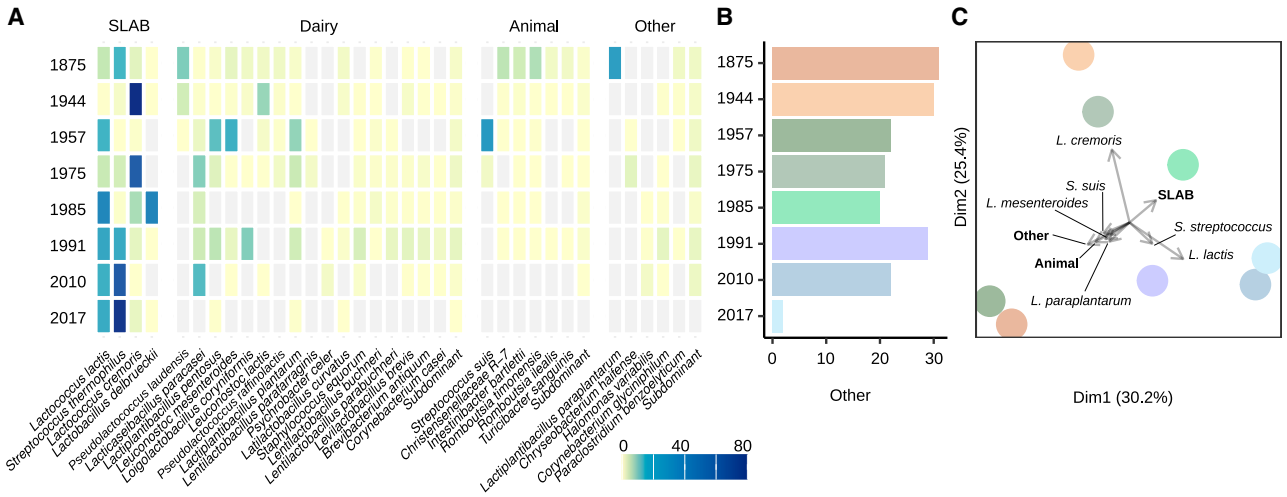


Figure 3. Temporal variations in the bacterial communities

(A) Relative abundance of the bacterial species grouped by SLAB, dairy bacteria (dairy), animal-associated bacteria (animal), or other bacteria (other).

(B) Family-level richness, colored by year of cheese production.

(C) PCA ordination biplot of samples based on relative abundance at species and category level. Colors of the objects refer to the year of cheese production, consistent with (B).

See also Figures S1–S3.

to the artisanal period. During the artisanal period, the mesophilic SLAB population, primarily composed of *L. lactis* and *L. cremoris*, clearly dominated over the thermophilic population, with *S. thermophilus* being nearly absent. This aligns with historical practices, where cheesemaking was initiated either with milk cultures obtained through natural fermentation at room temperature, akin to butter production,[38] or with natural whey starter cultures.[10] Despite the proximity of the 1957 and 1875 samples on the principal-component analysis (PCA) plot (Figure 3C), their bacterial communities differed markedly. The 1957 sample was dominated by *Streptococcus suis*, an animal-pathogenic species absent in 1875 and detected only at low relative abundances in all other samples. Conversely, *L. paraplantarum* was highly dominant in 1875 but absent in 1957. The high relative abundance of the mesophilic *L. lactis* in 1957 is consistent with the classification of this sample as being within the artisanal period.

From 1985 onward, the thermophilic SLAB population significantly increased, indicating the onset of the standardized period characterized by the use of commercial starter cultures, which could explain the reduced numbers of dominating bacterial species. Commercial cultures in use for Raclette du Valais production at that time combined mesophilic (*Lactococcus* spp.) and thermophilic (*Lactobacillus delbrueckii* subsp. *lactis* and *S. thermophilus*) strains. The detection of both lactococci and *L. delbrueckii* confirms commercial culture use in this Alpine dairy. The 2017 sample contained the dominant LAB species (*S. thermophilus*, *L. lactis*, and *L. cremoris*) typical of current cheeses of this type. However, the non-starter LAB characteristic of Raclette du Valais, such as *L. paracasei*, were present only in low relative abundances, highlighting differences from contemporary Raclette du Valais microbiomes.[29] Direct comparison with published datasets is further complicated by differences in the ripening stage, sampling strategy, and sequencing methodology.

These findings, together with historical records, illustrate the temporal evolution of cheesemaking practices from the late 19th century to today. These changes were driven by successive technological developments in cheesemaking: (1) the spontaneous period, which was characterized by spontaneous fermentation and milk coagulation using calf stomachs and reflected in the high relative abundances of species typically associated with the gut and plants; (2) the artisanal period, which was marked by the introduction of artisanal starter cultures produced through milk or whey fermentation at room temperature, combined with commercial rennets, and associated with increased relative abundances of mesophilic LAB species; and (3) the standardized period, which was defined by the use of commercial starter cultures and rennet and characterized by high relative abundances of a few mesophilic and thermophilic LAB species.

Conserved cheese-associated traits amid temporal fluctuations of ARGs

To explore functional changes in the cheese microbiome across time, we individually assembled and collectively binned the metagenomes of all samples. The total assembly length (ranging from 7.7 to 31.3 Mb) decreased in more recent samples (Figure 4A; Table S1). Most contigs were successfully binned into metagenome-assembled genomes (MAGs) across all time points (ranging from 48% to 88%; Figure 4A). These bins represent a broad range of taxa and include 37 high-quality MAGs (completeness > 90%, contamination < 5%; Figure 4B). Gene prediction within these MAGs revealed 96,070 gene families. Consistent with the reduction in assembly size, gene richness declined over time (Figure 4C). This loss suggests a progressive narrowing of functional potential, which was likely related to increasingly controlled conditions in the cheese favoring mesophilic LAB and *S. thermophilus*. Hierarchical clustering of log-transformed gene abundance profiles showed that successive samples generally clustered together (Figure S4).

The microbial functional gene content across all samples covered core cheese-associated traits such as acidification (e.g., lactate dehydrogenase genes) and casein degradation (e.g., extracellular proteinases), which are primarily associated with SLAB (Figure S5).[39] The temporal span of the cheese collection provided a unique opportunity to track the emergence of acquired antibiotic-resistance genes (ARGs). We hypothesized that ARGs detected using ResFinder[40] and fARGene[41] would appear following the introduction of antibiotics, increase in abundance and diversity during the 20th century, and persist as long as in use.

While most samples contained few or no detectable ARGs, certain time points, particularly in the second half of the 20th century, exhibited surges in specific resistance determinants (Figures 4D and 4E). MAGs from 1875 were devoid of acquired clinical ARGs. Interestingly, MAGs from 2017 were also free of such genes, which possibly reflects the effects of stricter regulatory measures implemented after 2012, when the European Food Safety Authority (EFSA) introduced microbiological cutoffs for clinically relevant antibiotics and issued recommendations to reduce ARG transmission through the food chain.[42] In contrast, MAGs from 1944 to 2010 contained six clinical ARGs targeting different antibiotic classes (Figures 4D and 4E): *tet(S)* (tetracycline resistance, tetracycline class), *cat* (chloramphenicol resistance, amphenicol class), a class *A1* β-lactamase (β-lactams class), *nar(A)* (narasin resistance, polyether ionophores class), *str_2* (streptomycin resistance, aminoglycosides class), and a class *A2* β-lactamase (β-lactams class). In the sample from 1944, only *nar(A)* was detected in the chromosome of *Enterococcus dispar*. Notably, polyether ionophores, the antibiotic class to which narasin belongs, were first isolated from *Streptomyces* spp. cultures in 1951.[43] Polyether ionophores were not introduced in agriculture to control coccidiosis in poultry until the 1970s, and narasin was only characterized in 1978.[44,45] The most abundant ARG in the MAGs, *tet(S)*, conferring resistance to tetracycline, was found exclusively in the sample from 1975 in an *L. lactis* MAG. *tet(S)* genes have been previously identified in both Gram-positive and -negative bacteria, including *L. lactis*.[46,47] Tetracyclines, first commercialized in 1948, gained rapid adoption in veterinary medicine due to their broad-spectrum activity and utility as growth promoters and prophylactics.[16,48] In Switzerland, they were used in veterinary medicine from the 1950s and authorized for growth promotion in livestock between 1956 and 1973.[49] The high abundance of *tet(S)* suggests that the strain harboring it was dominant, although its origin, milk or starter culture, remains unclear.

A gene conferring resistance to aminoglycosides, *str_2*, appeared in four consecutive samples, from 1957 to 1991.

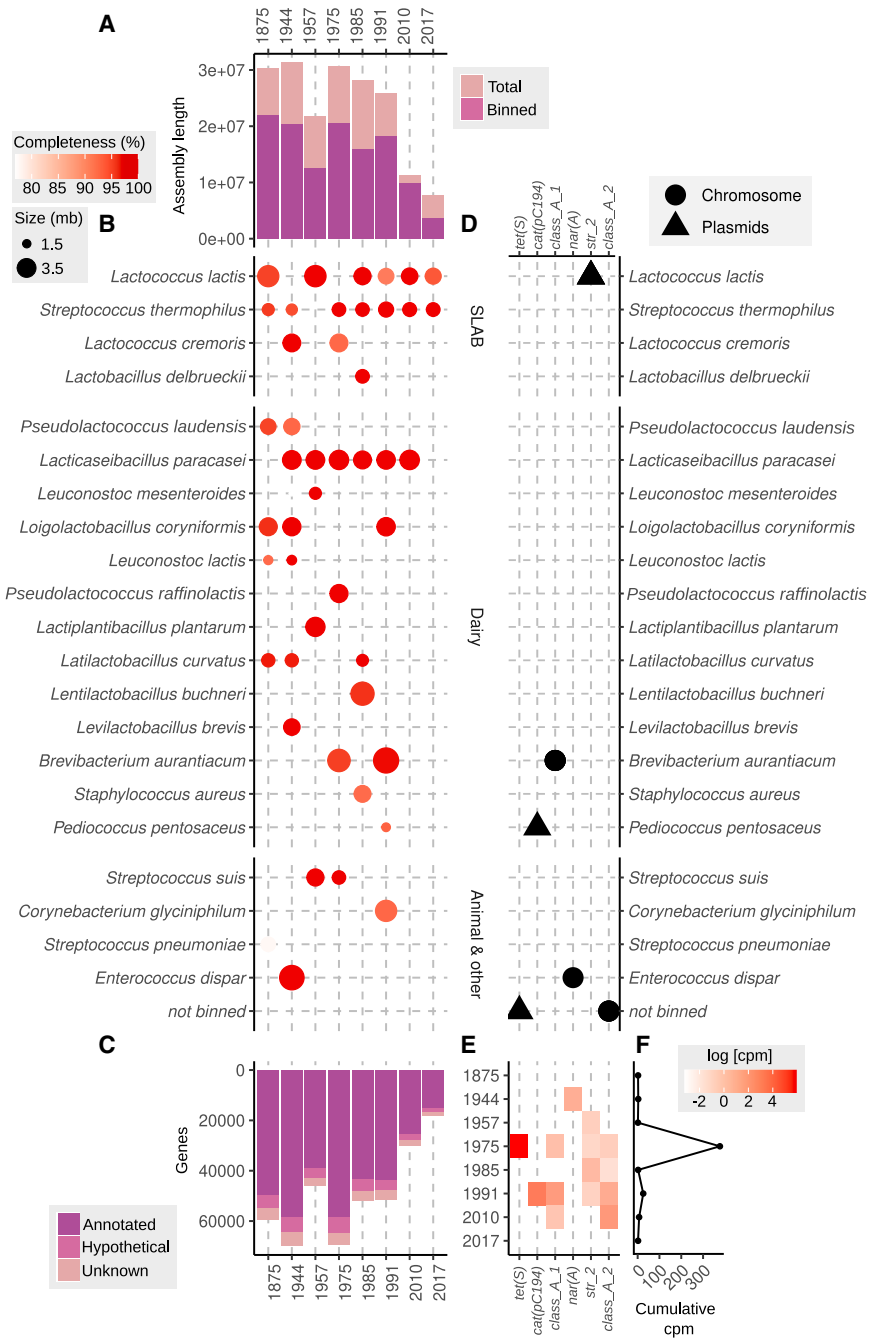


Figure 4. Functional diversity across samples

(A) Total assembly length for each sample, colored by contig binning status.

(B) MAGs, colored by completeness and sized by contig length.

(C) Number of annotated genes per sample.

(D) Genomic location of ResFinder- and fARGene-identified ARGs within MAGs.

(E) Normalized abundance (log(cpm)) of ARGs across samples.

(F) Cumulative normalized ARG counts per sample. cpm: counts per milion.

See also Figures S4 and S5 and Table S1.

The temporal pattern described here underscores the impact of antibiotic use in agriculture on the development and dissemination of ARGs in cheese production. Before the 1950s, antibiotics were not used in animal husbandry in Switzerland; accordingly, the samples from 1875 and 1944 contained either no ARGs or only one at low abundance. Although the limited number of samples prevents the reconstruction of continuous ARG dynamics, ARGs were detected more frequently in cheese wheels produced after the widespread introduction of antibiotics into livestock production. The occurrence and high abundance of *tet(S)* in the 1975 sample is consistent with the widespread use of tetracyclines as growth promoters from 1955 until their ban in 1973 and for mastitis treatment from the second half of the 20th century. The widespread use of antibiotics during this period likely exerted substantial selective pressure that drove the dissemination of ARGs even to remote environments such as Alpine pastures, where growth promoters were not used for dairy cattle. Finally, the introduction of recommendations and stricter measures in food and feed production coincides with the disappearance of ARGs in the most recent sample. The absence of *tet(S)* in the later samples is consistent with the possibility that regulatory measures contributed to reducing ARG dissemination, although the limited temporal resolution of our dataset does not allow the persistence of individual ARGs to be directly assessed.

Aminoglycosides, introduced in 1944, were widely used in agriculture, often alongside β-lactams.[50] Two genes predicted to confer resistance to β-lactams were detected in samples from 1975 to 2010. One (class_A1) shared 97% identity with a gene from *Brevibacterium aurantiacum* strain SMQ-1421, and the other (class_A2) showed 100% identity with a gene in *Staphylococcus* sp. OJ82. Finally, an ARG (*cat*) conferring resistance to chloramphenicol was identified in the sample from 1991. Although chloramphenicol was widely used to treat bovine infections since the 1940s, it was banned in the EU for food-producing animals in 1994.[51]

Major LAB domestication occurred prior to the period analyzed

Despite changes in the overall microbial community composition, the bacterial species responsible for milk acidification are consistently reused in cheesemaking, namely as SLAB. As a consequence, the starter bacteria bear genomic imprints of

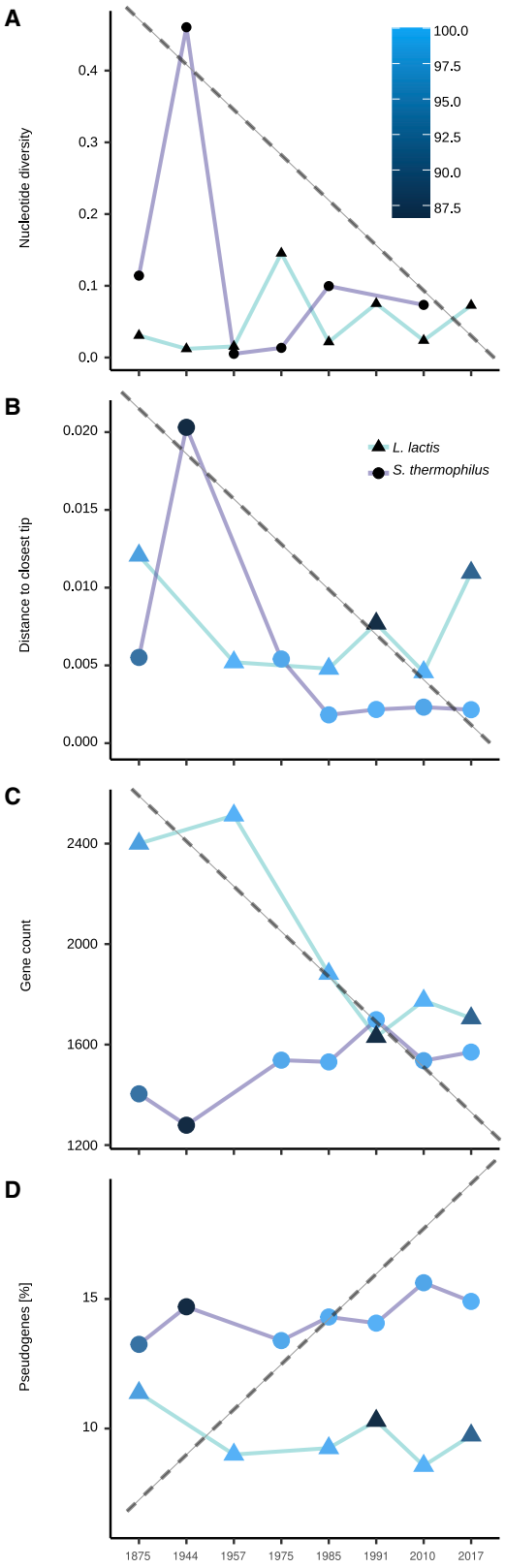


Figure 5. Four aspects of domestication

Expectations under domestication are illustrated as dashed gray lines.

(A) Nucleotide diversity of the samples for *Lactococcus lactis* and *Streptococcus thermophilus*.

(B) Distance to the closest non-MAG tip in the phylogenetic tree for the different MAGs.

(C and D) Pseudogene counts (C) and gene counts (D) of the *L. lactis* and *S. thermophilus* MAGs.

See also Figures S6–S8.

domestication.[52] While this process has been going on for millennia, we wanted to assess domestication imprints accumulating over the timescale of these samples. We examined key features at three levels: community, genome, and gene.

To assess whether industrialization has reduced functional gene diversity in the bacterial communities, particularly in cheese starter bacteria, which is analogous to the loss of diversity observed in crop monocultures,[53] we quantified temporal changes in core-genome nucleotide diversity (SNPs per 100 bp) in *S. thermophilus* and *L. lactis*. Overall, both species exhibited low nucleotide diversity, ranging between 0.005 and 0.1 SNPs per 100 bp. Low nucleotide diversity has previously been identified as a hallmark of traditional cheese starter cultures as well.[39] However, no significant decline in nucleotide diversity over the studied timeframe was observed (Figure 5A). We found no strong evidence that these species occupy different niches in the cheesemaking process, which may explain the generally limited standing strain variation.

Historically, dominant strains may have differed from those present today, analogous to the replacement of regionally adapted crop varieties by a few global breeds.[54] To test whether contemporary strains are more closely related to recent communities, we constructed a phylogeny of MAGs and reference isolates and quantified each MAG's phylogenetic distance to its nearest phylogenetic neighbor (Figures 5B, S6, and S7). In general, closely related reference strains were identified for all MAGs, with distances ranging from 0.002 to 0.01 SNPs per 100 bp. However, these phylogenetic distances remained stable over time, suggesting that strains recovered from historical cheeses were no more genetically divergent from their nearest modern relatives than strains recovered from modern cheeses. While this pattern is consistent with major divergence having occurred prior to the sampled period, it does not directly measure domestication because individual strain lineages were not followed through time.

Although we detected only single dominant strains in the historical samples, these species are known to have undergone gradual genetic erosion due to long-term, intensive use in cheese production.[52] To assess whether strains recovered from different time periods differed in their degree of genome erosion, we compared gene and pseudogene counts across MAGs. Complete genomes of *S. thermophilus* and *L. lactis* typically contain relatively low gene counts (e.g., *L. lactis*: 2,055 ± 374; *S. thermophilus*: 1,546 ± 94) but high pseudogene counts (9.58 ± 1.09 and 14.3 ± 0.905, respectively). Our MAGs showed slightly fewer genes and pseudogenes (Figure S8), likely reflecting incomplete assemblies.[55] However, we found no evidence of temporal genome reduction (Figure 5).

Overall, we detected genomic signatures consistent with domestication at the community, genome, and gene levels, which were already present in the first sample (1875). However, the lack of temporal trends in our dataset suggests that most

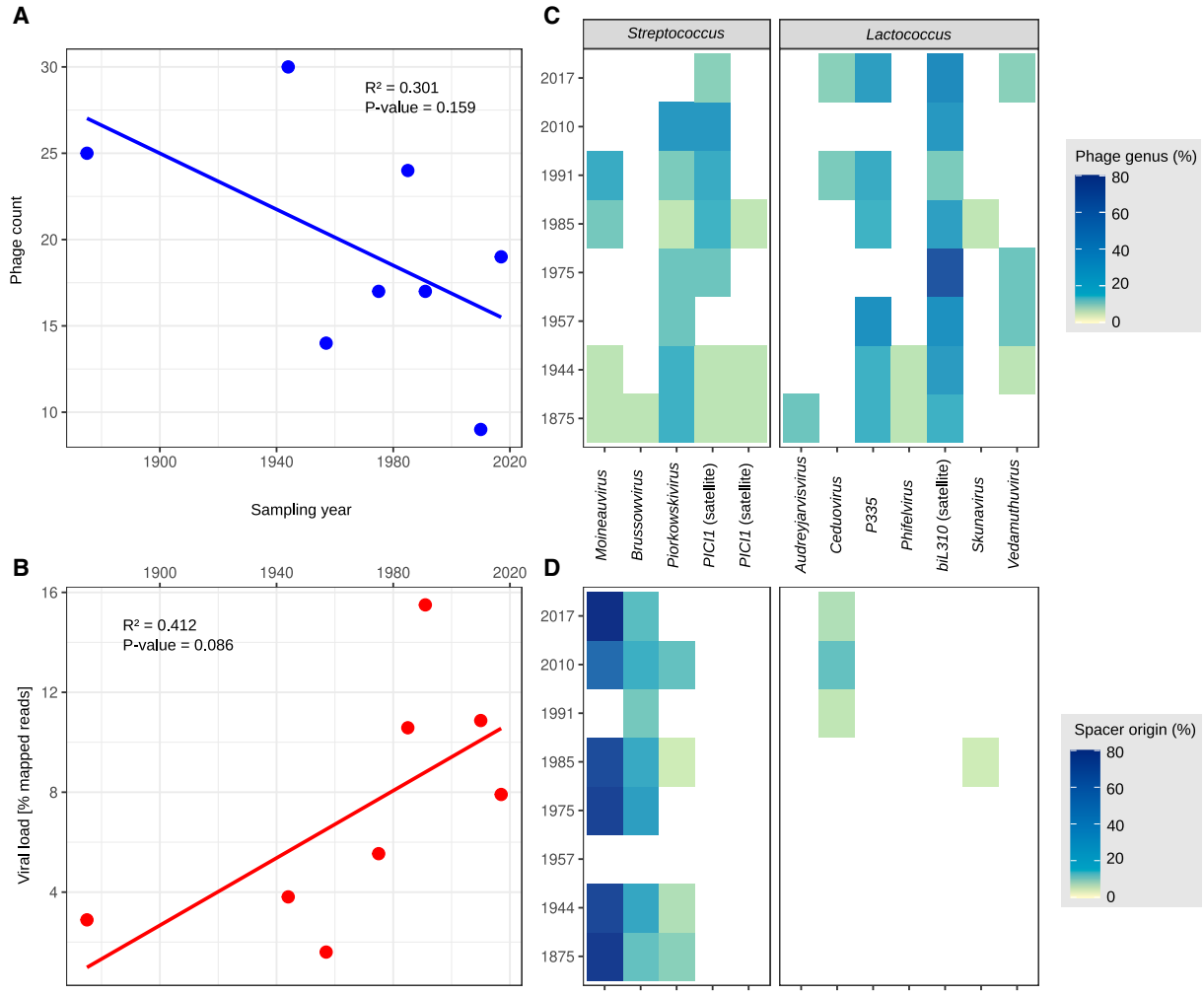


Figure 6. Phage diversity plot

(A and B) Phage count (A) and viral load (B) over time, with fitted linear regression lines. Both regression lines are statistically not significant (Pearson correlation, $p = 0.159$, A; $p = 0.086$, B).

(C) Fraction of clustered contigs assigned to viral genera (including satellite phages).

(D) Fraction of mapped CRISPR spacers, colored by viral genus.

See also Figures S9–S12.

domestication likely predates the time window captured here. This aligns with genomic evidence indicating that the main dairy-associated lineages of LAB originated 2,000–8,000 years ago, with most diversification and adaptation occurring during that time.[52] Given these long evolutionary timescales, the absence of measurable domestication signatures within the recent historical window examined here was not surprising; yet, to our knowledge, this lack of short-term evolutionary change has not yet been shown. Thus, the transition toward defined and repeatedly reused starter cultures likely imposed strong selection of specific strains rather than continued evolution within them, which may explain the limited genomic differences observed among strains sampled across the historical timescale examined here.

Stable phage communities

Phages remain a major risk in cheesemaking today, as they are capable of causing production failures or quality issues by infecting the SLAB.[56] To evaluate whether phage pressures have changed over the historical period covered by our samples, we characterized the phage communities by identifying phage contigs in the assemblies with geNomad.[57] In total, 68 distinct phages (≥99% identity and ≥85% coverage) were identified. While 24 phages (41%) were unique to individual samples, 8 phages (14%) were shared between 5 to 8 samples (Figure S9). Notably, the damage profiles of these contigs exhibited the same progressive reduction with sample age (Figure S10) as observed for bacterial contigs (Figure 2C), thus confirming they represent genuinely ancient phages rather than modern contaminants. Samples from consecutive time points were more similar in their phage community composition (Figure S11). Phage richness did not decline significantly over time (linear regression, $p = 0.159$, $R^2 = 0.301$; Figure 6A), and viral load showed only a modest increase once adjusted for contig coverage (linear regression,

p = 0.08, R^2 = 0.42; Figure 6B). Together, these results indicate that phage communities have remained relatively stable in these cheeses across the examined historical period.

To determine whether this stability also extended to phage identity and host range, we then clustered and identified the phage contigs using vContact2[58] and iPHoP.[59] Approximately 21% of the phage contigs were associated with streptococcal, 38% with lactococcal, and 2% with lactobacilli hosts (Figure 6C). While the remaining 40% did not cluster with any known phages, they were also shorter than 5,000 bp, indicating likely incomplete assemblies rather than novel phage groups. Consistent with the results above, we did not observe clear temporal shifts in specific phage genera (two-way ANOVA, $p > 0.05$), thus suggesting that the genera overall coexisted consistently over time. This includes *Piorkowskivirus*, a hybrid lineage between an *S. thermophilus* and an *L. lactis* phage considered increasingly common in modern dairy environments,[60] which was already consistently present in the older samples.

Given the importance of lactococcal phages in current cheese production, we examined how the three major contemporary taxonomic groups (*Skunavirus*, *Ceduovirus*, and P335) were represented over time. Members of the lactococcal P335 phage group were detected in nearly all cheese samples in the form of prophages. In contrast, the strictly lytic lactococcal phages belonging to the *Skunavirus* and *Ceduovirus* viral genera were detected only from 1985 onward, with *Skunavirus* present only in 1985 and *Ceduovirus* appearing in the 1991–2017 samples. Although modern cheesemaking environments are typically dominated by *Skunavirus*,[61,62] our results depict a slightly different situation in this specific cheese dairy, where *Ceduovirus* became more prominent in the later decades. Nevertheless, the fact that these phage genera were not detected in our metagenomic data does not imply that they were absent. Rather, they may have been present at sufficiently low titers to escape detection by metagenomic approaches. Such low abundance would be consistent with the absence of production defects, as high titers of these lytic phages are typically associated with production failures.

To further investigate changes in *S. thermophilus* phages over time, we analyzed phage-targeting CRISPR spacers. All known strains of *S. thermophilus* carry CRISPR-Cas systems.[63,64] Notably, fragments of lactococcal phage DNA were detected in CRISPR-Cas systems, although *L. lactis* and *L. cremoris* rarely possess this anti-phage defense system.[65] Across samples, we identified 1,179 *S. thermophilus* CRISPR spacers distributed across three prevalent CRISPR arrays (2 type II-A and 1 type III-A), averaging 182 spacers per sample (SD = 113; Figure 6D). Most spacers (85%) were unique to individual samples (Figure S12). When mapped to known phages from the IMG/VR phage database and our assembled phages, 14% of the spacers matched identifiable targets, predominantly streptococcal phages belonging to the *Moineauvirus* (69%) and *Brussowvirus* (20%) viral genera, which are also the two most predominantly found in the dairy industry today. Generally, spacer mapping is below 50%.[64] With the exception of 1957, when a single *S. thermophilus* spacer was recovered due to *S. suis* dominance, no significant pattern emerged regarding targeted phage genera. Thus, CRISPR evidence supports the same long-term stability observed at the level of phage abundance and taxonomy.

Overall, these results indicate that phage communities and their interactions with host bacteria have remained relatively stable over the past 150 years. Both metagenomic evidence, including phage abundance and identity, and CRISPR analyses reveal no signs of a major shift in phage prevalence or infectivity within this cheese production system.

Conclusions

To understand the anthropogenic impact on the microbes in fermented foods, we studied the microbial dynamics in cheese wheels of the same type and from the same geographic location across three centuries, a period marked by major scientific, technological, and industrial transformations in cheesemaking. Exploiting a unique regional tradition, where cheese wheels were kept as family heirlooms, we revealed a decline in taxonomic and functional diversity, along with broader compositional changes. Notably, both domestication and phage diversity predated the transitions in production practices. The limited sample size and lack of replication constrain inference about the persistence of individual taxa, genes, and strains, so our conclusions focus on broad trends rather than individual observations.

Phylogenetic inference offers a powerful window into the historical gene pool, but it provides only a coarse view of past microbial diversity. Resolving how microbiomes truly varied through time requires direct analysis of historical and archaeological specimens, despite the inherent limitations of such archives, including restricted sample availability, lack of biological replicates, and the possibility of sample-specific anomalies. For example, the 1957 cheese was dominated by *Streptococcus suis*, whereas the 1985 cheese reflected the introduction of a thermophilic starter culture, as is evident from the unusually high abundance of *Lactobacillus delbrueckii*. Nevertheless, museum collections, historical archives, and other preserved materials remain indispensable for reconstructing the evolutionary and ecological dynamics of fermented foods. Beyond changes in community composition, such microbial archives uniquely capture dynamics in the functional gene pool that cannot be inferred from phylogeny alone. In particular, they provide a rare opportunity to trace the emergence, spread, and decline of antimicrobial resistance genes in these microbiomes across the twentieth century, which was a period marked by the rapid expansion of antibiotic use in agriculture followed by increasing regulation.[16] The temporal trends in ARG presence observed in our dataset were broadly consistent with these regulatory and technological changes. While limited to a single time series, this observation suggests that fermented foods could serve as valuable recorders of anthropogenic selection pressures acting on microbial communities.

Across the full timeline, we observed a gradual reduction in overall taxonomic and functional diversity, which was likely driven by production standardization, improved hygiene, and the widespread adoption of defined starter cultures. Core functions essential for cheesemaking, such as acidification and casein degradation, remained stable, yet the loss of broader metabolic capabilities may influence flavor complexity, nutritional composition (e.g., via shifts in microbial protein profiles), and ecosystem resilience.[66,67] There is a clear lack of studies investigating the long-term changes in cheese microbiomes, as systematic sampling across years and decades has rarely

been conducted. While we were not able to measure sensory and biochemical effects, recent advances in metagenomics and experimental reconstruction now enable the recreation of historical or synthetic cheese communities.[68,69] Building on our metagenomic dataset, future work could attempt to reproduce cheeses resembling those produced 150 years ago, test whether similar trends occur in other cheese types, and experimentally re-establish historical production conditions to further clarify how human innovation has shaped, and continues to shape, the microbial ecosystems of fermented foods.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Noam Shani (noam.shani@agroscope.admin.ch).

Materials availability

This study did not generate any new, unique reagents. The historical and archaeological cheese specimens analyzed in this study are finite, non-renewable materials; no remaining sample material was left.

Data and code availability

- All raw sequencing reads generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject: PRJNA1419557 and are publicly available as of the date of publication. Accession numbers are also listed in the key resources table. Processed and intermediate analysis files have been deposited at Zenodo (DOI: 10.5281/zenodo.18504637) and are publicly available as of the date of publication.
- All original code used for data processing, analysis, and figure generation is publicly available on GitHub (https://github.com/Freevini/Old_Raclette), with a permanent release archived on Zenodo (DOI: 10.5281/zenodo.18504637) as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

V.S., M.M., A.N.-R., M.T., P.L., V.J.S., and N.S. designed research; V.S., M.M., A.N.-R., M.H., H.B., P.L., and N.S. performed research; V.S., M.M., A.N.-R., J.B.-P., J.N., K.M., M.P.-D., and U.v.A. analyzed data; V.S., M.M., and N.S. wrote the paper; and J.B.-P., S.M., U.v.A., V.J.S., V.S., M.M., and N.S. revised the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw metagenomic and 16S rRNA amplicon sequencing reads	This paper; NCBI SRA	BioProject: PRJNA1419557
Processed data, intermediate analysis files, and original code	This paper; Zenodo	Zenodo: https://doi.org/10.5281/zenodo.18504637
Original code (GitHub repository)	This paper; GitHub	https://github.com/Freevini/Old_Raclette
Software and algorithms		
ATLAS (metagenomics pipeline)	Kieser et al.[70]	V2.5.0; https://github.com/metagenome-atlas/atlas
SPAdes	Nurk et al.[71]	v3.13.1; RRID:SCR_000131
MaxBin	Wu et al.[72]	v2.2.7
MetaBAT	Kang et al.[73]	v2.14
KneadData	Huttenhower Lab[74]	v0.7.7-alpha
DADA2	Callahan et al.[75]	v1.28.0; RRID:SCR_023519
IDTAXA (DECIPHER)	Murali et al.[76]	N/A
R	R Core Team (2021)	V4.0.3; https://www.R-project.org/; RRID:SCR_001905
RStudio Pro	RStudio Team (2020)	v1.4.1103-4
Phyloseq (R package)	McMurdie and Holmes[77]	v1.42.0
Vegan (R package)	Oksanen et al.[78]	v2.6-4
Factoextra (R package)	Kassambara and Mundt[79]	v1.0.7
Castor (R package)	Louca and Doebeli[80]	v1.7.4
geNomad	Camargo et al.[57]	v1.7.4
Usearch	Edgar[81]	https://www.drive5.com/usearch/
fARGene	Berglund et al.[41]	N/A
OrthoFinder	Emms and Kelly[82]	v2.5.5
iTOL	Letunic and Bork[83]	https://itol.embl.de/; RRID:SCR_018174
Bakta	Schwengers et al.[84]	v1.9.2
PseudoFinder	Syberg-Olsen et al.[85]	v1.1.0
Aniclust	Nayfach et al.[86]	v1.0.2
Seqkit	Shen et al.[87]	v2.6.1
Phyloplan	Asnicar et al.[88]	N/A
CheckV	Nayfach et al.[86]	v1.0.1
iPHoP	Roux et al.[59]	v1.3.3
BACPHLIP	Hockenberry and Wilke[89]	v0.9.6
CoverM	Aroney et al.[90]	v0.6.1
Pharokka	Bouras et al.[91]	v1.7.2
vConTACT2	Bin Jang et al.[58]	v0.11.3
Cutadapt	Martin[92]	v4.6
Other		
Lactococcus lactis reference genome	NCBI RefSeq	GenBank: NC_002662.1
Lactococcus cremoris reference genome	NCBI RefSeq	GenBank: NZ_BCVK01000000
Streptococcus thermophilus reference genome	NCBI RefSeq	GenBank: NC_017581.1
Lacticaseibacillus paracasei reference genome	NCBI RefSeq	GenBank: NZ_CP007122
Host-removal reference genomes (*Bos taurus*, *Capra hircus*, *Ovis aries*, *Sus scrofa*)	NCBI	GenBank: NC_006853.1, GCF_002263795.1, NC_005044.2, NC_030808.1, GCF_000298735.2, KX094894.1, GCF_000003025.6

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
DAIRYdb 16S rRNA taxonomic reference database	Meola et al.[93]	DAIRYdb v1.2.5
ResFinder antibiotic resistance database	Bortolaia et al.[40]	https://cge.food.dtu.dk/services/ResFinder/
RefSeq viral reference database	NCBI	RefSeq viral (downloaded 20241201)

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Cheese samples

The cheese wheels investigated in this study were produced at the same Alpine dairy in the canton of Valais, Switzerland (Alpine pasture of Moiry, former pasture of Torrent). This is the highest cheese dairy in Switzerland (2497 m ASL, GPS coordinates 46.131, 7.557). The only exception was the cheese wheel from 1944, manufactured on the Alpine dairy of Châteaupré (J.-J. Zufferey, personal communication) at ca. 2250 m ASL, which was subsequently flooded as the Moiry dam was commissioned in 1958. This alp pasture was located in the same valley as the dairy of Moiry. The cheese wheels were preserved in the cellar of the Zufferey family, in Grimentz (Switzerland). Samples were selected based on having a known production date between 1875 and the present. The selection comprised 8 cheese wheels from the years 1875, 1944, 1957, 1975, 1985, 1991, 2010, and 2017.

Sample collection and preparation

Samples of the 1875 cheese were taken using a sterilized scalpel in a dedicated ancient DNA clean laboratory at the Institute of Evolutionary Medicine, University of Zurich.

For the cheeses 1944, 1957, 1975, 1985, 1991 and 2017 two samples were collected. The rind with the first 1 cm ("plug") was excluded from sampling for these cheeses. The sample .1 was 1–4 cm and .2 was 4–8 cm into the cheese wheel. Sample 2010.1 included the plug and 2010.2 was 4–6 cm in depth. Samples from the 2017 cheese were taken using a sterile biopsy needle (BMN "J" Type 8Gx150mm, HS Hospital Services SpA, Italy) in a dedicated modern DNA extraction laboratory at the Institute of Evolutionary Medicine, University of Zurich. All other samples were taken using a sterile biopsy needle (ibid) *in situ* in the cheese cellar, Grimentz, Switzerland. All samples were stored at 4 °C after sampling.

METHOD DETAILS

DNA extraction

For the pre-1970 samples, DNA extractions and sequencing library preparations were performed in the ancient DNA clean laboratory at the Institute of Evolutionary Medicine, University of Zurich[94] following standard anti-contamination protocols[95–97] with parallel non-template controls. For the post-1970 samples, the same anti-contamination procedures were enforced in a dedicated modern DNA extraction laboratory at the Institute of Evolutionary Medicine, University of Zurich. Approximately 100–150 mg of cheese were homogenized using a sterile 200 μl pipette tip and incubated at 55 °C and 600 rpm for 4 hours, then overnight at room temperature at 600 rpm in 1.25 ml of extraction buffer (160 mM ethylenediaminetetraacetic acid, 2% sodium dodecyl sulfate, 4 μg/μl proteinase K). A total of 1 ml was transferred to 15 ml of binding buffer (5 M GuHCl, 40% (v/v) isopropanol, 400 μl of 3 M sodium acetate; UV-treated before use). The mixture was then transferred to a high pure extender assembly (Roche Applied Science, UV treated) modified with a MinElute column (QIAGEN), centrifuged until all the sample solution was passed through, and washed twice with 700 μl PE buffer (QIAGEN) before elution in 100 μl of TET (10 mM Tris-HCl, 1 mM EDTA, 0.05% Tween-20, pH 8.0).

Shotgun metagenomic sequencing

Metagenomic sequencing libraries were generated from 10 μl of extract or extraction blank as described previously[98,99] with modifications. The re-amplification step was performed with 1 unit Herculase II DNA polymerase (Agilent), 5× Herculase II reaction mix, 0.3 μM primers IS5 and IS6,[98] and 4–7 μl library template with the following thermal profile: initial denaturation at 95 °C for 2 min, 10 to 25 cycles of denaturation at 95 °C for 30 sec, annealing at 60 °C for 30 sec, and elongation at 72 °C for 30 sec, followed by a final elongation at 72 °C for 5 min. Libraries were purified with MinElute spin columns following the manufacturer's instructions. Quantitative PCR (qPCR) analyses on an Agilent 2200 TapeStation were used to assess the quality and concentration of the libraries. Libraries were pooled and sequenced on the Illumina platforms NextSeq500 2x75 bp and NovaSeq 2x150 bp at the Functional Genomics Center Zurich (Switzerland). The raw reads were mapped, quantified, and removed against reference genomes of the dairy species *Bos taurus* mt genome NC_006853.1 and whole genome GCF_002263795.1_ARS-UCD1.2, *Capra hircus* mt genome NC_005044.2 and whole genome NC_030808.1, *Ovis aries* whole genome GCF_000298735.2, *Sus scrofa* mt genome KX094894.1 and whole genome GCF_000003025.6 and the human genome with kneaddata (v0.7.7-alpha).[74] Additionally, all reads were clipped by 6 bp to remove potential age damage (Figure 2C).

Metagenomics data processing

Shotgun metagenomic reads were assembled, binned, annotated, and quantified with ATLAS v.2.5.0[70] using default parameters and kmers of 21, 33, and 55 for assembly with spades v.3.13.1,[71] MaxBin v.2.2.7,[72] MetaBAT v.2.14[73] using minContig 1500, minCV 1.0, minCVSum 1.0, maxP 95%, minS 60, and maxEdges 500. Other tools used in ATLAS were: diamond v0.9.29.130, blastp 2.9.0+, Prodigal v.2.6.3, ruby v.2.6.5p114. Quality of the MAGs was assessed with checkM v.1.1.1[100] and BUSCO v.5.8.0.[101]

DNA damage analysis

The raw sequencing data were mapped against the complete reference genomes of *L. lactis* (RefSeq ID: NC_002662.1), *L. cremoris* (RefSeq ID: NZ_BCVK01000000) *S. thermophilus* (RefSeq ID: NC_017581.1) and *L. paracasei* (RefSeq ID: NZ_CP007122). The mapping and further processing was conducted as described above using the EAGER pipeline v.1.92.55.[102] In brief, the DNA fragments were mapped using BWA aln[103] with a mapping quality of 25 and a maximum edit distance of n = 0.1. All other parameters were used as default by EAGER.[102] Duplicates were removed with MarkDuplicates (https://broadinstitute.github.io/picard) and Qualimap v.2.2.1[104] was employed to evaluate the mapping. Finally, DamageProfiler v.1.0[105] was used to investigate the damage patterns.

16S rRNA-based bacterial community analyses

16S rRNA gene amplicon sequencing

Amplicon sequencing of the 16S rRNA gene was performed as described previously[29] with some modifications. Briefly, libraries were prepared by PCR amplification of the V1-V2 region of the 16S rRNA gene using primers 8F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 355R (5′-GCWGCCTCCCGTAGGAGT-3′) with 35 to 40 cycles at 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 30 s after an initial denaturation step at 94 °C for 2 min. The amplicons were sequenced on an Ion S5™ System with an Ion530 Chip (Thermo Fisher Scientific, Waltham, MA, USA).

Bacterial composition

The single-end (SE) raw reads (mean length = 310 bp; sd = 32 bp) were primer trimmed and quality filtered (maxEE = 15, truncQ = 6, maxN = 0, *n* = 1e+06, minLen = 100, max-Len = 460) in DADA2.[75] Amplicon sequence variances (ASVs) were obtained in DADA2 with the parameter POOL = ‘‘pseudo’’. Taxonomic annotation was performed using DAIRYdb v1.2.5[93] with IDTAXA.[76] ASVs assigned to *L. lactis* were further resolved to *L. lactis*, *L. cremoris* or *P. laudensis* by blastn v.2.13.0+ and the command: blastn -query [query_file.fasta] -db [16S_ribosomal_RNA.fasta] -out [output_file.txt] -outfmt "6 qseqid sseqid pident length mismatch gapopen qstart qend sstart send evalue bitscore salltitles" -num_alignments 5.

The bacterial communities were analyzed at the species level. Multivariate statistical analyses were performed using RStudio Pro v.1.4.1103-4 (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/) with the R software v.4.0.3 (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/) and the Phyloseq package v.1.42.0.[77]

Bacterial community changes

The barchart was generated with normalized compositional samples. For clarity reasons, all species with abundances lower than 1% in all samples were removed. The richness and diversity plot was performed using the *plot_richness* function in Phyloseq with the not normalized counts at species level.

The PCA biplot was performed using the normalized species abundance table. The values were Hellinger-transformed using the *decostand* function in the vegan package v.2.6-4.[78] The Principal Component Analysis (PCA) was calculated with the *prcomp* function in stats package with parameter scale=FALSE. The PCA biplot was visualized using the function *fviz_pca_biplot* from the factoextra package v.1.0.7.[79]

Functional composition

The functional composition analysis was based on outputs generated by the ATLAS pipeline (v.2.5.0).[70] We quantified the number of SPAdes-assembled contigs assigned to each DASTool bin to assess genome-resolved functional potential. ATLAS additionally enumerated gene families by first clustering all annotated genes across all samples at 90% identity and 90% coverage. Reads from each sample were subsequently mapped to the resulting gene catalogue, and abundances were normalized as counts per million (CPM). Plasmid contigs were identified using geNomad.[57] Antibiotic resistance genes were annotated using Usearch[81] against the ResFinder database[40] using a 70% identity and 80% target coverage cutoff. In addition, fARGene[41] was used to identify potentially novel ARGs.

Genomic evolution of key species

To quantify nucleotide diversity in *L. lactis* and *S. thermophilus*, quality-filtered reads were mapped to a reference collection of SLAB species genomes using BWA-MEM v.0.7.17.[106] Species-level mappings were included only when the relative abundance of the species exceeded 0.01%. Single-nucleotide polymorphisms (SNPs) were identified with FreeBayes,[107] and only positions with >10× coverage, located within the core genome, and exhibiting an allele frequency between 0.2 and 0.8, were retained. For phylogenetic analysis, all available RefSeq proteomes for *L. lactis* and *S. thermophilus* were downloaded. A core-proteome phylogeny was inferred using OrthoFinder v2.5.5[82] and phyloplan[88] and visualized with iTOL.[83] Distances between MAGs and their nearest phylogenetic

neighbors were calculated in R using the *find_nearest_tips* function from the castor package v.1.7.4.[80] To further characterize genome integrity, gene sequences were extracted with Bakta v1.9.2,[84] and pseudogene counts were identified using PseudoFinder v.1.1.0.[85]

Bacteriophage communities

Bacteriophage contigs were identified from the ATLAS assemblies using geNomad v.1.7.4.[57] Only contigs longer than 1 kb were retained. Contigs were de-replicated at 99% nucleotide identity and 85% alignment coverage using a combination of BLASTn v.2.14.1,[108] aniclust v.1.0.2,[86] and seqkit v.2.6.1.[87] Following de-replication, only phage clusters with a total length of at least 5 kb were retained for downstream analyses. Genome completeness was assessed using CheckV v.1.0.1,[86] bacterial host prediction was performed with iPHoP v.1.3.3,[59] and phage lifestyle was inferred using BACPHLIP v.0.9.6.[89] Phage prevalence and abundance were estimated by mapping metagenomic reads to the phage reference collection using BWA-MEM v.0.7.17,[106] and coverage was calculated with CoverM v.0.6.1.[90] Phage genomes were annotated with Pharokka v.1.7.2.[91] Viral clustering and taxonomic assignment were performed using vContact2 v.0.11.3[58] with the RefSeq viral reference database (downloaded 20241201). CRISPR spacers from *S. thermophilus* were extracted directly from metagenomic raw reads as previously described.[64] Briefly, conserved CRISPR repeat sequences were used to identify and excise CRISPR arrays from raw reads using Cutadapt v.4.6.[92] Extracted spacers were then de-replicated using DADA2 v.1.28.0.[75] To infer spacer origins, the non-redundant spacer sequences were mapped against the bacterial reference database.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed in R v.4.0.3 (R Core Team, 2021) within RStudio Pro v.1.4.1103-4 (RStudio Team, 2020), unless otherwise stated. Bacterial community composition was analyzed at the species level. Alpha diversity (richness and diversity) was estimated from counts using the *plot_richness* function in the Phyloseq package v.1.42.0.[77] For beta-diversity, species abundance tables were Hellinger-transformed using the *decostand* function in the vegan package v.2.6-4,[78] and a PCA was computed with the *prcomp* function (scale = FALSE) in the stats package and visualized with the *fviz_pca_biplot* function in the factoextra package v.1.0.7.[79] For genomic evolution analyses, single-nucleotide polymorphisms were retained only at positions with >10x coverage located within the core genome and exhibiting an allele frequency between 0.2 and 0.8; species-level mappings were included only when the relative abundance of the species exceeded 0.01%. The specific software packages, versions, and parameters used for each analysis, together with the number of samples (n = 8 cheese wheels) analyzed, are detailed in the corresponding subsections above and in the key resources table.