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Deciphering microbial communities of three Savoyard raw milk cheeses along ripening and regarding the cheese process

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ABSTRACT

Different Savoyard cheeses are granted with PDO (Protected Designation or Origin) and PGI (Protected Geographical Indication) which guarantees consumers compliance with strict specifications. The use of raw milk is known to be crucial for specific flavor development. To unravel the factors influencing microbial ecosystems across cheese making steps, according to the seasonality (winter and summer) and the mode of production (farmhouse and dairy factory ones), gene targeting on bacteria and fungus was used to have a full picture of 3 cheese making technologies, from the raw milk to the end of the ripening. Our results revealed that Savoyard raw milks are a plenteous source of biodiversity together with the brines used during the process, that may support the development of specific features for each cheese. It was shown that rinds and curds have very contrasted ecosystem diversity, composition, and evolution. Ripening stage was selective for some bacterial species, whereas fungus were mainly ubiquitous in dairy samples. All ripening stages are impacted by the type of cheese technologies, with a higher impact on bacterial communities, except for fungal rind communities, for which the technology is the more discriminant. The specific microorganism's abundance for each technology allow to see a real bar-code, with more or less differences regarding bacterial or fungal communities. Bacterial structuration is shaped mainly by matrices, differently regarding technologies while the influence of technology is higher for fungi. Production types showed 10 differential bacterial species, farmhouses showed more ripening taxa, while dairy factory products showing more lactic acid bacteria. Meanwhile, seasonality looks to be a minor element for the comprehension of both microbial ecosystems, but the uniqueness of each dairy plant is a key explicative feature, more for bacteria than for fungus communities.

1. Introduction

Raw milk is defined by the European Food Safety Authority (EFSA) as "milk produced by farmed animals which has not been heat treated to more than 40°C nor had any equivalent treatment" (Andreoletti et al., 2015).

Raw milk cheeses are an important part of Savoie's (France) cultural heritage and a significant source of income. Some of them have been granted with either the Protected Designation of Origin (PDO) or the Protected Geographical Indication (PGI). These designations protect

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indigenous food products based on geographical and biotechnological features. It is currently estimated that raw milk cheeses have an organoleptic richness due to the microbial richness and diversity (Duthoit et al., 2005; Frétin et al., 2022; Montel et al., 2014) and the specific flavors developed by wild strains (raw milk native and environmental microbiota) in comparison to industrial starters (Ayad et al., 1999; Wouters et al., 2002). Empirically, it is believed that wild strains are essential to produce traditional cheeses that stand out from industrial ones.

Lactic acid bacteria (LAB) are commonly added as starters in milk to produce cheeses mainly due to their ability to consume lactose to produce lactic acid. Depending on the type of cheeses (Nutrition Division, 2011) and the selected transformation process, different types of starters can be used: mesophilic and/or thermophilic cultures. Most of the cheeses are then ripened under the action of some LAB, non-starter bacteria, yeasts and molds either naturally present (raw milk, brine, environment) or intentionally added (commercial solution). Ripening is a slow process during which a succession of microorganisms occurs (Irlinger and Mounier, 2009). Starters acidify the dairy matrix, decreasing the pH.

During the following days, in surface ripened cheese, yeasts and molds colonize the cheese, use lactate and then leads to the deacidification of the cheese allowing the development of less acid tolerant communities of bacteria which may include the *Brevibacterium, Glutamicibacter* and *Corynebacterium* genera (Irlinger and Mounier, 2009). Ripening bacteria, yeasts and molds contribute to the development of texture and flavor characteristics in particular thanks to lipolysis and proteolysis mechanisms (Smit et al., 2005). The latter is responsible for peptides and amino acids released from caseins (Law, 2010). Amino acids are then the substrates for transamination, dehydrogenation, decarboxylation and reduction, providing a wide variety of flavor compounds including alcohols, aldehydes and organic acids. Balanced lipolysis can be the way to produce a large diversity of desirable aromatic compounds including secondary alcohols and esters. (Marilley, 2004).

For a long time, most of the studies aiming at to assessing the diversity of dairy microbial communities have been made using traditional cultivation-based methods including microbiological counts, biochemical characterization, isolation, and identification of specific colonies. The non-cultivated or rare taxa have thus rarely been considered (Feurer et al., 2004). Now, culture independent methods such as metabarcoding allows an in-depth view and a better understanding of food microbiota (Sabater et al., 2021). Some studies highlighted the richness, and the diversity of microbial communities present in raw milks or in dairy products produced with raw milk (Bettera et al., 2023; Biçer et al., 2021; Egger et al., 2021; Papadimitriou et al., 2022; Yap et al., 2021). Studies have already revealed that milk composition fluctuates because of many factors, including season, climatic conditions, lactation stage, farm system, indoor versus outdoor housing, cleaning process and feed (Nikoloudaki et al., 2021; Parente et al., 2020; Yap et al., 2021).

Few studies investigated microbial dynamics during ripening. These dynamics are suspected to be multifactorial and impacted in particular by biotic and abiotic elements such as the milk primary production conditions, raw milk and environmental microbiota, technological process (temperature, salt content, cooking, washing, duration of ripening), selection drivers (pH, water activity) and duration of ripening (Dugat-Bony et al., 2019, Dugat-Bony et al., 2015; Frétin et al., 2022, Frétin et al., 2018; Irlinger et al., 2015). Some technologies induce heat to cook the coagulum, others do not. Brine baths or direct addition of salt contribute to microbiota selection during ripening. In particular, it has been shown that salt and moisture content of the rind are the major drivers of fungal microbiota development (Spinnler, 2017).

There is a lack of knowledge regarding how the native microbiota of raw milk develops during ripening (Gatti et al., 2014; Poznanski et al., 2004). This last point is very important for cheese makers and the whole dairy sector and could promote the use of raw milk to produce traditional cheeses having particular aromatic features. Thanks to metabarcoding analysis, this project aims to establish a full picture of the microbial communities and diversities of three different French raw milk cheeses from Savoie granted with either PDO/PGI designation considering different parameters of which the season and the type of production, farmhouse or factory production type, the farmhouse type is made up with the milk of a single herd. The evolution of microbiota during ripening has been meticulously decrypted.

2. Material and methods

2.1. Sample collection & microbial enumeration

A large collection of samples was performed in a total of 12 different cheeses dairy plants. Three different French cheese (Savoie) technologies granted by PDO were studied: one smear-ripened hard cheese type (H), one semi-hard (SH) and one soft cheese type (S) (Andreoletti et al., 2015).

Starters are selected and traditional in H technology, and only selected in SH and S technologies. In H technology, fresh curd is cooked until 50 °C, 36 °C in SH, and not cooked in S. All technologies use pressing and brine baths. The ripening time is 100 days for H, 30 days for SH, and 20 for S.

Cheese making technologies are schematize on Fig. A.a (S), Fig. A.b, (SH) Fig. A.c (H) in Supplementary section. All cheeses were made using cow milk. Sampling was performed on raw milk, starters, milk containing a pool of starters, brine, curd and rind. Curd and rind were sampled at different stages of ripening, for all technologies the first day of the curd ripening was sampled, others depended on the technology: 7, 30, 60, 100 days for H technology, 8, 12, 20 days for SH technology and 7, 15, 20, 30 days for S technology.

Except for the samples taken when no rind had formed, the rind was gently separated from the core using sterile knives and both rind and core fractions were analyzed to obtain a more detailed picture of the microbial diversity for those cheeses. For each cheese technology, samples were carried out in three repetitions (three successive days of sampling), in 12 different cheese dairy plants either in summer or winter. They were chosen for the diversity of their processes; two were farmers (milk from a single farm) and two were dairy factories (milk collected from many farms). It represents a total of 923 samples processed in this study.

When needed, the samples (5 g) were processed in a homogenizer for 2 min (AES Chemunex, Easy Mix) and diluted up to 10–8 by 10-fold serial dilutions. Total microbiota was enumerated according to NF EN ISO 4833. Briefly, samples were inoculated (counting in mass) on agar plates containing 5 nnn of casein enzyme digestate, 2,5 g/L of yeast extract, 1 g/L of anhydrous glucose and 1 g/L of skim milk powder. Plates were incubated aerobically during 72 h at 30 °C.Yeasts and molds were counted according to NF V 08–059. Briefly, samples were plated on agar plates containing 5 g/L of yeast extract, 20 g/L of glucose and 0,1 g/L of chloramphenicol. Plates were incubated aerobically during 5 days at 25 °C.

2.2. DNA extraction

All samples were prepared to extract DNA from the microorganisms in each sample. Briefly, samples were homogenized in a stomacher for 2 min (AES Chemunex, Easy Mix) after being resuspended in a citrate buffer (20 g/L). The suspensions were centrifuged at 6000 xg and washed twice using citrate buffer to remove impurities. DNA extraction and purification were performed following the recommendations of the provider (DNeasy Blood & Tissue Kits, Qiagen). DNA integrity assessment was done by visualization after electrophoresis and the DNA concentration was evaluated using spectrophotometric method.

2.3. Bacterial 16S rRNA genes (S) and fungal ITS2 gene amplification and amplicon sequencing

Bacterial analysis targeted the V4 region of the 16S rRNA genes (S) using the Bact02 primer pair (Taberlet et al., 2018) (5'-GCCAGCMGCCGCGGTAA-3' and 5'-GGACTACCMGGGTATCTAA-3'). The fungal analysis targeted the fungal ITS1 region, using the Fung02 primer pair (Taberlet et al., 2018) (5'-CAAGAGATCCGTTGYTGAAAGTK-3' and 5'-GGAAGTAAAAGTCGTAACAAGG-3'). These primer pairs were selected based on their in silico coverage and taxonomic resolution, as evaluated on the EMBL public database version 133 by running an in silico PCR using the ecoPCR program (Ficetola et al., 2010). Bact02 and Fung02 amplicons were 254 bp and 225 bp long on average without primers (Taberlet et al., 2018). PCR was done following the recommendations of the provider (AmpliTaq Gold, Applied BioSystems). The PCR mix contained 10 µL of AmpliTaq Gold 360 Master Mix 2× (Applied Biosystems), 2 µL of primers (5 µM each), 0.16 µL of bovine serum albumin (3.2 µg; Thermo Scientific™) and 2 µL of DNA template, in a final volume of 20 µL. PCR amplifications consisted of a 10 min denaturation step at 95 °C, followed by 33 (Bact02) or 40 (Fung02) cycles of a 30s denaturation at 95 °C, a 30s annealing at 53 °C (Bact02) or 56 °C (Fung02), and a 90s elongation at 72 °C; and finally a 7 min elongation step at 72 °C.

Four independent PCR replicates were carried out for each DNA extract. To allow bioinformatic discrimination of PCR replicates after sequencing, eight-nucleotide tags were added on the 5′ end of both the forward and reverse primers, so that each PCR replicate was represented by a unique combination of forward and reverse tags. Tags had at least five nucleotide differences among them (Coissac, 2012).

The success of amplification was checked for a subset of randomly selected PCR products by running 5ul aliquots on a Qiaxcel (Qiagen). PCR products from the same marker were pooled together in equi-volumes and purified using the MinElute PCR Purification kit (Qiagen) following the manufacturer's protocol. Purified amplicons were sent to Fasteris (Geneva, Switzerland; https://www.fasteris.com/en-us/) for library preparation using the MetaFast protocol (https://www.fasteris.com/en-us/NGS/DNA-sequencing/Amplicons-sequencing/Short-Amplicon-sequencing). Finally, two libraries were sequenced per marker on a MiSeq Illumina platform using a 2 × 250 bp paired-end approach.

2.4. Metabarcoding data processing

The OBITools (Boyer et al., 2016) software suite was used to process raw sequencing data. Paired-end reads were first assembled with the program *illuminapairedend*, keeping only sequences with an alignment score higher than 40. Aligned sequences were assigned to the corresponding sample using the program *ngsfilter*, by allowing two and zero mismatches on primers and tags, respectively. After sequence dereplication using *obiuniq*, sequences observed <1000 times overall and sequences whose length fell outside the expected size interval [200-300 bp] for Bact02 and [60-500 bp] for Fung02 were discarded.

Clustering of sequences was done using a minimum threshold of 97 % of sequence identity. Taxonomic assignment was performed with the *ecotag* program from the OBITools using either a reference database built from GenBank or the Silva 126 database. The GenBank database was built by performing an in silico PCR with EMBL version 133, using the ecoPCR program (Ficetola et al., 2010) and the Bact02 or Fung02 primer pair, and allowing three mismatches at most per primer. The GenBank reference database was further curated by keeping only sequences assigned at the species, genus and family levels. The obtained taxonomic assignment was manually corrected and validated using nBlast solution (NCBI) and with the support of scientific literature.

Phyloseq package (McMurdie and Holmes, 2013) was used to determine Shannon diversity index, (Shannon, 1948) specific richness while Vegan package (Oksanen et al., 2015) was used to evaluate Bray-Curtis dissimilarities (Bray and Curtis, 1957) and to realize NMDS.

2.5. Statistical analysis

All statistical analysis were made using the 4.2.3 R version (R Core Team, 2023). Also, PERMANOVA (Anderson, 2001) and pairwise PERMANOVA were calculated with the Bray-Curtis distance, both with 999 permutations using vegan (Oksanen et al., 2022) and pairwiseAdonis packages (Pedro, 2017). The post-hoc test krukalmc was made after a Kruskal-Wallis test, using the pgirmess package (Giraudoux, 2023). When parametric conditions were met for an ANOVA, the post-hoc test used was the Tukey-HSD test of the stats package of the R base.

ANCOM-BC model (Lin and Peddada, 2020) was used to classify bacterial and fungal species through ripening. For the elaboration of models, starter species as well as species which were found to be present in raw milk but not in curd and rind were not considered. Bacterial species used as starters during cheese making composition were known, but it was not the case for fungal species. It was then estimated using the 25 most abundant taxa found in starter samples, which represent between 50 and 98 % of the composition. Usually, 3 to 5 species of fungus are added in starters, the fact that so many are found in starters indicates probable contamination during sampling.

For the bacterial model, matrices only were included in the model, whereas for the fungal model, matrices and technologies were put in the formula. ANCOM-BC was first run with the non-normalized abundances tables, considering structural zeros, and assuming a negative binomial distribution of the data. The sample biases were calculated, and the abundances tables were changed by log-transforming the count and adding a pseudocount of 1. The sample biases were subtracted from the news abundances tables to correct them.

ANCOM-BC was run a second time on the new abundance tables, and coefficients were obtained. Depending on their value (see in Results section) they allow taxa to be classified: starter, ubiquitous, promoted by process, promoted by environment, less than milk or not detected during ripening.

To calculate the influences of season and farmhouse/factory different models were tested regarding on the kingdom bacteria or fungus. Heatmap realized next to the classification were made with the unifrac distance, from a phylogenetic tree realized with the function rtree with the ape package (Paradis and Schliep, 2019).

3. Results and discussion

3.1. Microbial diversity of dairy samples

A rough estimate of the diversity is the specific richness that is a count of all the species present in a sample, regardless of their abundance. The Shannon diversity also considers the relative abundance of each species (Feranchuk et al., 2018). It is zero when there is only one species present and maximal when all the species are present at equal abundances.

Microbial diversity has been observed in all the different dairy samples collected (Fig. 1). Notably, results revealed a tremendous microbial diversity in raw milk in which respectively 164 and 105 different bacterial and fungal species (for respectively 141 and 74 genus) were found. Of particular interest, brines exhibited an unexpected microbial richness, respectively 124 and 101 bacterial and fungal species. Concomitantly, raw milks and brines are samples containing the smallest concentration of revivable aerobic mesophilic bacteria (Fig. 2. a). Kruskal-Wallis test detected a significant difference between the Shannon indices of different sample types (bacteria: p-value $<2.2.10^{-16}$, fungi: p-value $= 6.957.10^{-13}$), and the richness of the different matrices (bacteria: p-value $<2.2.10^{-16}$, fungi: p-value $<2.2.10^{16}$). The post-hoc test detected several significant differences by pairs for bacterial communities (Table A Supplementary section) and fungal communities (Table B Supplementary section).

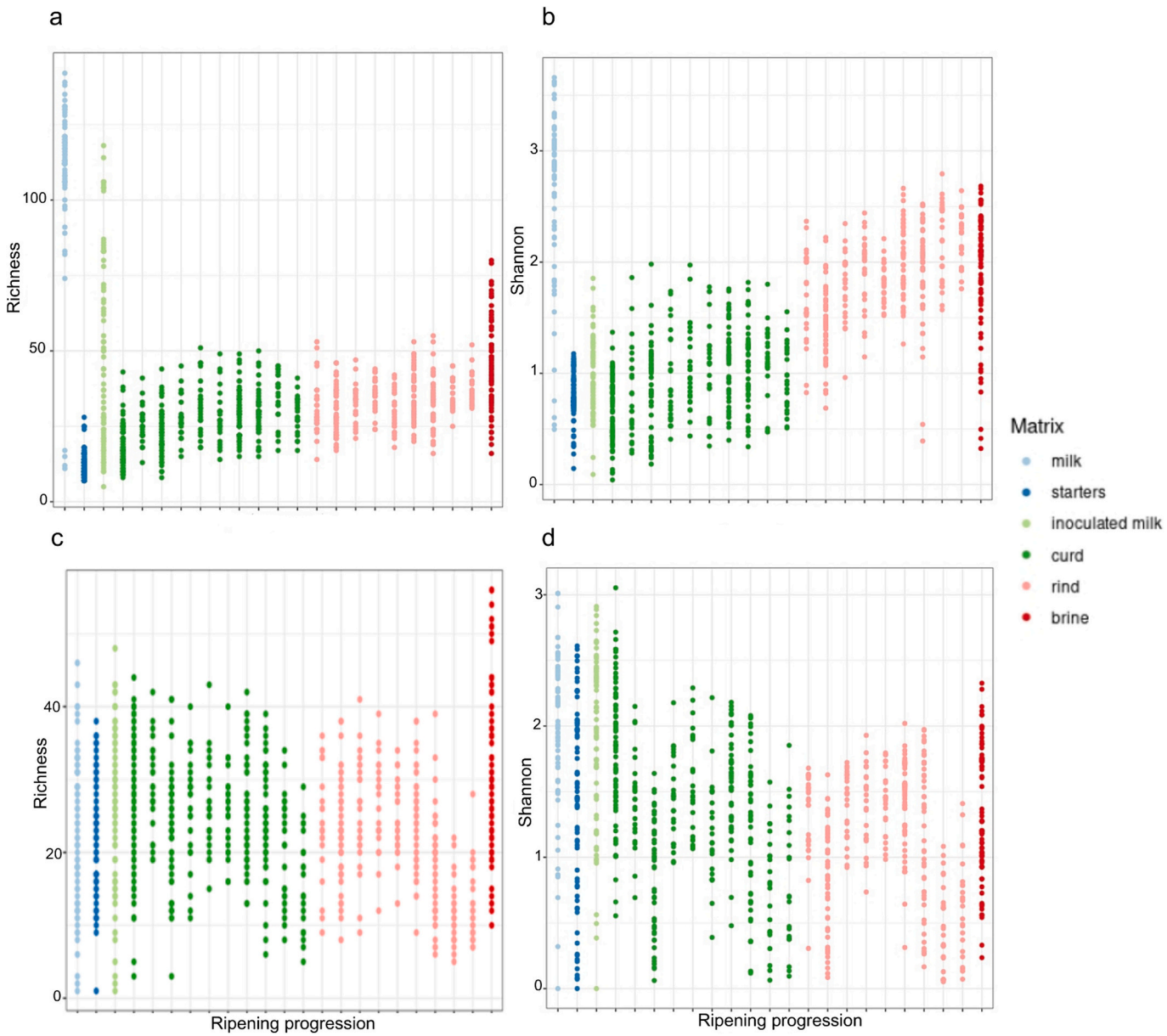

Fig. 1. Overview of the microbial α-diversity. Specific richness (a and c) as well as Shannon index on calculated on species (b and d) are represented for all samples collected: milk samples are represented in light blue, starters in blue, inoculated milk in light green, curd in green, rind in pink and brine in red. Samples of curd and rind are represented according to increasing ripening time. Results of samples collected in summer and in winter are shown. A and b represent results obtained for bacteria while c and d figures represent those obtained for fungi.

Log levels of bacteria and fungus (Fig. 2) also showed differences (p-value $<2.10^{-16}$), details are presented in Table C Supplementary section. As expected, (Levante et al., 2023), raw milks exhibit higher bacterial diversity and richness according to both measures (Fig. 1.a and b; Table A Supplementary section). There is no significant difference in bacterial specific richness between starters and inoculated milk (Fig. 1.a, Table A Supplementary section), but the diversity of the latter is higher than those of starters, as revealed by Shannon index (Fig. 1.b). LAB starters are composed of mesophilic and/or thermophilic bacteria and mostly comprised few species representatives (either *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* or other *Lactobacillus* sp.) while inoculated milk is the result of the addition of starters to raw milk.

There is thus a recovery in bacterial diversity following the addition of starters in milk until the end of the ripening, which is more strongly obvious with respect to the Shannon Index. The bacterial diversity significantly increases all along the ripening, in the rind and the curd, in contrast to the specific richness (Fig. 1.b). In parallel, the concentration of revivable aerobic mesophilic microbiota also increases (Fig. 2.a). This indicates that bacterial species develop in a way that leads to a greater homogeneity in proportions.

Conversely, the fungal specific richness and the diversity does not change so drastically during ripening with a pattern even reversed (Fig. 1.c and d, Table B Supplementary section). Fungal counts showed an increase during ripening. As envisaged, there is a higher bacterial richness and diversity in the rind than in the curd, indicating a greater number of species on the surface and a greater homogeneity in the species proportions.

Physicochemical characteristics of rind and curd differ significantly and can explain to some extent a part of this variability (Beresford et al., 2001). Indeed, curd is an environment that favors mainly microorganisms able to grow in anaerobic conditions. LAB are then the main

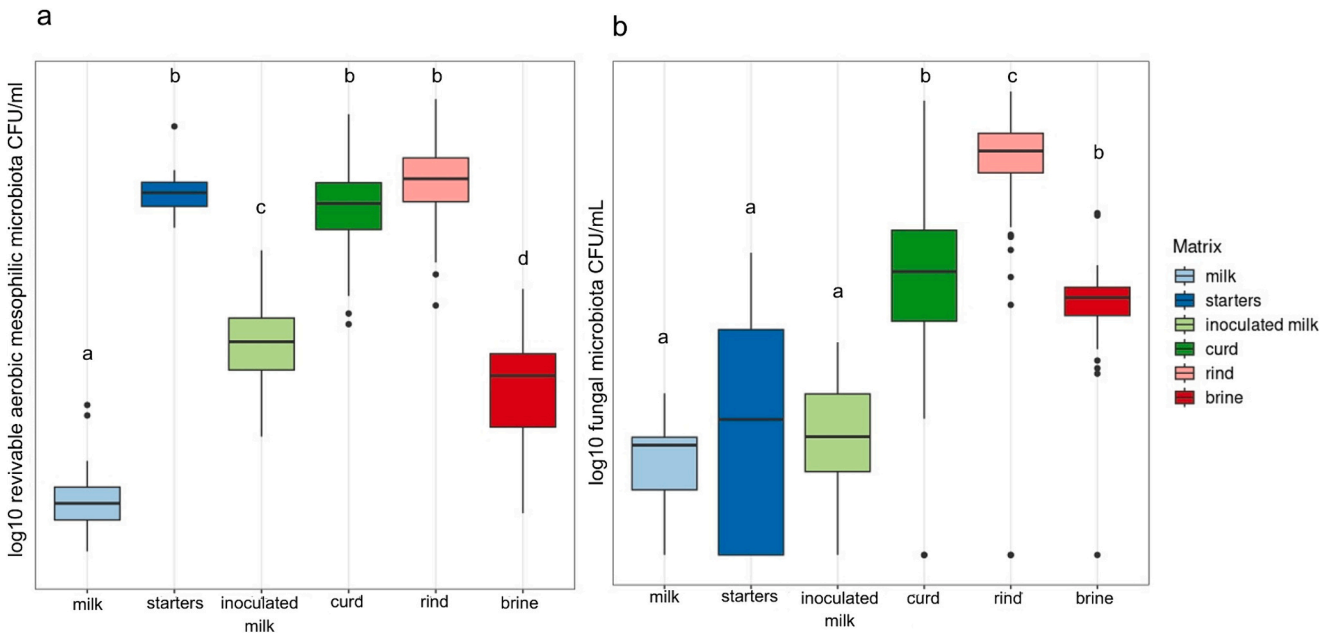

Fig. 2. Abundance of revivable aerobic mesophilic bacteria (a), and fungi (b) in dairy products. Tukey-HSD statistical test with a threshold of 0,05 was used on data. The results of the test are indicated by letters, e.g. samples with identical letters are not statistically different. The left and right sides of the box are the first and third quartiles. The box thus covers the interquartile range, where 50 % of the data lie. The vertical line dividing the box in two represents the median.

bacteria of the curd. More contact with the external environment, use of brine, cheese washes as well as different conditions of oxygenation promote the development at the surface of the cheese of other micro-organisms belonging to *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroides*, yeasts and molds (Irlinger et al., 2015).

Given the high concentration of fungi in brines (Fig. 2.b), it could represent an important reservoir for further development in cheese rind. The presence of a high quantity of yeasts in the brines is hypothetically related to their halotolerance as well as their ability to grow at low temperatures. Use of brines is the most common method during cheese making. Brine is a salty biome and selects mostly halophilic species among them the genus *Staphylococcus, Debaryomyces, Clavispora, Torulaspora* are well represented (Hammer et al., 2019; Innocente et al., 2023; Levante et al., 2023). During this processing step, Na + and Cl- transfer from the brine into the outer layers of curd driven by osmolarity (Fox et al., 2017). Few are studies reporting about brine's microbial

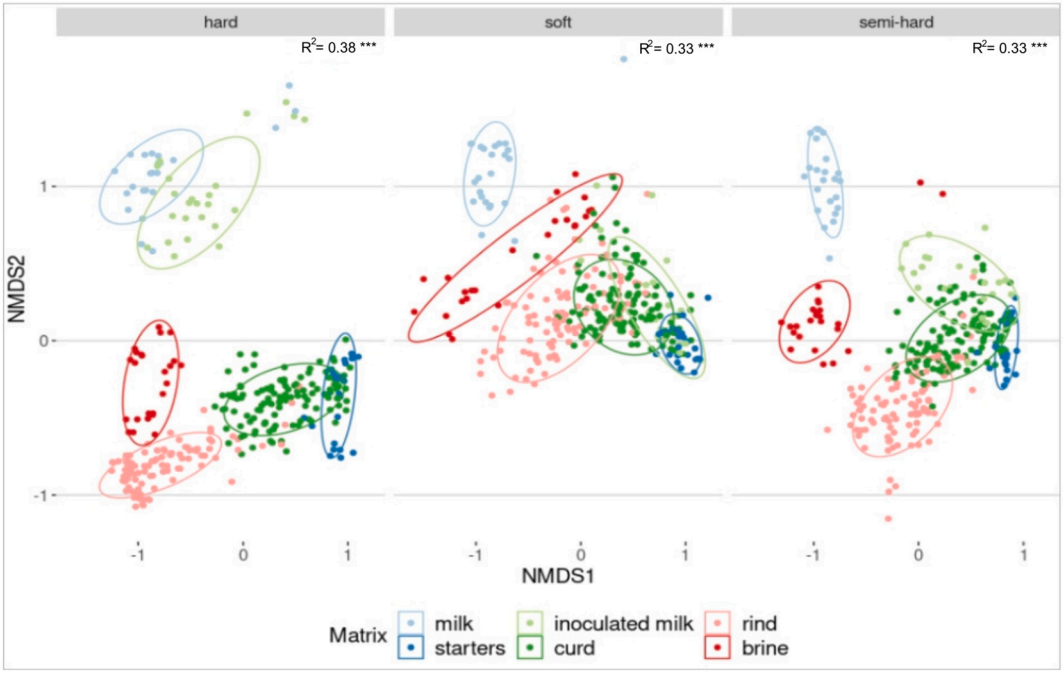

Fig. 3. Multidimensional representation (NMDS) of bacterial microbiota's samples depending on matrices. Samples have been separated by technology (hard cheese: left frame, soft cheese: middle frame and semi-hard cheese: right frame). The NMDS was realized after an ordination on the Bray-Curtis distances matrix. The ellipses cover 80 % of a multi-variate t distribution. Stress = 0.21. R2 with significance were obtained using a Permanova test with 999 permutations. p-value: (0.05–0.1]., (0.01–0.05]*, (0.001–0.01]**, (0–0.001]***.

communities, but it has been shown that microbial strains in brines could support further aromatic richness development in cheeses (Innocente et al., 2023), in addition to that from the raw milks.

3.2. Microbial dynamics during cheese process

The compositional dissimilarities of the bacterial communities were investigated between the type of dairy samples according to the different technologies (Fig. 3). Figs. 3 and Fig. B. (Supplementary section) reveal the distribution of respectively bacterial and fungal communities according to matrices and grouped by technologies. Results revealed that raw milk and starters bacterial samples form tight and well separated groups, showing that their ecosystems are very different (Fig. 3). A pairwise adonis test (alpha = 0.05) shows that starters are significatively different according to technologies (p-adjusted = 0.012), because technologies require certain species capable of growing and acting according to different temperatures and needs for each final product. The curd and rind samples follow a perpendicular path to the milk/starter axis, due to the influence of processing and the cheese environment. Finally, the brine is also off the milk/starter axis, but closer to milk, thus remaining a putative reservoir of biodiversity, shaped by salinity.

In addition, multiple pairwise adonis tests realized on the matrices show that, in the hard cheese technology, all bacterial communities are different depending on the matrices, but those of inoculated milk are closer to the raw milk's ones ($R^2 = 0.11$, p-adjusted = 0.015), compared to the other technologies. This certainly is because the Shannon diversity of starters is the highest in hard and SH technologies without being different from each other (H-S: obs.dif = 15.30, *p*-value <0.05; S-SH: obs.dif = 25.39, p-value <0.05), and that the starter quantity in H technology is lower than this in SH which is the highest (SH vs H: obs. diff = 28.82, SH vs S: obs.dif = 35.71, p-value <0.05). Starter's diversity and quantity partly explain that milk and inoculate milk are closer together in hard technology, and in an intermediate distance in SH technology.

In contrast, starters and inoculated milk bacterial communities look similar both in semi hard and soft cheeses (respectively $R^2 = 0.06$, p-adjusted = 0.360 and $R^2 = 0.04$, p-adjusted = 1). This indicates that the biodiversity of the raw milk is better retained following the first steps of hard cheese making.

In hard cheese technology, the rind bacterial ecosystem is far from that of raw milk ($R^2 = 0.20$, p-adjusted = 0.015), indicating that it is in this technology that the process and the environment have the greatest effect. In soft technology, the rind remains close to the milk/starter axis, so process and environment seem to have less impact on the microbiota during the ripening in this technology. Finally, SH technology shows an intermediate case for both the impact of starters on the inoculated milk communities and the effects of environment and process. It could be explained both regarding the ripening time and the steps used during the ripening (cheese washes using smear).

Fungal communities show a totally different pattern (Fig. B, Supplementary section). First, no major difference was observed between raw milk, inoculated milk and starters for none of the technologies tested. Only starters from SH technology are different from milk ($R^2 = 0.06$, p-adjusted = 0.015). The quantity of bacterial starters used is higher than that of fungal strains added during the process (Fig. 2). Bacterial starters quantity can differentiate the milk communities from the inoculated milk communities, which isn't the case with the fungal communities. Moreover, all fungal community matrices are differentiated by the technology (pairwise adonis, p-adjusted = 0.003). Those results are certainly explained by the fact that fungi develop during the ripening, when the pH is lower than those of the raw milk or inoculated milk and start to show differences in curd and rind. To see the evolution of bacterial communities during the process, the abundance of species depending on the technology was observed (Fig. 4). In raw milk, a wide variety of species has been detected. It included genus already described in the literature and are among others *Acinetobacter*, *Lactococcus*, *Streptococcus*, *Corynebacterium*, *Brevibacterium* and *Pseudomonas* (W. Donnelly, 2014).

Then, LABs are the major component of the curd regardless of the technology. The LAB composition in the curd for each technology is correlated to the composition of respective starters. LAB are selected for their role in acidification and in the synthesis of specific molecules involved in corresponding organoleptic qualities (Coelho et al., 2022). Thermophiles *Streptococcus thermophilus* as well as *Lactobacillus delbrueckii* are found in all technologies but *Lactobacillus helveticus* is only found in hard cheese curd which can be cooked until 50 °C (Fig. 4.a) while mesophilic LAB as *Lactococcus lactis* and *Leuconostoc mesenteroides* look of high importance in soft and semi hard cheeses (Fig. 4.b and c).

During ripening, the abundance of native microbiota (eg. whose origin is raw milk) increase for all technology but never represents >30 % in the curd. This microbiota, depending on the technology, is mainly composed of ripening bacteria including genus belonging to *Brevibacterium*, *Brachybacterium*, *Arthrobacter*, *Staphylococcus*, *Enterococcus* and *Corynebacterium.* It also includes marine-inhabiting microorganisms as *Alkalybacterium*, *Halomonas*, *Vibrio*, *Marinomonas*, *Psychrobacter* and *Facklamia.* Our results confirm a very contrasted picture of bacterial composition in the rind in comparison of the curd for all the cheeses, as noted by other authors (Frétin et al., 2022). Indeed, at the beginning of the ripening (either day 1 or 7 depending on the technology), the rind is also composed mainly of LAB but the native microbiota develops and even becomes the major community at the end of ripening. Their abundance reaches 80 %, 85 % and 95 % for respectively soft, semi-hard and hard cheese rinds.

The presence of *Faklamia* sp. and *Alkalibacterium* sp. have been already documented on Raclette type cheeses (Roth et al., 2011). *Alkalibacterium* as well as *Facklamia* are halophilic and alkaliphilic lactic acid bacteria (HALAB). HALAB were found to be present at very high concentration on soft, semi soft, semi hard and unripened cheese rinds reaching often 10^7 CFUs g^{-1} and in particular, *Alkalibacterium* was widely recovered (Ishikawa et al., 2007). The increase of the abundance of HALAB and other halophilic organisms looks to be related to the increase of pH and salt concentration during the ripening process leading to a more convenient environment for their growth. Strong positive relations between fungi and halophilic bacteria have been suggested, the deacidification of the firsts supporting the growth of the others (Wolfe et al., 2014).

The role of HALAB and other halophilic bacteria during ripening has been hypothesized as important but it remains a lack of knowledge. In a more general perspective, a recent study highlights the diversity of halophilic and halotolerant bacteria composing the rinds of a wide diversity of cheeses including soft, semi-hard, hard, blue cheeses with either washed, natural or bloomy rind type (Kothe et al., 2021). It included the genus found in our study as the Proteobacteria halophilic bacteria *Vibrio*, *Psychrobacter* and *Halomonas* as well as the gram positive *Brevibacterium*, *Brachybacterium*, *Arthrobacter* and *Staphylococcus*. The recent availability of additional genomes (Kothe et al., 2021) will support the technological characterization of these microorganisms.

While the introduction of some species is assumed to be correlated with the use of brine baths, some others may have other origins. *Brevibacterium* and *Corynebacterium* can inoculate the raw milk from the mammalian cutaneous microbiota (Aoki et al., 2021; Eidensohn et al., 2021). These two genera are of particular interest for ripening and are known to be responsible for particular flavor development and color (Bay et al., 2023; Rattray and Fox, 1999), as same as *Enterococcus* (Rodriguez et al., 2019). The sources of *Enterococcus* are not well known, but they are found in the farm environment, soil, bulk tank, and milking machine (Gelsomino et al., 2002). *Enterococcus* are in low levels in cheeses, and cannot change volatile compounds profiles in specific conditions tested (Bouton et al., 2009), or in the opposite, being involved on interesting aroma production, or even in enterocins productions against food spoilage organisms or pathogens (Giraffa, 2003).

An increase of the abundance of *Bifidobacterium mongoliens* is also

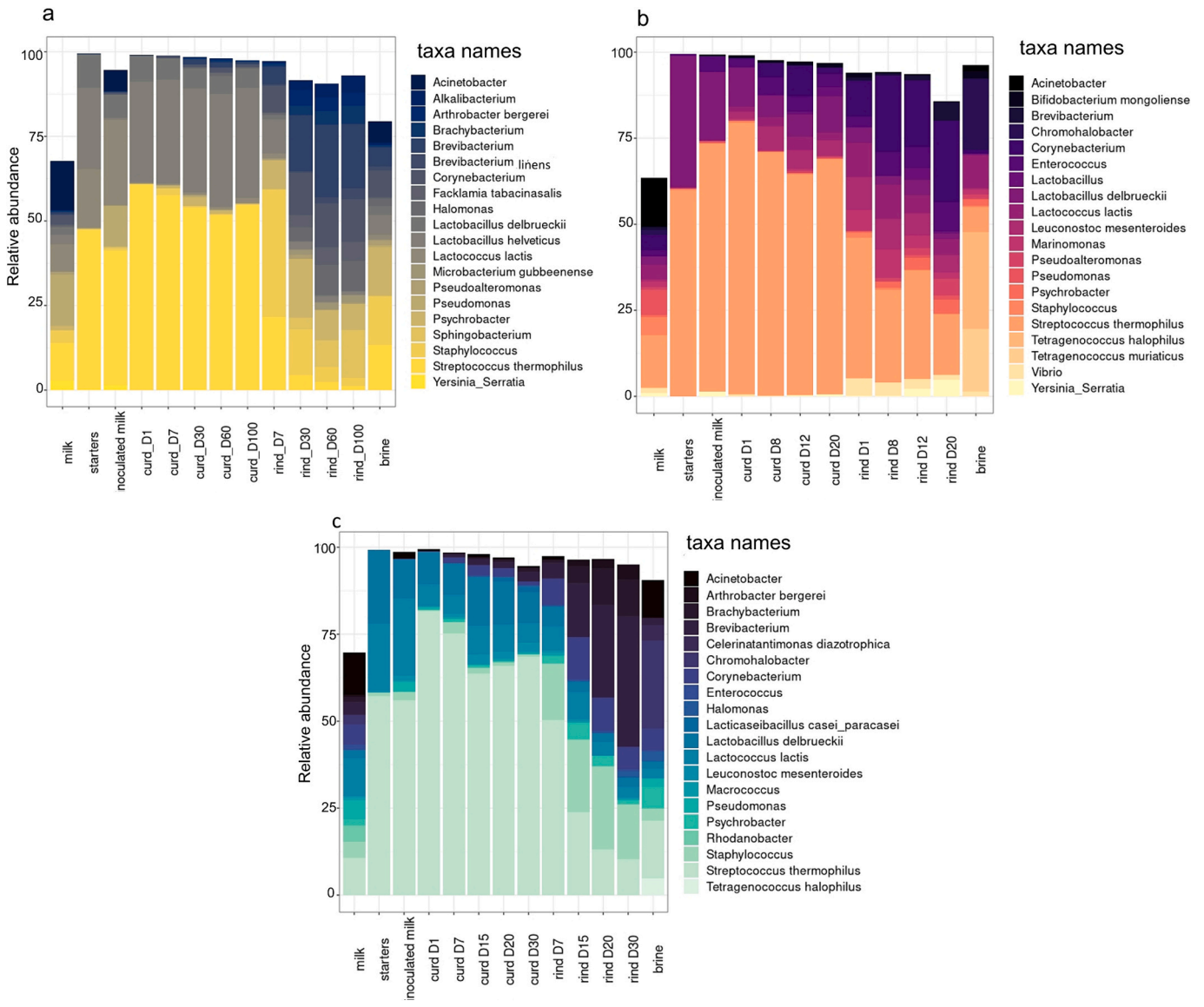

Fig. 4. Abundances of the 20 most abundant taxa in the hard cheese (a), semi-hard cheese (b) and soft cheese (c) technologies.

observed in soft cheese during ripening. This last species could have an interest for human health, by the production of antivirulent metabolites (Bondue et al., 2020).

We combine the data for bacteria from all types of cheese and seasons to get an overall picture of the abundance of each species. Fig. 5 shows a visualization of the density of the 164 bacterial species found in raw milk. We classified species by using the ANCOM-BC model, to determine which species were significantly greater or lower in abundance between matrices (Table D Supplementary section). All bacterial species were classified as follow: (i) Ubiquitous; species whose abundance is not significantly different between raw milk and curd and also between raw milk and rind, (ii) starters; species added during the process (*Streptococcus*, *Streptococcus thermophilus*, *Leuconostoc mesenteroides*, *Lactococcus*, *Lactococcus lactis*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*), (iii) promoted by environment; species whose abundance is significantly higher in rind in comparison to raw milk, (iv) promoted by process; species whose abundance is significantly higher in curd in comparison to raw milk, or higher in rind and curd than in raw milk, or not different between curd and raw milk but lower in rind, or not different between rind and raw milk but lower in curd. They are therefore favored by the cheesemaking process to different degrees. These are putative markers of typicity, (v) less than milk; species whose abundance is significantly lower in rind and curd than in raw milk and (vi) not detected; species present in raw milk, but not detected in rind and curd. It is remarkable that all the bacterial species detected in cheese are naturally present in milk, including those in starters (Fig. 4; Fig. 5). This finding demonstrates that “inoculation” not introduce new biodiversity, but simply allows specific species to prevail in the ecosystem at the desired time and to introduce a particular strain, selected for its technological abilities. It should be noted that metabarcoding cannot differentiate strains of the same species, in particular, between commercial strains and wild types. The number of microorganisms whose growth seems favored by process with a great abundance is relatively important, representing 53 taxa, and shows that specific microbial profile is produced by two inextricable factors: the native microbiota on the one hand and cheesemaking technology on the other. This microbiota is specially composed of: *Corynebacterium.sp*, *Brevibacterium.sp*, *Psychrobacter.sp*, *Brachybacterium.sp*, *Halomonas.sp*, *Sphingobacterium.sp*, *Lacticaseibacillus casei/paracasei*.

Some of them were found to be largely present in cheeses during ripening and seem of particular interest for the forthcoming of the final cheese product. *Corynebacterium*, *Brachybacterium* and *Brevibacterium*

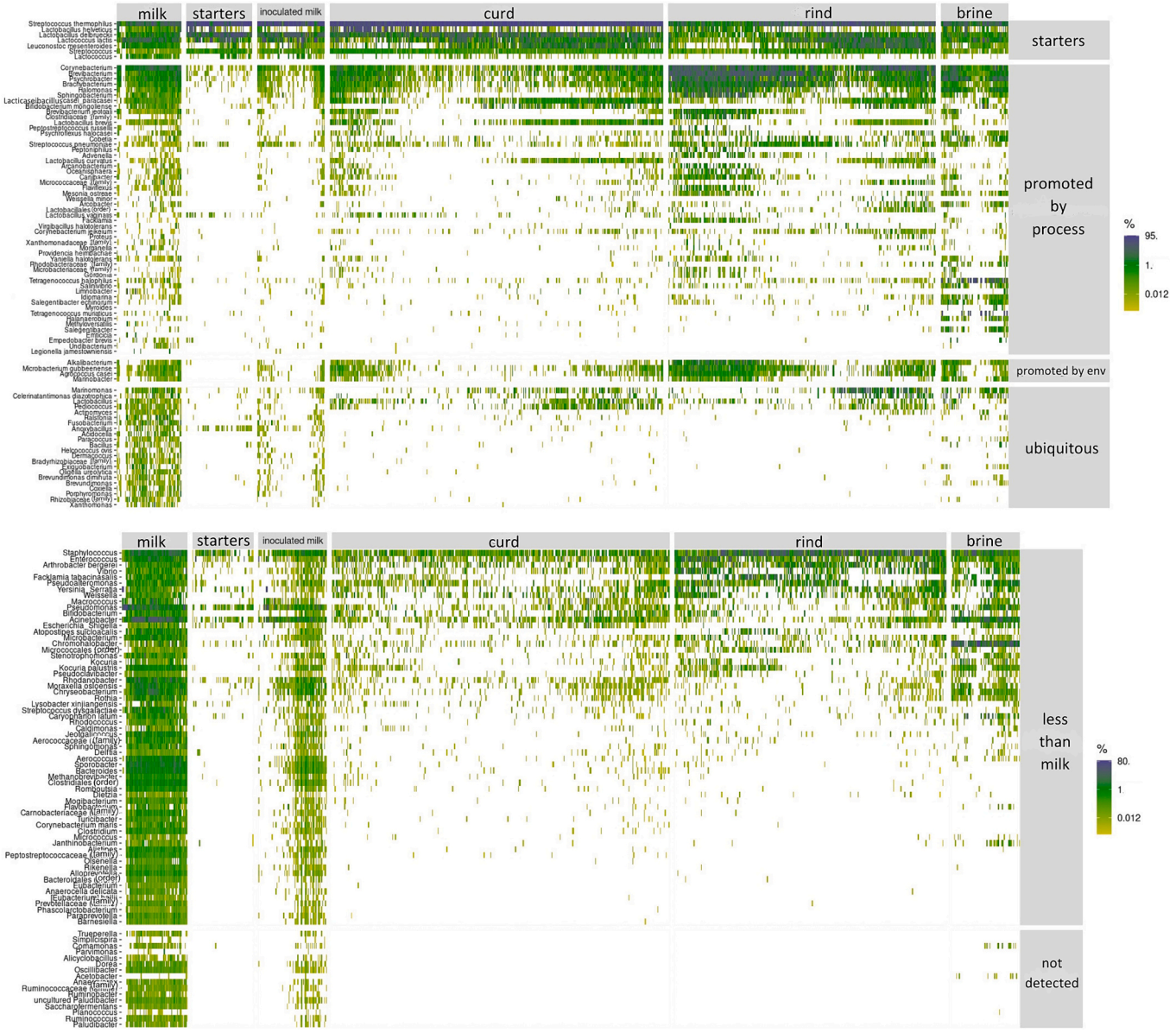

Fig. 5. Heatmap of all 164 bacterial species found in raw milk, sorted by decreasing abundances in rind and curd. All samples are presented from milk to brine, and each raw corresponds to a species. Sample ordination, within the groups resulting of the ANCOM-BC test, was based on clustering using NMDS, based on ecological distance calculated with Unifrac. The color indicates the percentage of the total counts in a sample represented by the species.

are aerobic coryneforms bacteria, developing during the ripening on the surface after the deacidification, and contribute to the synthesis of aroma and flavor compounds (Brennan et al., 2004). *Lacticaseibacillus casei/paracasei* is often found on surface ripened cheeses, and is a mesophilic non starter LAB. The source of this species is the raw milk or the dairy plants environment (Beresford et al., 2001; Fitzsimons et al., 1999) *Psychrobacter* are psychrophilic bacteria, halotolerant and aerobic, contribute to cheese ripening and aromatic compounds production (Irlinger et al., 2012). *Sphinobacterium* have already be found on hard cheese rind (Wolfe et al., 2014). *Sphingobacteria* has been described in the raw milk and the dairy environment (Quigley et al., 2013), but their role in cheese ripening is always unknown. *Halomonas* is a halotolerant genera, coming from the brine, which can be involved in the ripening process and aroma compounds production, notably in smear hard rind cheeses or in curd short ripened cheeses (Schornsteiner et al., 2014).

Some species decreased in the rind and the curd, but are always of a great abundance in the rind, without being in the starter, like *Staphylococcus.sp*, *Enterococcus.sp,* while others develop more in rind like *Alkalibacterium.sp*, *Agrococcus casei*, *Marinobacter.sp*, *and Microbacterium gubbeenense* which are halotolerant and/or halophilic species (Brennan et al., 2001), indicating that their provenance may be the brine., *M. gubbeenense* was already described in cheese surface (Mounier et al., 2009), like *A.casei* (Bora et al., 2007).

Interestingly, a few taxa present in raw milk are no longer detected during ripening. It includes: *Ruminococcaceae*, *Ruminobacter*, *Acetobacter*, *Simplicispira*, *Comamonas*, *Alicyclobacillus*, *Dorea*, *Oscillibacter*, *Paludibacter*, *Parvimonas*, *Anaerovorax*, *Planococcus*. While some genera such as *Acetobacter*, *Ruminococcaceae* and *Ruminobacter* include species commonly found on cow skin (Pham et al., 2017), most of the others are almost not described in the literature. It is still difficult to conclude why they are not detected during the process, but the reason could be related to their nutritional requirements that do not meet those found in milk, or because of the competition during the process, or the presence of phages that would have eliminated them.

All these results indicate that raw milk could be a purveyor of typicity and the growth of some of them can be favored or not depending on

the cheese process. Fig. 6 below presents the fungal classes abundances through the different matrices. Details are shown in Table E Supplementary section. Remarkably, fungi do not follow the same pattern as bacteria. Indeed, 71 % of fungi that are present in raw milk are ubiquitous and their abundance does not evolve significatively during the process from raw milk. Only few species are favored by process, they are *Cladosporium herbarum*, *Acremonium chrysogenum* and *Neurospora.*sp. *Cladosporium herbarum* is the most common species in the environment (Schubert et al., 2007), it is not surprising to see its development in the curd and the rind. *Acremonium chrysogenum* is known to produce cephalosporin C, which is a powerful antibiotic (Liu et al., 2018), its presence can certainly impact bacterial development. There is only one *Penicillium* species for which the growth is favored in the rind. Using our model, it looks like the abundance of this species is correlated with its abundance in other sources than milk and then could be brought by the environment. Only *Aspergillus chevalieri* decreased significantly in the rind and in the curd. No information has been found about this species in dairy products. Three species are "unclassified", because their origin could be the brine, but they are not sufficiently developed to be in the "favored by process" class, compared to the raw milk abundance.

All classes except the "unclassified" class, contain species in relatively large abundances in comparison to the starters. Raw milk and environment (such as the ambiance of the ripening cave) are once again a source of specificity in raw milk cheeses. Furthermore, the most abundant species in cheese do not only come from the starter, but they are also found in milk in important abundances. These species are: *Debaryomyces hansenii*, *Fusarium domesticum*, *Geotrichum candidum*, *Kluyveromyces lactis*, *Mucor fuscus*, *Clavispora lusitaniae* depending on the technologies.

It was already described that a part of the fungal community in Feta is less controlled by technological steps, suggesting that diversity may arise from raw material, the brine and/or the environment in which the production takes place. These routes for yeast inoculation/contamination of the final product have been suggested before (Papadimitriou et al., 2022).

3.3. Impact of cheese technology on microbial communities

One of our goals was to determine to what extent the microbial communities differed from one technology to another to determine their respective role to drive the fungal and bacterial communities.

Ordination method (NMDS) was then used to visualize bacterial and fungal communities according to (i) cheese technologies and (ii) matrices (Fig. C, Supplementary section).

Results show that both cheese technologies and matrices represent factors responsible for the structuration and the composition of bacterial and fungal communities (Fig. C and Table 1). Interestingly, the matrix is the most discriminant variable for shaping the bacterial communities, while it is the technology which discriminates the most the fungal communities.

Indeed, as early as the selection and the introduction of starters during the process, technology has the higher impact on the bacterial communities shaping (Fig. C.a, $R^2 = 0.19$, p-value = 0.001). This impact does not evolve drastically afterwards. Despite a similar shaping effect between the rind and curd ($R^2 = 0.17$, p-value = 0,001), the rind has one of the higher dissimilarities between technologies and looks to be the results of both selected technological and biological (chosen starters used) pathways.

For fungi, the scenario is quite different (Fig. C.b). The fashion of communities regarding technologies is obvious only in the curds and in the rinds even if the discrimination is significant as soon as in the raw milk ($R^2 = 0.05$, p-value = 0.001). The fungal communities in soft cheeses in curd and rind during ripening differ more deeply compared to those in semi hard and hard cheeses (in curd: $R^2 = 0.11$ & 0.12, p-adjusted = 0.003, in rind: $R^2 = 0.25$ & 0.21, p-adjusted = 0.003, test: pairwise. Adonis).

Less attention to the selection and the intentional introduction of fungi seems paid in the 3 technologies studied in comparison to the bacterial starters. It can explain that the path of the fungal communities is less pronounced than bacterial communities. Notably, house microbiota could count for a large part of the explanation.

To explain these first observations, shared and specific taxa through cheesemaking technologies were looked at carefully (Fig. 8). First, the overall profile of abundance of both bacterial and fungal species is specific to each technology. This tool can be powerful to discriminate between different types of cheeses.

First, there is a lot of shared bacterial taxa, and the most abundant are known for their properties on acidification and/or cheese ripening steps (contribution to the flavor, texture and the coloration) (Fig. 7.a). It includes the most widespread *L. lactis*, *S. thermophilus, L. delbrueckii*, *Brevibacterium* sp., *Arthrobacter* sp., *Brachybacterium* sp., *Staphylococcus* sp. and *Corynebacterium* sp. As already explained above, ripening bacteria are prevalent in the rind and are already very well described elsewhere (Irlinger et al., 2015; Wolfe et al., 2014).

There is also a lot of shared fungal taxa (Fig. 7.b). It included species involved in cheese ripening by producing proteases and lipases, and using lactic acid produced by LAB (Marcellino et al., 2013) leading to the deacidification of the cheeses. Those taxa are *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Geotrichum candidum*, which are of interest in cheese ripening, by producing flavor compounds (Martin et al., 2002). The impact of some others abundant shared taxa are less known even if they are frequently found in cheeses: *Diutina catelunata*, *Clavispora lusitaniae*, *Trichosporon coremiforme* (Ceugniez et al., 2017; Martin and Cotter, 2023). The last three were recovered from different brines and members of those species are able to develop lipolytic and/or proteolytic activities. Their growth could be related to the production of some interesting alcohols supporting aromatic richness development in cheeses (Innocente et al., 2023).

Some shared taxa show proportional differences across technologies. For example, the abundance of *Leuconostoc mesenteroides* is higher in soft and semi-hard cheeses in comparison to hard cheese (lfc = 1.53, qval = $1.86.10^{-96}$; lfc = 1.07; qval = 1.80.10–^{47}). It is mainly due to the starters used in each technology; *L. mesenteroides* being mesophilic, it is inoculated and favored only in the two first cheeses. The smear-ripened hard cheese technology differs from the two other technologies and seem to promote a higher development of some specific *Proteobacteria* (*Halomonas* and *Psychrobacter*), and *Actinobacteria* (*Brevibacterium linens*, *Canibacter*, *Microbacterium gubbeenense and Yaniella halotolerans)* (Table F Supplementary section). The development of these species may be promoted by the conditions of ripening which are very different from the two other technologies (long ripening, frequent washings). These bacteria are frequently found in smear-ripened cheeses (Irlinger et al., 2015; Larpin-Laborde et al., 2011). *Proteobacteria* can be on one hand very interesting for flavor development but their growth needs to be well balanced as they can be involved in spoilage of the final product, with smell and taste defaults (Desmasures et al., 1997) or in the production of biogenic amines (Delbès-Paus et al., 2012). Regarding fungi species, soft cheese technology is correlated with a higher abundance of *Geotrichum candidum* and *Kluyveromyces lactis* (lfc = 1.48, qval = 0; lfc = 1.26, qval = 0) while semi-hard cheese has higher abundance of *Mucor racemosus* and *Mucor fuscus* (lfc = 0.94, qval = 0; lfc = 1.51, qval = 0). The discrepancies of abundance of these taxa are directly linked to their intentional introduction during the process. Hard cheese technology differs from soft technology with a higher abundance of *Fusarium domesticum* (lfc = 0.83, qval = 0). This fungi is sometimes used during cheese-making for its "*anti-adhesive*" property that prevents the sticky smear defect of some cheeses including some washed rind cheeses like hard cheeses, in addition to being used as a ripening culture (Savary et al., 2023). An other important species added in starters is more abundant in hard technologies than in soft and semi-hard, *Kluyveromyces marxianus* (lfc = 0.43, qval = 0; lfc = 0.32, qval = 0) (Geronikou et al., 2022). At last, *Torulaspora delbrueckii* and *Dipodascus australiensis* are

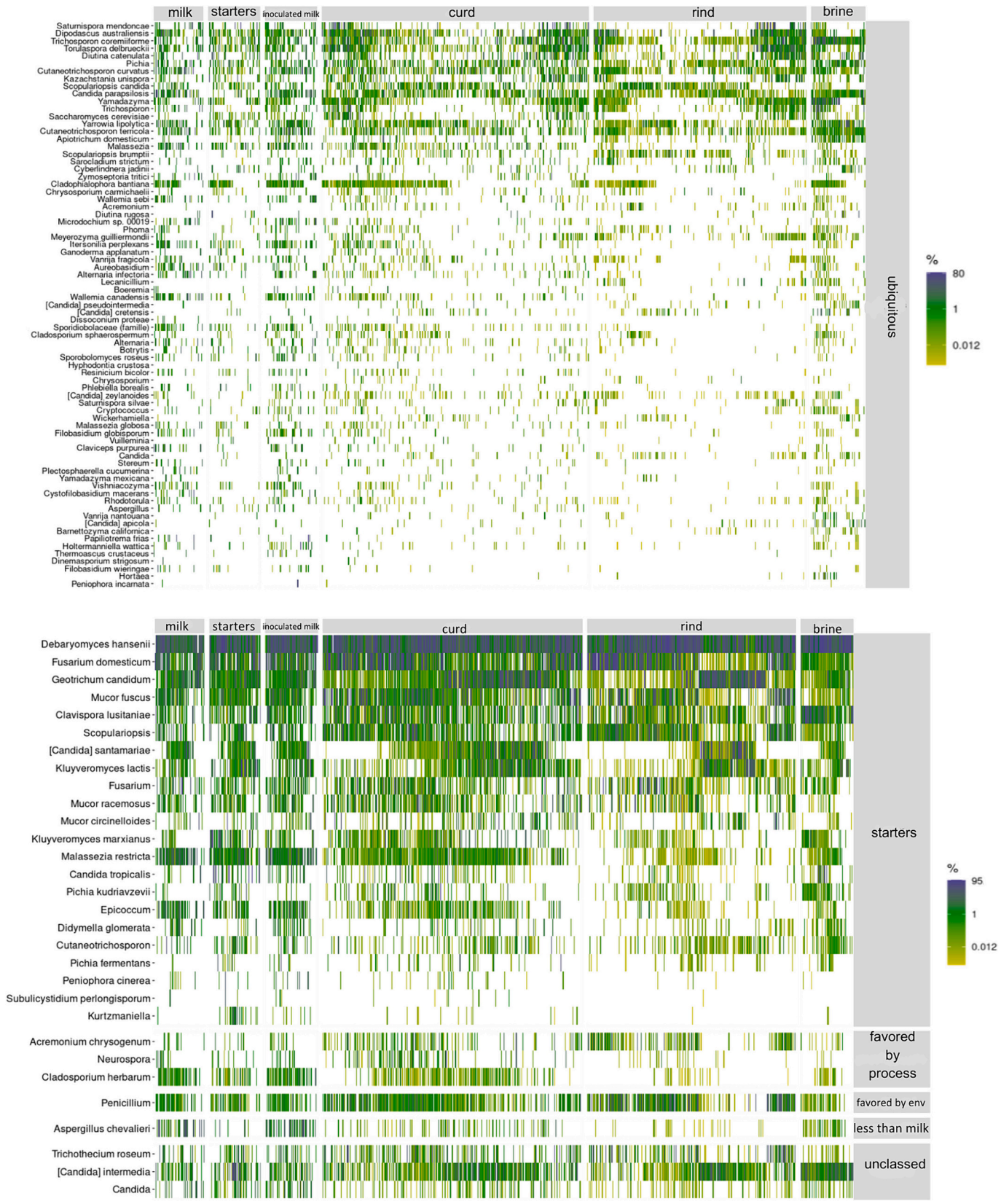

Fig. 6. Heatmap of all 105 fungal species found in raw milk, sorted by decreasing abundances in rind and curd. All samples are presented from milk to brine, and each raw corresponds to a species. Sample ordination was based on clustering using NMDS, based on ecological distance calculated with Unifrac. The color indicates the percentage of the total counts in a sample represented by the species. All species were classified as explained in Fig. 5.

Table 1
Permanova results in bacterial microbiota are above, and fungal microbiota are below. 999 permutations were made with an alpha of 0,05.

Row	Df	SumOfSqs	R2	F	Pr < F
Bacteria					
Technology	2	17.35	0.065	48.89	0.001
Matrix	5	68.62	0.258	77.36	0.001
Technology:matrix	10	19.06	0.072	10.75	0.001
Residual	905	160.55	0.605		
Total	922	265.58	1		
Fungi					
Technology	2	34.87	0.095	55.34	0.001
Matrix	5	28.86	0.078	18.30	0.001
Technology:matrix	10	20.05	0.054	6.37	0.001
Residual	904	284.77	0.773		
Total	921	368.52	1		

more abundant in soft cheeses technology (lfc = 1.60, qval = 0, lfc = 1.51, qval = 0). *T. delbrueckii* is frequently identified in cheeses overworld (Martin et al., 2023). Its role for wine fermentation is well described but its putative impact on cheeses needs to be elucidated. There is a considerable lack of knowledge regarding *Dipodascus australiensis*. Only a few studies have been interested in this salt tolerant yeast (Sajbidor et al., 1994).

Very interestingly, there is almost no taxa specific to one of the three technologies. Some are found to be present almost exclusively in the inoculated milks of hard cheese technology (Eg. *Rikenella* sp., *Brevundimonas* sp.) but only a few sequences have been recovered and they are not detected thereafter. Their role would then be limited. Remarkably, *Chrysosporium carmichaelii* has been found to be specific to the semi-hard cheese technology and could be considered as a marker.

Details of differences in fungal taxa between technologies are shown in Table G Supplementary section.

3.4. Influences of season, production type and dairy plant on microbial species

Samplings were performed both in winter and in summer. Of the four dairy plants studied for each technology, two different production types were chosen: farmhouse or factory type. Statistical analysis revealed that seasonality was a factor influencing the structuration of both microbial communities (Table 2). However, the R^2 are only 0.019 and 0.015, respectively for bacterial and fungal communities. In addition, the dairy plants factor seems to have a higher impact on bacterial communities (R^2 = 0.108 and 0.063, p-value = 0.010) than for fungal communities (R^2 = 0.076, p-value = 0.001). The ANCOM-BC model was then used to compare the seasonality effect as well as the impact of the type of production. In addition, interactions between the variables were also integrated. Fig. 9 shows the differences in bacterial taxa regarding the production type. Results did not reveal obvious evidence concerning the influence of seasonality on bacterial and fungal communities neither influence of the type of production on fungal communities. However, without regards to the technology, ten bacterial taxa have shown statistical different abundances between farmhouse and industrial dairy plants (Fig. 8, Table H, Supplementary section).

Notably, *Corynebacterium*, *Brevibacterium* sp. (including the species *Brevibacterium linens)*, *Agrococcus casei* and *Lactobacillus* sp. are found to be more present in farmhouse products than in industrial ones. These species are found to be involved in typic flavor development and coloration of cheeses (Bora et al., 2007; Mounier et al., 2009). *Brevibacterium linens* is a halotolerant bacteria, which have a proteolytic activity on the cheese surface. This activity is involved in the flavor compounds of the final product by the production of sulfur compounds, ammoniac and aromatic compounds (Boyaval and Desmazeaud, 1983). Also, this species has a significant level of lipolysis during ripening (Brennan et al., 2004). We found that this species is specific to hard technology. *Lactococcus lactis* and *Lactobacillus helveticus*; species commonly used as starters, are more abundant in industrial productions than in farmhouses ones. It could be related to the quantity of starters used during cheese making that are higher in some factory dairy plants, according to a Dunn test (chi-squared = 0.2396, p-value = $1.54.10^{-6}$). In addition, relative abundance of *Lactococcus lactis* was higher in industrial starters of SH technology after a Wilcoxon Mann-Whitney test (p-value = $1.47.10^{-6}$).

To go more deeply on the analysis of factors influencing composition and structuration of the ecosystems, we then pay attention to the impact of house microbiota found in each dairy plants on the global ecosystem found in dairy samples going to raw milk to ripened cheeses. The differences of bacterial communities were higher than for fungal communities. Hard cheese technology is the one for which this factor is the fewer, both for fungal and bacterial communities.

Dairy plants structured much more bacterial communities than fungal ones (Fig. 9.a, Fig. 9.b). Soft technology is the one whose dairy plants structure the most bacterial communities, explaining up to 29 % of deviation between communities for rind and brine. Effect of dairy plants in bacterial communities seems to be more important after the initiation of the ripening (curd, rind and brine samples). The hard cheese technology differs from the others as it is in the rind and brine that the dairy plants impact is the higher. The importance of dairy plants and associated house microbiota in the structuration of global communities depends essentially on the technology for fungal communities.

The higher dairy plant effect on bacterial communities can be explained by the dairy plant's practices, which are mainly aimed at selecting bacterial species, not using the same bacterial starters, neither the same quantity, and by adapting practices for the best coagulation. Interestingly, for each technology, rind's fungus are less related to dairy plants than rind's bacteria, allowing to say that aerobic bacteria shape more communities than fungus on the rind.

Despite our lack of differences on the seasonal effect, 27 bacterial species have already been detected as markers for raw milk samples across months (Yap et al., 2021). For example, *Ralstonia insidiosa* and *Microbacterium esteraromaticum* were found associated to raw milks in spring while species as *Corynebacterium xerosis, Psychrobacter sp, Microbacterium maritypicum*, and *Facklamia* were found to be specific to the fall season (Yap et al., 2021). Moreover, the relative abundance of *Acidobacteria*, *Actinobacteria*, *Bacteroides* and *Firmicutes* in raw cow milks during summertime was significantly higher in compared with that in winter (Nikoloudaki et al., 2021).

4. Conclusion

This project allows us to access the microbiota of a large number of dairy samples, and to have a better view of Savoyard cheeses PDO product: more comparative, more global and more complex.

We saw that raw milk is a major source of biodiversity, in the same way as brine, and that microbiota evolved during ripening to an equilibrium. Bacterial communities are, in this project, mainly structured by dairy matrices, then by cheese technology, whereas it is the opposite for fungal microbiota. Also, the way the raw milk microbiota is conserved in inoculated milks depends on the technology, as does the way the process selects the curd and rind microbiota. Fungal one is much less impacted by the ripening and the addition of fungal starters.

Bacterial dynamics picture shows us how the curd microbiota is impacted by starters, and how the rind microbiota is different and certainly dependent on the original raw milk microbiota, and on the environment and brines ‘one.

A global view of microbiota shows that all microbiota contained in different matrices can also be found in raw milk, proving its reservoir role. In addition, processes reinforce the bacteria microbiota, which explains that a lot of species are favored by process. However fungal microbiota is mostly ubiquist and does not seem to respond to the same factors at all. An amplification bias could be a part of the explanation of this result because of the large heterogeneity of gene copy variations for

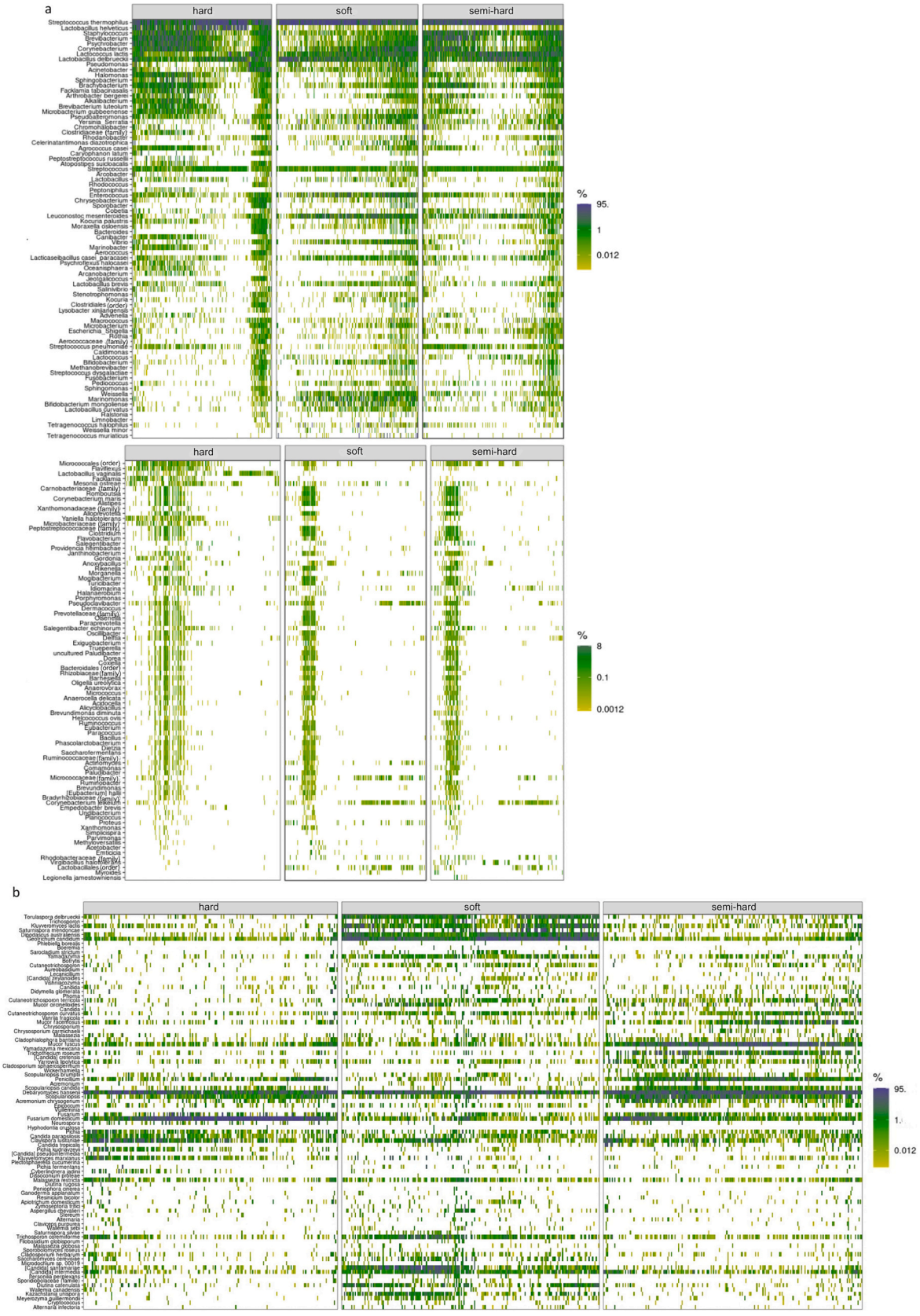

Fig. 7. Comparison of all bacterial taxa (a) and fungal taxa (b), in the 3 different cheesemaking technologies. The distance used was the Bray-Curtis distance.

Table 2
Descriptive statistics of the Permanova test for bacterial communities (left) and fungal communities (right) structuration depending on matrix, technology, dairy plants and season. Bacterial formula: Matrix*Technology + Dairy plants * Matrix + Season. Fungal formula: Matrix + Technology * Dairy plants + Season.

Row	Df	SumOfSqs	R2	F	Pr < F
Bacteria					
Matrix	5	68.64	0.259	106.28	0.001
Technology	2	17.32	0.065	67.05	0.001
Dairy plants	9	16.78	0.063	14.44	0.001
Season	1	5.18	0.019	40.14	0.001
Matrix:Technology	10	19.06	0.072	14.73	0.001
Matrix:Dairy plants	45	28.82	0.108	4.96	0.001
Residual	850	109.80	0.413		
Total	922	265.58	1		
Fungi					
Matrix	5	28.94	0.079	19.31	0.001
Technology	2	34.75	0.094	57.95	0.001
Dairy plants	9	28.23	0.077	10.46	0.001
Season	1	5.55	0.015	18.50	0.001
Residual	904	271.05	0.736		
Total	921	368.52	1		

this kingdom (Lavrinienko et al., 2021; Lofgren et al., 2019).

Abundance differences among technologies mainly concern proportions rather than the presence or absence of species, which shows the importance of certain species for different technologies. Technologies have a greater impact on bacterial communities as soon as starters are added in the raw milk, whereas fungal microbiota is more shaped by technologies in the rind.

Further investigations about dairy plants showed that bacterial communities depend strongly on the uniqueness of each dairy plant while this factor accounts less for fungal communities.

It seems that cheese's singularity and individuality is the result of multiple factors of which the type of production, the technology, the native and house microbiota, and the specificity of parameters used by each dairy plant.

More investigations need to be done about the impact of specific process parameters (strains used as starters, starter and rennet dose, maturation time, temperature used during the process, cleaning, and disinfection procedures, etc.) to better understand their impact on the final product. Moreover, it could be very interesting to study positive or negative interactions between fungal and bacterial microbiota.

Finally, this study highlights several species little or no described so far in dairy samples and in cheeses and it can help to support the fulfillment of the inventory of microbial food cultures.

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CRediT authorship contribution statement

Cresciense Lecaudé: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis. **Nicolas Orieux:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Sarah Chuzeville:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Alicia Bertry:** Investigation, Writing – review & editing. **Eric Coissac:** Methodology, Resources, Software. **Frederic Boyer:** Methodology, Resources, Software. **Aurélie Bonin:** Investigation, Methodology, Resources, Software. **Nelly Colomb-Boeckler:** Data curation, Resources. **Bruno Mathieu:** Supervision, Conceptualization, Methodology. **Manon Recour:** Writing – review & editing. **Joël Vindret:** Conceptualization, Resources. **Céline**

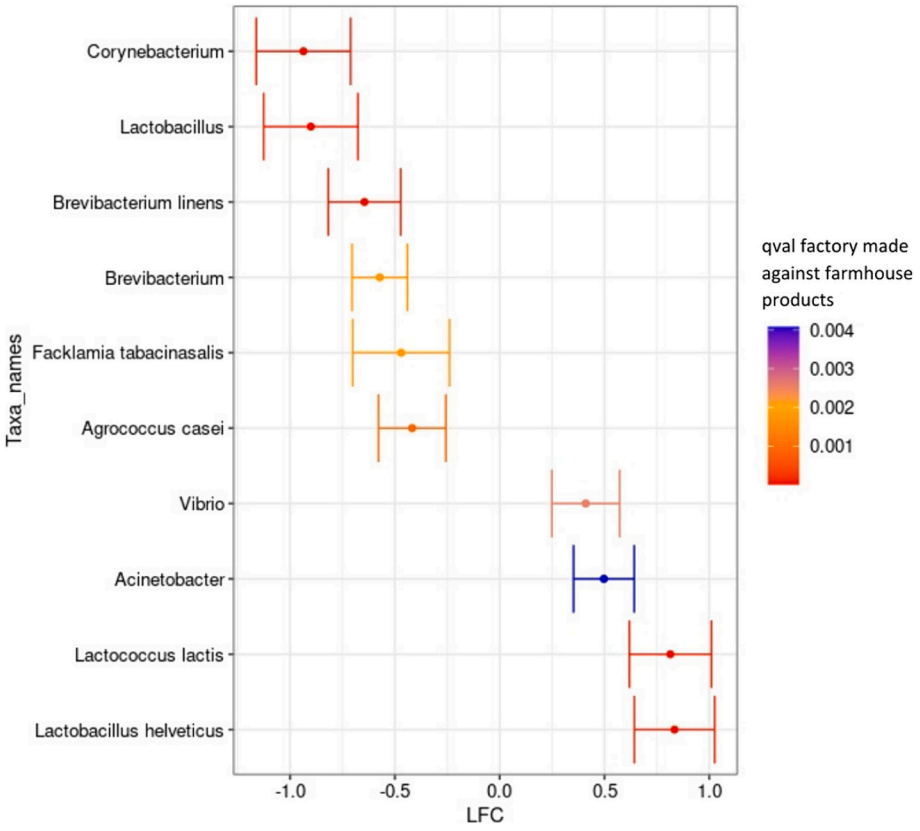

Fig. 8. logFoldChange of ANCOM-BC model, of differential taxa between farmhouse and factory productions type. Differential bacteria are presented on the top. Differential analysis was performed only on rind and curd. LFC >0 indicates that the taxa is more abundant in factory products. The qval is the correction of the p-val regarding the number of tests realized.

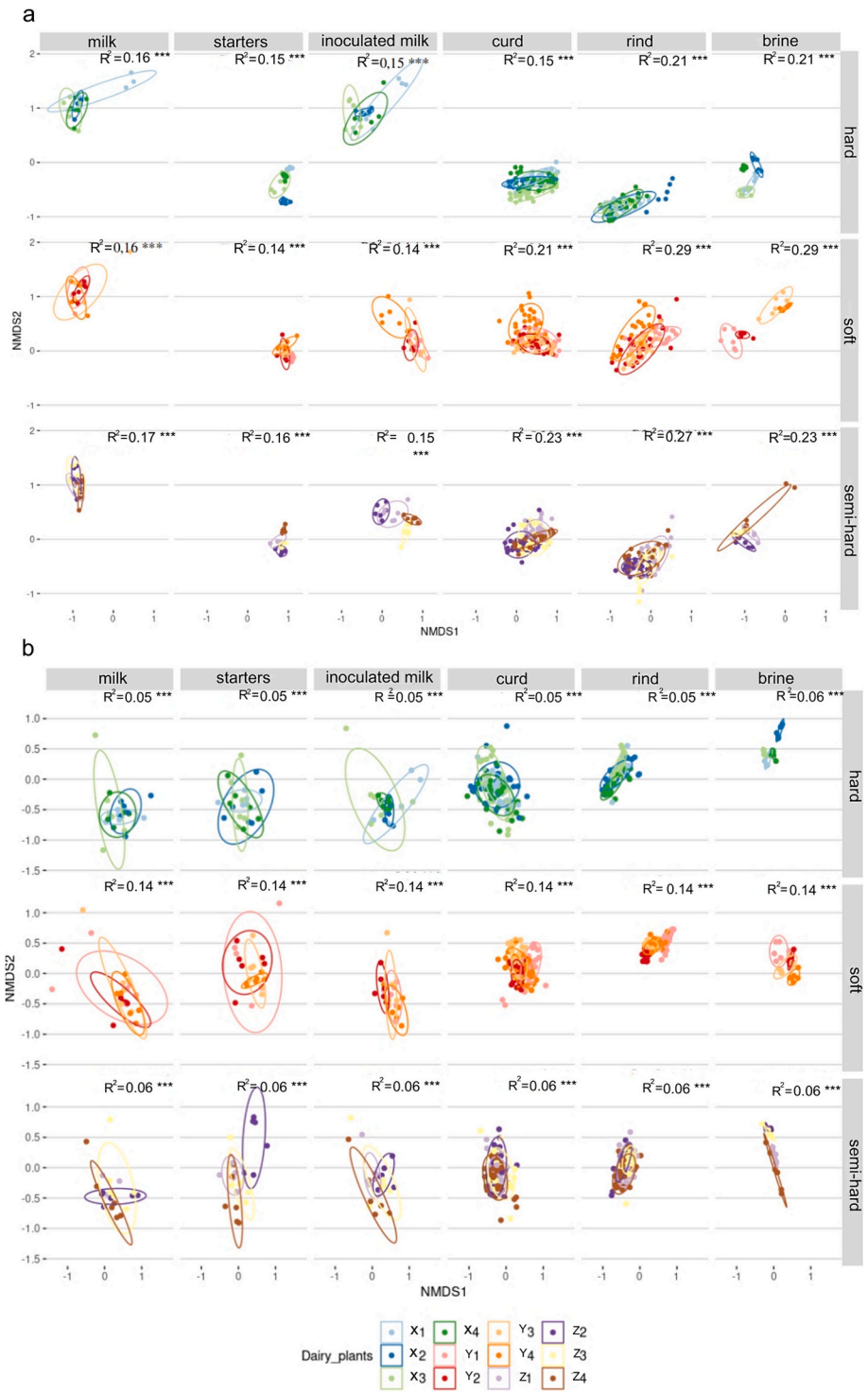

Fig. 9. Representation of the structure of communities according to technologies, matrices, and dairy plants. a. NMDS structuring bacterial communities Stress = 0.22 b. NMDS structuring fungal communities. Stress = 0.25. Each community for the 12 dairy plants were indicated using different colors. All dairy plants of the same technology have the same letter, X, Y, or Z, the number refers to the identification of the dairy plant. The NMDS were performed after an ordination on the Bray-Curtis distances matrix. The ellipses cover 80 % of a multivariate t distribution. R2 with significance were obtained with a Permanova test using 999 permutations. p-value: (0.05–0.1]., (0.01–0.05]*, (0.001–0.01]**, (0–0.001]***.

Pignol: Methodology, Resources. **Stéphane Romand:** Investigation, Writing – review & editing. **Caroline Petite:** Conceptualization, Resources, Supervision. **Pierre Taberl:** Methodology, Conceptualization. **Cécile Charles:** Supervision, Methodology, Conceptualization. **Nadège Bel:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Agnès Hauwuy:** Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Andreoletti, O., Lau Baggesen, D., Bolton, D., Butaye, P., Cook, P., Davies, R., Fernández Escámez, S., Griffin, J., Hald, T., Havelaar, A., Koutsoumanis, K., Lindqvist, R., McLauchlin, J., Nesbakken, T., Prieto Maradona, M., Ricci, A., Ru, G., Sanaa, M., Simmons, M., Sofos, J., Threlfall, J., 2015. Scientific opinion on the public health risks related to the consumption of raw drinking milk. EFSA J. https://doi.org/10.2903/j.efsa.2015.3940.

Aoki, T., Kitazawa, K., Deguchi, H., Sotozono, C., 2021. Current evidence for Corynebacterium on the ocular surface. Microorganisms 9, 254. https://doi.org/10.3390/microorganisms9020254.

Ayad, E.H.E., Verheul, A., de Jong, C., Wouters, J.T.M., Smit, G., 1999. Flavour forming abilities and amino acid requirements of Lactococcus lactis strains isolated from artisanal and non-dairy origin. Int. Dairy J. 9, 725–735. https://doi.org/10.1016/S0958-6946(99)00140-5.

Bay, V., Gillespie, A., Ganda, E., Evans, N.J., Carter, S.D., Lenzi, L., Lucaci, A., Haldenby, S., Barden, M., Griffiths, B.E., Sánchez-Molano, E., Bicalho, R., Banos, G., Darby, A., Oikonomou, G., 2023. The bovine foot skin microbiota is associated with host genotype and the development of infectious digital dermatitis lesions. Microbiome 11, 4. https://doi.org/10.1186/s40168-022-01440-7.

Beresford, T.P., Fitzsimons, N.A., Brennan, N.L., Cogan, T.M., 2001. Recent advances in cheese microbiology. Int. Dairy J. 11, 259–274. https://doi.org/10.1016/S0958-6946(01)00056-5.

Bettera, L., Levante, A., Bancalari, E., Bottari, B., Gatti, M., 2023. Lactic acid bacteria in cow raw milk for cheese production: which and how many? Front. Microbiol. 13, 1092224. https://doi.org/10.3389/fmicb.2022.1092224.

Biçer, Y., Telli, A.E., Sönmez, G., Telli, N., Uçar, G., 2021. Comparison of microbiota and volatile organic compounds in milk from different sheep breeds. J. Dairy Sci. 104, 12303–12311. https://doi.org/10.3168/jds.2021-20911.

Bondue, P., Milani, C., Arnould, E., Ventura, M., Daube, G., LaPointe, G., Delcenserie, V., 2020. Bifidobacterium mongoliense genome seems particularly adapted to milk oligosaccharide digestion leading to production of antivirulent metabolites. BMC Microbiol. 20, 111. https://doi.org/10.1186/s12866-020-01804-9.

Bora, N., Vancanneyt, M., Gelsomino, R., Swings, J., Brennan, N., Cogan, T.M., Larpin, S., Desmasures, N., Lechner, F.E., Kroppenstedt, R.M., Ward, A.C., Goodfellow, M., 2007. Agrococcus casei sp. nov., isolated from the surfaces of smear-ripened cheeses. Int. J. Syst. Evol. Microbiol. 57, 92–97. https://doi.org/10.1099/ijs.0.64270-0.

Bouton, Y., Buchin, S., Duboz, G., Pochet, S., Beuvier, E., 2009. Effect of mesophilic lactobacilli and enterococci adjunct cultures on the final characteristics of a microfiltered milk Swiss-type cheese. Food Microbiol. 26, 183–191. https://doi.org/10.1016/j.fm.2008.09.002.

Boyaval, P., Desmazeaud, J., 1983. Le point des connaissances sur Brevibacterium linens. Lait 63, 187–216.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. OBITOOLS: a UNIX -inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 16, 176–182. https://doi.org/10.1111/1755-0998.12428.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349. https://doi.org/10.2307/1942268.

Brennan, N.M., Brown, R., Goodfellow, M., Ward, A.C., Beresford, T.P., Vancanneyt, M., Cogan, T.M., Fox, P.F., 2001. Microbacterium gubbeenense sp. nov., from the surface of a smear-ripened cheese. Int. J. Syst. Evol. Microbiol. 51, 1969–1976. https://doi.org/10.1099/00207713-51-6-1969.

Brennan, N.M., Cogan, T.M., Loessner, M., Scherer, S., 2004. Bacterial surface-ripened cheeses. In: Cheese: Chemistry, Physics and Microbiology. Elsevier, pp. 199–225. https://doi.org/10.1016/S1874-558X(04)80045-9.

Ceugniez, A., Taminiau, B., Coucheney, F., Jacques, P., Delcenserie, V., Daube, G., Drider, D., 2017. Fungal diversity of "Tomme d'Orchies" cheese during the ripening process as revealed by a metagenomic study. Int. J. Food Microbiol. 258, 89–93. https://doi.org/10.1016/j.ijfoodmicro.2017.07.015.

Coelho, M.C., Malcata, F.X., Silva, C.C.G., 2022. Lactic acid bacteria in raw-milk cheeses: from starter cultures to probiotic functions. Foods 11, 2276. https://doi.org/10.3390/foods11152276.

Coissac, E., 2012. OligoTag: a program for designing sets of tags for next-generation sequencing of multiplexed samples. Methods Mol. Biol. Clifton NJ 888, 13–31. https://doi.org/10.1007/978-1-61779-870-2_2.

Delbès-Paus, C., Pochet, S., Helinck, S., Veisseire, P., Bord, C., Lebecque, A., Coton, M., Desmasures, N., Coton, E., Irlinger, F., Montel, M.-C., 2012. Impact of Gram-negative bacteria in interaction with a complex microbial consortium on biogenic amine content and sensory characteristics of an uncooked pressed cheese. Food Microbiol. 30, 74–82. https://doi.org/10.1016/j.fm.2011.12.008.

Desmasures, N., Opportune, W., Guéguen, M., 1997. Lactococcus spp., yeasts and Pseudomonas spp. on teats and udders of milking cows as potential sources of milk contamination. Int. Dairy J. 7, 643–646. https://doi.org/10.1016/S0958-6946(97)00042-3.

W.Donnelly, C., 2014. Cheese and Microbes. ASM Books. American Society for Microbiology. https://doi.org/10.1128/9781555818593.

Dugat-Bony, E., Straub, C., Teissandier, A., Onésime, D., Loux, V., Monnet, C., Irlinger, F., Landaud, S., Leclercq-Perlat, M.-N., Bento, P., Fraud, S., Gibrat, J.-F., Aubert, J., Fer, F., Guédon, E., Pons, N., Kennedy, S., Beckerich, J.-M., Swennen, D., Bonnarme, P., 2015. Overview of a surface-ripened cheese community functioning by meta-omics analyses. PLoS One 10, e0124360. https://doi.org/10.1371/journal.pone.0124360.

Dugat-Bony, E., Bonnarme, P., Fraud, S., Catellote, J., Sarthou, A.-S., Loux, V., Rué, O., Bel, N., Chuzeville, S., Helinck, S., 2019. Effect of sodium chloride reduction or partial substitution with potassium chloride on the microbiological, biochemical and sensory characteristics of semi-hard and soft cheeses. Food Res. Int. Ott. Ont 125, 108643. https://doi.org/10.1016/j.foodres.2019.108643.

Duthoit, F., Callon, C., Tessier, L., Montel, M.-C., 2005. Relationships between sensorial characteristics and microbial dynamics in "registered designation of origin" Salers cheese. Int. J. Food Microbiol. 103, 259–270. https://doi.org/10.1016/j.ijfoodmicro.2004.11.040.

Egger, L., Ménard, O., Abbühl, L., Duerr, D., Stoffers, H., Berthoud, H., Meola, M., Badertscher, R., Blaser, C., Dupont, D., Portmann, R., 2021. Higher microbial diversity in raw than in pasteurized milk Raclette-type cheese enhances peptide and metabolite diversity after in vitro digestion. Food Chem. 340, 128154 https://doi.org/10.1016/j.foodchem.2020.128154.

Eidensohn, Y., Wei, A., Sirkin, M., Dever, L.L., 2021. Brevibacteria Tibial Osteomyelitis. IDCases, 23, e01046. https://doi.org/10.1016/j.idcr.2021.e01046.

Feranchuk, S., Belkova, N., Potapova, U., Kuzmin, D., Belikov, S., 2018. Evaluating the use of diversity indices to distinguish between microbial communities with different traits. Res. Microbiol. 169, 254–261. https://doi.org/10.1016/j.resmic.2018.03.004.

Feurer, C., Irlinger, F., Spinnler, H.E., Glaser, P., Vallaeys, T., 2004. Assessment of the rind microbial diversity in a farmhouse-produced vs a pasteurized industrially produced soft red-smear cheese using both cultivation and rDNA-based methods. J. Appl. Microbiol. 97, 546–556. https://doi.org/10.1111/j.1365-2672.2004.02333.x.

Ficetola, G.F., Coissac, E., Zundel, S., Riaz, T., Shehzad, W., Bessière, J., Taberlet, P., Pompanon, F., 2010. An in silico approach for the evaluation of DNA barcodes. BMC Genomics 11, 434. https://doi.org/10.1186/1471-2164-11-434.

Fitzsimons, N.A., Cogan, T.M., Condon, S., Beresford, T., 1999. Phenotypic and genotypic characterization of non-starter lactic acid bacteria in mature cheddar cheese. Appl. Environ. Microbiol. 65, 3418–3426. https://doi.org/10.1128/AEM.65.8.3418-3426.1999.

Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., 2017. Salting of cheese curd. In: Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. (Eds.), Fundamentals of Cheese Science. Springer US, Boston, MA, pp. 251–277. https://doi.org/10.1007/978-1-4899-7681-9_9.

Frétin, M., Martin, B., Rifa, E., Isabelle, V.-M., Pomiès, D., Ferlay, A., Montel, M.-C., Delbès, C., 2018. Bacterial community assembly from cow teat skin to ripened cheeses is influenced by grazing systems. Sci. Rep. 8, 200. https://doi.org/10.1038/s41598-017-18447-y.

Frétin, M., Gérard, A., Ferlay, A., Martin, B., Buchin, S., Theil, S., Rifa, E., Loux, V., Rué, O., Chassard, C., Delbès, C., 2022. Integration of multiomic data to characterize the influence of milk fat composition on cantal-type cheese microbiota. Microorganisms 10, 334. https://doi.org/10.3390/microorganisms10020334.

Gatti, M., Bottari, B., Lazzi, C., Neviani, E., Mucchetti, G., 2014. Invited review: microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. J. Dairy Sci. 97, 573–591. https://doi.org/10.3168/jds.2013-7187.

Gelsomino, R., Vancanneyt, M., Cogan, T.M., Condon, S., Swings, J., 2002. Source of enterococci in a farmhouse raw-milk cheese. Appl. Environ. Microbiol. 68, 3560–3565. https://doi.org/10.1128/AEM.68.7.3560-3565.2002.

Geronikou, A., Larsen, N., Lillevang, S.K., Jespersen, L., 2022. Occurrence and identification of yeasts in production of white-brined cheese. Microorganisms 10, 1079. https://doi.org/10.3390/microorganisms10061079.

Giraffa, G., 2003. Functionality of enterococci in dairy products. Int. J. Food Microbiol. 88, 215–222. https://doi.org/10.1016/S0168-1605(03)00183-1.

Giraudoux, P., 2023. pgirmess: Spatial Analysis and Data Mining for Field Ecologists.

Hammer, P., Jordan, J., Jacobs, C., Klempt, M., 2019. Characterization of coagulase-negative staphylococci from brining baths in Germany. J. Dairy Sci. 102, 8734–8744. https://doi.org/10.3168/jds.2018-15610.

Innocente, N., Renoldi, N., Moret, E., Maifreni, M., Marino, M., 2023. Volatilome of brine-related microorganisms in a curd-based medium. J. Dairy Sci. https://doi.org/10.3168/jds.2022-23051.

Irlinger, F., Mounier, J., 2009. Microbial interactions in cheese: implications for cheese quality and safety. Curr. Opin. Biotechnol. 20, 142–148. https://doi.org/10.1016/j.copbio.2009.02.016.
Irlinger, F., In Yung, S.A.Y., Sarthou, A.-S., Delbès-Paus, C., Montel, M.-C., Coton, E., Coton, M., Helinck, S., 2012. Ecological and aromatic impact of two Gram-negative bacteria (Psychrobacter celer and Hafnia alvei) inoculated as part of the whole microbial community of an experimental smear soft cheese. Int. J. Food Microbiol. 153, 332–338. https://doi.org/10.1016/j.ijfoodmicro.2011.11.022.
Irlinger, F., Layec, S., Hélinck, S., Dugat-Bony, E., 2015. Cheese rind microbial communities: diversity, composition and origin. FEMS Microbiol. Lett. 362, 1–11. https://doi.org/10.1093/femsle/fnu015.
Ishikawa, M., Kodama, K., Yasuda, H., Okamoto-Kainuma, A., Koizumi, K., Yamasato, K., 2007. Presence of halophilic and alkaliphilic lactic acid bacteria in various cheeses. Lett. Appl. Microbiol. 44, 308–313. https://doi.org/10.1111/j.1472-765X.2006.02073.x.
Kothe, C.I., Bolotin, A., Kraïem, B.-F., Dridi, B., Renault, P., 2021. Unraveling the world of halophilic and halotolerant bacteria in cheese by combining cultural, genomic and metagenomic approaches. Int. J. Food Microbiol. 358, 109312 https://doi.org/10.1016/j.ijfoodmicro.2021.109312.
Larpin-Laborde, S., Imran, M., Bonaïti, C., Bora, N., Gelsomino, R., Goerges, S., Irlinger, F., Goodfellow, M., Ward, A.C., Vancanneyt, M., Swings, J., Scherer, S., Guéguen, M., Desmasures, N., 2011. Surface microbial consortia from Livarot, a French smear-ripened cheese. Can. J. Microbiol. 57, 651–660. https://doi.org/10.1139/w11-050.
Lavrinienko, A., Jernfors, T., Koskimäki, J.J., Pirttilä, A.M., Watts, P.C., 2021. Does intraspecific variation in rDNA copy number affect analysis of microbial communities? Trends Microbiol. 29, 19–27. https://doi.org/10.1016/j.tim.2020.05.019.
Law, B.A., 2010. Cheese-ripening and cheese flavour technology. In: Law, Barry A., Tamime, A.Y. (Eds.), Technology of Cheesemaking. Wiley-Blackwell, Oxford, UK, pp. 231–259. https://doi.org/10.1002/9781444323740.ch7.
Levante, A., Bertani, G., Marrella, M., Mucchetti, G., Bernini, V., Lazzi, C., Neviani, E., 2023. The microbiota of Mozzarella di Bufala Campana PDO cheese: a study across the manufacturing process. Front. Microbiol. 14, 1196879. https://doi.org/10.3389/fmicb.2023.1196879.
Lin, H., Peddada, S.D., 2020. Analysis of compositions of microbiomes with bias correction. Nat. Commun. 11, 3514. https://doi.org/10.1038/s41467-020-17041-7.
Liu, J., Gao, W., Pan, Y., Liu, G., 2018. Metabolic engineering of Acremonium chrysogenum for improving cephalosporin C production independent of methionine stimulation. Microb. Cell Factories 17, 87. https://doi.org/10.1186/s12934-018-0936-5.
Lofgren, L.A., Uehling, J.K., Branco, S., Bruns, T.D., Martin, F., Kennedy, P.G., 2019. Genome-based estimates of fungal rDNA copy number variation across phylogenetic scales and ecological lifestyles. Mol. Ecol. 28, 721–730. https://doi.org/10.1111/mec.14995.
Marcellino, O.S.B., S.N, Benson, D.R., 2013. The good, the bad, and the ugly: tales of mold-ripened cheese. Microbiol. Spectr. 1, 1.1.12. https://doi.org/10.1128/microbiolspec.CM-0005-12.
Marilley, L., 2004. Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. Int. J. Food Microbiol. 90, 139–159. https://doi.org/10.1016/S0168-1605(03)00304-0.
Martin, J.G.P., Cotter, P.D., 2023. Filamentous fungi in artisanal cheeses: a problem to be avoided or a market opportunity? Heliyon 9, e15110. https://doi.org/10.1016/j.heliyon.2023.e15110.
Martin, N., Berger, C., Spinnler, H.E., 2002. Sensory and instrumental flavor analyses of cheese curd cocultured with selected yeast and BACTERIA. J. Sens. Stud. 17, 1–17. https://doi.org/10.1111/j.1745-459X.2002.tb00328.x.
Martin, J.G.P., Silva, J.M.M., César, I.C.D.R., Da Silva, M., Santana, S.A., Veloso, T.G.R., Silva, J.G.E., Ferreira, C.L.D.L.F., Leech, J., Cotter, P.D., 2023. Seasonal variation in the Canastra cheese mycobiota. Front. Microbiol. 13, 1076672. https://doi.org/10.3389/fmicb.2022.1076672.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.
Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, N., Berthier, F., 2014. Traditional cheeses: rich and diverse microbiota with associated benefits. Int. J. Food Microbiol. 177, 136–154. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019.
Mounier, J., Monnet, C., Jacques, N., Antoinette, A., Irlinger, F., 2009. Assessment of the microbial diversity at the surface of Livarot cheese using culture-dependent and independent approaches. Int. J. Food Microbiol. 133, 31–37. https://doi.org/10.1016/j.ijfoodmicro.2009.04.020.
Nikoloudaki, O., Lemos Junior, W.J.F., Borruso, L., Campanaro, S., De Angelis, M., Vogel, R.F., Di Cagno, R., Gobbetti, M., 2021. How multiple farming conditions correlate with the composition of the raw cow's milk lactic microbiome. Environ. Microbiol. 23, 1702–1716. https://doi.org/10.1111/1462-2920.15407.
Nutrition Division, 2011. Milk and Milk Products, Second. ed, Codex Alimentarius - Joint FAO/WHO Food Standards Programme. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), Rome.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B., Simpson, G., Solymos, P., Stevens, H., Wagner, H., 2015. Vegan: Community Ecology Package. R Package Version 22-1 2, pp. 1–2.
Oksanen, J.L., Simpson, G., Blanchet, F.G., Kindt, R., Legendre, P.R., Minchin, P., O'Hara, R.B., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Beatriz Antoniazi Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G.O., Hill, M., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. vegan: Community Ecology Package.
Papadimitriou, K., Anastasiou, R., Georgalaki, M., Bounenni, R., Paximadaki, A., Charmpi, C., Alexandraki, V., Kazou, M., Tsakalidou, E., 2022. Comparison of the microbiome of artisanal homemade and industrial feta cheese through amplicon sequencing and shotgun metagenomics. Microorganisms 10, 1073. https://doi.org/10.3390/microorganisms10051073.
Paradis, E., Schliep, K., 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in {R}. Bioinformatics 35, 526–528. https://doi.org/10.1093/bioinformatics/bty633.
Parente, E., Ricciardi, A., Zotta, T., 2020. The microbiota of dairy milk: a review. Int. Dairy J. 107, 104714 https://doi.org/10.1016/j.idairyj.2020.104714.
Pedro, M.A., 2017. pairwiseAdonis: Pairwise Multilevel Comparison using Adonis.
Pham, N.-P., Layec, S., Dugat-Bony, E., Vidal, M., Irlinger, F., Monnet, C., 2017. Comparative genomic analysis of Brevibacterium strains: insights into key genetic determinants involved in adaptation to the cheese habitat. BMC Genomics 18, 955. https://doi.org/10.1186/s12864-017-4322-1.
Poznanski, E., Cavazza, A., Cappa, F., Cocconcelli, P.S., 2004. Indigenous raw milk microbiota influences the bacterial development in traditional cheese from an alpine natural park. Int. J. Food Microbiol. 92, 141–151. https://doi.org/10.1016/j.ijfoodmicro.2003.09.006.
Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F., Cotter, P.D., 2013. The complex microbiota of raw milk. FEMS Microbiol. Rev. 37, 664–698. https://doi.org/10.1111/1574-6976.12030.
R Core Team, 2023. R: A Language and Environment for Statistical Computing.
Rattray, F.P., Fox, P.F., 1999. Aspects of enzymology and biochemical properties of Brevibacterium linens relevant to cheese ripening: a review. J. Dairy Sci. 82, 891–909. https://doi.org/10.3168/jds.S0022-0302(99)75308-7.
Rodriguez, M.D., Yu, K.K., Paul, Z.S., Keller-Wood, M., Wood, C.E., Triplett, E.W., 2019. Contamination is not linked to the gestational microbiome. Appl. Environ. Microbiol. 85 https://doi.org/10.1128/AEM.01127-19 e01127-19.
Roth, E., Schwenninger, S.M., Eugster-Meier, E., Lacroix, C., 2011. Facultative anaerobic halophilic and alkaliphilic bacteria isolated from a natural smear ecosystem inhibit Listeria growth in early ripening stages. Int. J. Food Microbiol. 147, 26–32. https://doi.org/10.1016/j.ijfoodmicro.2011.02.032.
Sabater, C., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., Ruas-Madiedo, P., Ruiz, L., Margolles, A., 2021. Novel methods of microbiome analysis in the food industry. Int. Microbiol. 24, 593–605. https://doi.org/10.1007/s10123-021-00215-8.
Sajbidor, J., Lamačka, M., Breirerová, E., Chrastina, A., Pokreisz, P., Certík, M., 1994. Effect of salt stress on fatty acid alterations in some strains of Dipodascopsis and Dipodascus spp. World J. Microbiol. Biotechnol. 10, 184–186. https://doi.org/10.1007/BF00360883.
Savary, O., Coton, E., Maillard, M.-B., Gaucheron, F., Le Meur, C., Frisvad, J., Thierry, A., Jany, J.-L., Coton, M., 2023. Functional diversity of Bisifusarium domesticum and the newly described Nectriaceae cheese-associated species. Food Res. Int. 168, 112691 https://doi.org/10.1016/j.foodres.2023.112691.
Schornsteiner, E., Mann, E., Bereuter, O., Wagner, M., Schmitz-Esser, S., 2014. Cultivation-independent analysis of microbial communities on Austrian raw milk hard cheese rinds. Int. J. Food Microbiol. 180, 88–97. https://doi.org/10.1016/j.ijfoodmicro.2014.04.010.
Schubert, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., Starink, M., Hill, C.F., Zalar, P., De Hoog, G.S., Crous, P.W., 2007. Biodiversity in the Cladosporium herbarum complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosporium taxonomy and diagnostics. Stud. Mycol. 58, 105–156. https://doi.org/10.3114/sim.2007.58.05.
Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.
Smit, G., Smit, B.A., Engels, W.J.M., 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiol. Rev. 29, 591–610. https://doi.org/10.1016/j.fmrre.2005.04.002.
Spinnler, H.-E., 2017. Surface mold–ripened cheeses. In: Cheese. Elsevier, pp. 911–928. https://doi.org/10.1016/B978-0-12-417012-4.00036-3.
Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: For Biodiversity Research and Monitoring, 1st ed. Oxford University PressOxford. https://doi.org/10.1093/oso/9780198767220.001.0001.
Wolfe, B.E., Button, J.E., Santarelli, M., Dutton, R.J., 2014. Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. Cell 158, 422–433. https://doi.org/10.1016/j.cell.2014.05.041.
Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J., Smit, G., 2002. Microbes from raw milk for fermented dairy products. Int. Dairy J. 12, 91–109. https://doi.org/10.1016/S0958-6946(01)00151-0.
Yap, M., Gleeson, D., O'Toole, P.W., O'Sullivan, O., Cotter, P.D., 2021. Seasonality and geography have a greater influence than the use of chlorine-based cleaning agents on the microbiota of bulk tank raw milk. Appl. Environ. Microbiol. 87, e0108121 https://doi.org/10.1128/AEM.01081-21.