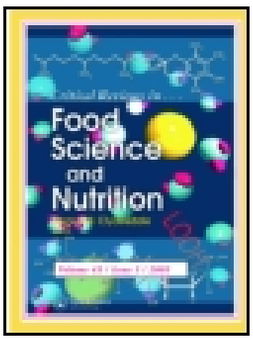

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An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far?

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ABSTRACT

The emergence of next-generation sequencing (NGS) technologies has revolutionized the way to investigate the microbial diversity in traditional fermentations. In the field of food microbial ecology, different NGS platforms have been used for community analysis, including 454 pyrosequencing from Roche, Illumina's instruments and Thermo Fisher's SOLiD/Ion Torrent sequencers. These recent platforms generate information about millions of rDNA amplicons in a single running, enabling accurate phylogenetic resolution of microbial taxa. This review provides a comprehensive overview of the application of NGS for microbiome analysis of traditional fermented milk products worldwide. Fermented milk products covered in this review include kefir, buttermilk, *koumiss, dahi, kurut, airag, tarag, khoormog, lait caillé,* and *suero costeño*. *Lactobacillus*—mainly represented by *Lb. helveticus*, *Lb. kefiranofaciens,* and *Lb. delbrueckii*—is the most important and frequent genus with 51 reported species. In general, dominant species detected by culturing were also identified by NGS. However, NGS studies have revealed a more complex bacterial diversity, with estimated 400-600 operational taxonomic units, comprising uncultivable microorganisms, sub-dominant populations, and late-growing species. This review explores the importance of these discoveries and address related topics on workflow, NGS platforms, and knowledge bioinformatics devoted to fermented milk products. The knowledge that has been gained is vital in improving the monitoring, manipulation, and safety of these traditional fermented foods.

KEYWORDS

Kefir; probiotic; lactic acid bacteria; food safety; microbial diversity

Introduction

Fermented milk products have been a vital component in the daily diet of ethnic groups all around the world, and play an important nutritional role in modern life (Granato et al. 2010). At early times, fermented milk products were produced spontaneously by the action of indigenous microorganisms present in the raw milk or from the environment. Subsequently, spontaneous fermentations were replaced by the "backslopping" technique, which involves inoculating milk with a small amount of a precedent successful fermentation (Shrivastava and Ananthanarayan 2015). This procedure naturally selects well-adapted microorganisms reducing fermentation time and increasing predictability and quality. Nowadays, several naturally fermented milk products are produced by backslopping, including kefir, *koumiss, dahi, doogh, mohi, chhurpi, kashk, somar, philu, shyow*, buttermilk, *airag, tarag, khoormog, lait caillé* and *suero costeño* (Kim et al. 2018; Li et al. 2020; Dewan and Tamang 2007; Oki et al. 2014; Owusu-Kwarteng et al. 2017; Meybodi et al. 2016; Motato et al. 2017; Uchida et al. 2007).

The evolution of studies on microbial diversity of naturally fermented milk products started at the end of the 19th century, when Grigoroff (1905) isolated *Lactobacillus bulgaricus* from Bulgarian fermented milk (Oberman and Libudzisz 1998). Thenceforth, various culture-dependent-based studies reported lactic acid bacteria (LAB) as the predominant microbiota present in natural milk fermentation, mostly represented by *Lactobacillus*, *Lactococcus, Streptococcus,* and *Enterococcus* genera (Shangpliang et al. 2018; Akabanda et al. 2013; Savadogo et al. 2004). The early studies were based on the cultivation, isolation, and identification of microorganisms according to their morphological or biochemical characteristics and, posteriorly, through the sequencing of the ribosomal RNA gene (rDNA amplicons) (Ray and Bhunia 2007; De Melo Pereira et al. 2020). The culture-dependent approach drove advances in microbiology, despite its well-known serious limitations (Al-Awadhi et al. 2013). In this methodology, microbial groups that appear in small numbers compete for growth with abundance populations (Hugenholtz, Goebel, and Pace 1998), and many

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fastidious microorganisms may be unable to grow in vitro by the difficulty in simulating the natural habitat conditions (Gatti et al. 2004). Thus, the major limitation of classical cultivation techniques is to drastically underestimate the number and microbial composition in the samples under study (Cao et al. 2017; Al-Awadhi et al. 2013).

In recent decades, culture-independent methodologies were developed to overcome the limitations of conventional microbiology testing, through DNA analyses without any culturing step. These include, for example, denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient gel electrophoresis (TTGE), single-stranded conformation polymorphism (SSCP), real-time quantitative PCR (qPCR), automated PCR-based techniques (PCR-ARDRA, ARISA-PCR, AP-PCR, and AFLP), and terminal restriction fragment length polymorphism (T-RFLP) (Giraffa and Neviani 2001; Ercolini 2004; Fusco and Quero 2014; Mayo et al. 2014). Studies applied to natural milk fermentation using these techniques revealed a more accurate analysis of the microbial composition, diversity, and dynamics, uncovered by traditional cultivation. A complete list of microbial groups identified by these culture-independent methods is shown in the supplementary material (Table S1). PCR-DGGE is the most widely applied technique, although it has provided uncertain results, not being able to reveal many species identified by cultivation (Ercolini 2004).

The emergence of next-generation DNA sequencing (NGS) methodology, and the first application of the pyrosequencing platform in kefir samples from Ireland (Dobson et al. 2011), produced exceedingly high numbers of DNA sequences and allowed an in-depth characterization of the microbial constituents of this ecosystem. To date, the 454 pyrosequencing, Illumina, and PacBio platforms revealed a diverse community in naturally fermented milk products, with the estimated average ranging from 400 to 600 operational taxonomic units (OTUs), represented by Proteobacteria, Bacteroides, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, Deinococcus-Thermus, TM7, and Spirochetes (Marsh et al. 2013; Gesudu et al. 2016; Liu, Xi, et al. 2015; Jayashree et al. 2013; Sun et al. 2014; Wurihan et al. 2019; Gao et al. 2013; Liu, Zheng, et al. 2015; Oki et al. 2014). This review aims to provide an update into the current knowledge of the microbial composition of traditional fermented milk products after a short introduction to the most common NGS platforms.

NGS and workflows in fermented milk microbiomes

Fermented milk' microbiome is the aggregate of all the microbes that reside in this ecosystem. Bacteria are the core microbiota components, comprising commensal, symbiotic and pathogenic microorganisms (Quigley et al. 2013). Recent advances in sequencing technologies have allowed an increasing number of microbiome studies into popular and ethnic fermented milks. Figure 1 illustrates standard NGS workflow for microbiome analysis for these products, including (1) sampling, (2) DNA extraction, (3) library preparation, (4) sequencing, and (5) data analysis.

Sampling

There is no standardized sampling strategy for fermented milk, which sample volumes ranging from one to 30 mL are withdrawn at various time points, representing the beginning, middle, and end of fermentation (Hong et al. 2019; Marsh et al. 2013; Shangpliang et al. 2018). The sampled liquid fraction is subsequently centrifuged under varied parameters (16,000 × g for 10 min; 10,000 g for 15 min; 5,444 × g for 30 min; 8,000 g for 10 min; 16,000 × g for 10 min) and the DNA is extracted from the resulting pellet (Nalbantoglu et al. 2014; Walsh et al. 2016; Walsh et al. 2017; Jayashree et al. 2013; Liu, Xi, et al. 2015). In the case of solid matrices such as kefir grains, a pre-phase of sample preparation is usually performed before DNA extraction. For instance, while Nalbantoglu et al. (2014) manually macerated kefir grains in 0.9% NaCl, Walsh et al. (2016) ground them into a fine powder using PowerBead tube on the TissueLyser II from Qiagen company. The resulting extracts are homogenized to remove microorganisms from the kefir matrix, releasing them into suspension. Some solutions used for this purpose included CTAB pre-heated at 60 °C (Zamberi et al. 2016), 0.9% NaCl (Nalbantoglu et al. 2014), 0.1% peptone (Gao and Zhang 2019), and 1.2 M sorbitol (Wang et al. 2018).

DNA extraction

Following sampling, a variety of DNA extraction methods has been utilized to isolate DNA from fermented milk samples (Table 1); however, no studies are focusing on different DNA protocols designed for subsequent NGS approaches. In general, four common steps are followed, including mechanical homogenization, cell lysis, removal of cell fragments, and precipitation and purification of nucleic acids. When reviewing DNA extraction procedures for fermented milk samples (Table 1), cell lysis is the most variable procedure. This is a critical step, as microbial taxa within a community have different cell wall compositions (Quigley et al. 2012). Bacterial cell lysis is usually performed by either chemical, enzymatic, and physical methods, or even a combination of different principles (Table 1). Generally, LAB are more sensitive to enzymatic methods, while microorganisms of the Bacillaceae, Acetobacteraceae, and Clostridiaceae families are more susceptible to physical and chemical methods (Keisam et al. 2016). Buffers, such as sodium citrate (2%) and trisodium citrate (2%), are generally used to improve lysis procedure by removing lipids, proteins, and salts (Jatmiko, Mustafa, and Ardyati 2019; Shangpliang et al. 2018). After cell lysis, the following steps for DNA separation, precipitation, and purification are usually performed using commercial DNA extraction kits, including PowerFood™ Microbial DNA Isolation Kit, FastDNA® Spin Kit for Soil, Qiagen DNA Stool Mini Kit, Wizard Genomic DNA Purification Kit, and GeneMATRIX Food-Extract DNA Purification Kit (Dertli and Çon 2017; Gao and Zhang 2019; Gesudu et al. 2016; Wurihan et al. 2019; Nalbantoglu et al. 2014). However, some studies have shown a low efficiency of commercial kits—based on the amount and the purity of the recovered DNA— when compared to in-house protocols (Hurt et al. 2001; Luna, Dell'Anno, and Danovaro 2006;

Table 1. List of DNA extraction methods used in NGS fermented milk studies.

Cell lysis principles	Lysis agents	Sample	Reference
Chemical	50 mM EDTA, 0.1 M NaCl, 10 mM Tris-HCl, 25 mM sucrose, 20% SDS		(Nalbantoglu et al. 2014)
Physical	Three freeze-thawing cycles	Kefir grains	
Enzymatic	30 mg/mL lysozyme, 5000 µL/mL mutanolysin, 10 mg/mL proteinase K, 10 mg/mL RNAase		
Chemical	CTAB, 1.4 M NaCl, ß-mercaptoethanol	Kefir grains	(Zamberi et al. 2016)
Physical	Heating at 60 °C		
Chemical	20 mM Tris-HCl, 2 mM sodium EDTA, 1.2% Triton-100	Kefir grains	(X. Wang et al. 2018)
Enzymatic	50 U lyticase, 20 mg/ml lysozyme, 20 mg/mL proteinase K		
Physical	Heating at 90 °C	Kefir grain	(Dertli and Çon 2017)
Chemical	Genematrix food-extract DNA purification kit (Eurx, Poland)		
Chemical	DNeasy Blood & Tissue Kits Print	Kefir grain	(Korsak et al. 2015)
Chemical	Power Food® Microbial DNA Isolation Kit (Mo Bio, USA)	Kefir milk	(Marsh et al. 2013)
Physical	Qiagen TissueLyser II		
Enzymatic	100 U/ml mutanolysin, 50 mg/ml, lysozyme, 250 mg/mL proteinase K		
Automated	NucliSENS easyMAG system (BioMérieux, France)	Kefir milk	(Kim, Kim, and Seo 2020)
Chemical	PowerSoil DNA isolation kit (Mo Bio)	Kefir milk	(Hong et al. 2019)
Physical			
Chemical	Power Food® Microbial DNA Isolation Kit (Mo Bio)	Kefir grains and milk	(Gao and Zhang 2019)
Enzymatic	50 mg/mL lysozyme, 100 U/mL mutanolysin, proteinase K	Kefir grains and milk	(Walsh et al. 2016)
Physical	PowerBead tube on the TissueLyser II		
Enzymatic	50 U lysozyme, 25 U mutanolysin, 20 U lyticase, 25 mg/mL proteinase-K	*Dahi*	(Shangpliang et al. 2018)
Chemical	50 mM Tris, 1 mM EDTA, 8.7% sucrose		
Chemical	10% SDS, 5 M NaCl, CTAB/NaCl	*kurut*	(Liu, Zheng, et al. 2015)
Mechanical	glass beads (Mini-Beadbeater-8)		
Physical	FastDNA® Spin Kit for Soil (MP Biomedicals, USA)	*kurut*	(Jiang et al. 2020)
Chemical	Tris-EDTA, 10% SDS	Buttermilk	(Jayashree et al. 2013)
Enzymatic	20 mg/mL lysozyme, 20 mg/mL proteinase K		
Chemical	100 mM Tris–HCl, 40 mM EDTA, benzyl chloride, 10% SDS	*Airag, Khoormog* and,*Tarag*	(Oki et al. 2014)
Mechanical	glass beads (FastPrep FP120)		
Chemical	0.5 M EDTA	*Nunu*	(Walsh et al. 2017)
Physical	five freeze-thawing cycles		
Not mentioned	Qiagen DNA Stool Mini Kit*	*Koumiss*	(Gesudu et al. 2016)
Physical	FastDNA® Spin Kit for Soil (MP Biomedicals)	*Koumiss*	(Wurihan et al. 2019)
Physical	FastDNA® Spin Kit for Soil (MP Biomedicals)	Fermented mare's milk	(Jatmiko, Mustafa, and Ardyati 2019)
Not mentioned	Qiagen DNA Stool Mini Kit*	Naturally fermented cow's milk	(Liu, Zheng, et al. 2015)b
Physical	Frozen in liquid nitrogen, thawed in a water bath at 60 °C	*Kurut*	(Liu et al. 2012)
Chemical	10% SDS, 10 mg /mL proteinase K		
Not mentioned	Qiagen DNA Stool Mini Kit*	*Suero costeño*	(Motato et al. 2017)

* - Out-of-circulation extraction kits.

Keisam et al. 2016; Quigley et al. 2012). A particularly important and limiting factor in NGS investigations is the usual small amount of suitable starting DNA or too much DNA degradation, which underestimates the OTUs in the sample (Lienhard and Schäffer 2019). Inhibitors within environmental samples, such as DNase and excess protein, may create similar problems (Ariefdjohan, Savaiano, and Nakatsu 2010). Therefore, DNA extraction optimization is a further important factor in gaining reliable results for NGS (Lamble et al. 2013; Arseneau, Steeves, and Laflamme 2017).

Library preparation

Library construction prepares DNA into a form that is compatible with the sequencing system to be used (Figure 1). The core steps in preparing DNA for NGS analysis are: (*i*) fragmenting or sizing the target DNA to the desired length, (*ii*) converting target to single-stranded DNA, (*iii*) attaching oligonucleotide adapters, and (*iv*) quantitating the final library product for sequencing (Head et al. 2014). Physical, enzymatic, and chemical processes can perform the DNA fragmentation. Physical methods include acoustic shearing and sonication, enzymatic fragmentation uses nonspecific endonuclease cocktails and transposase tagmentation reactions, and chemical process involves PCR amplification of a single taxonomically informative "marker gene" from organisms of interest (Ari and Arikan 2016; Hennig et al. 2018).

The chemical process targeting rRNA gene is more widely used for microbiome studies. This process, also called metagenetic, increases the depth of taxonomic information

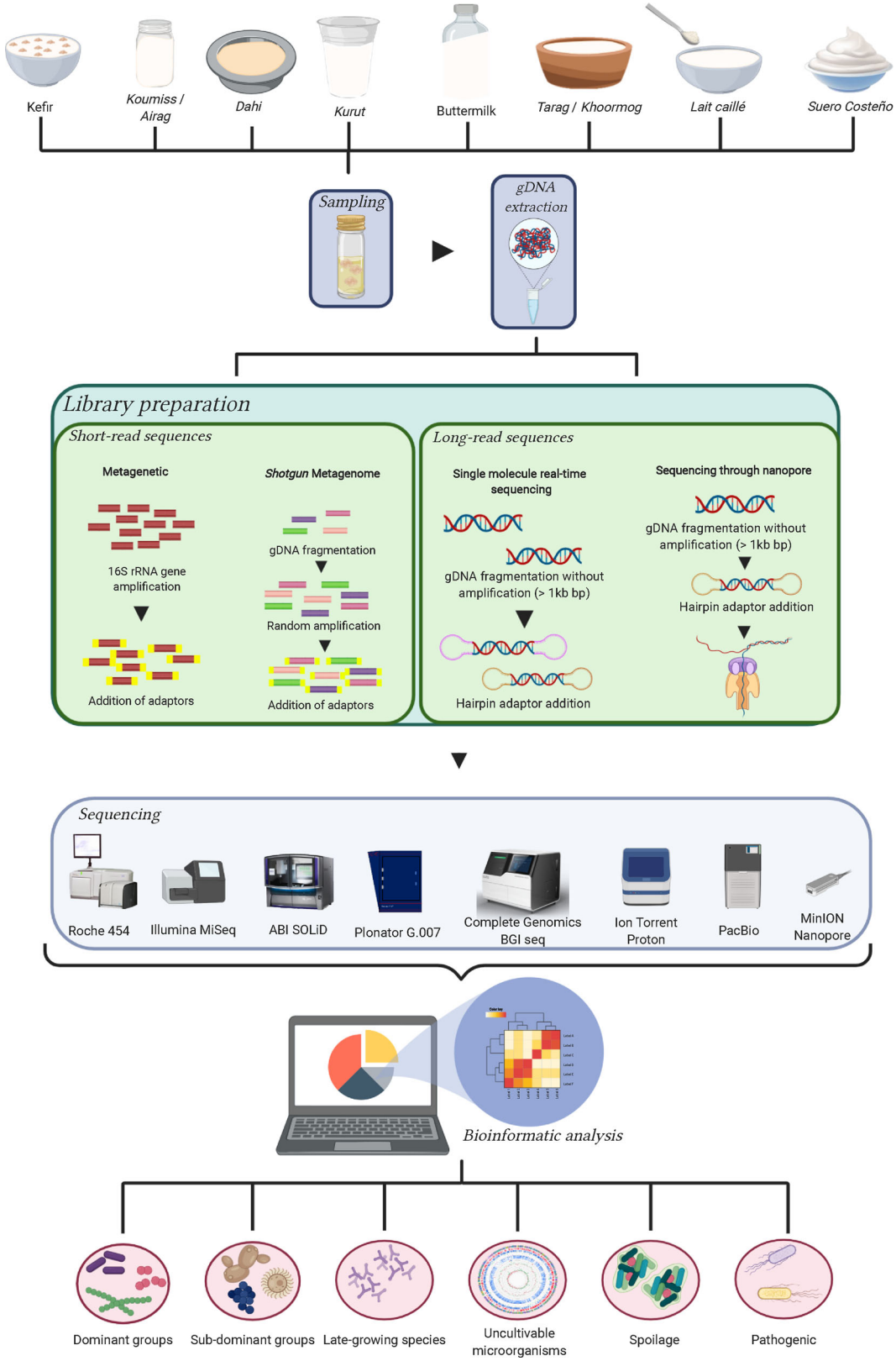

Figure 1. Schematic workflow for the implementation of NGS-based studies in fermented milk microbiome analysis.

(Beiko, Hsiao, and Parkinson 2018). The bacterial small subunit ribosomal RNA gene (16S rRNA) is the most popular genomic region for profiling bacterial communities. This locus presents a series of characteristics that make it particularly suited for bacteria analysis, including its universal distribution in prokaryotic species, its high number of copies making it easy for isolation and purification, and its intrinsic constitution with conserved regions (used for primer annealing) and hypervariable regions (used for phylogenetics comparison) (Hodkinson and Grice 2015; Silva, de Oliveira, and

Table 2. Overview of the metagenetic strategy (16S rRNA gene amplification) used to evaluate the bacterial community composition of natural fermented milk products.

Sample	Hypervariable site	Platform	Database	Taxonomic resolution	Reference
Kefir grains	V1-V2	Pyrosequencing	RDP	Family	(Leite et al. 2012)
Kefir grains	V1-V2	Pyrosequencing	NCBI	Genus/species	(Nalbantoglu et al. 2014)
Kefir grains	V3-V4	Illumina MiSeq	Silva	Genus/species	(Zamberi et al. 2016)
Kefir grains	V3-V4	Illumina MiSeq	Silva	Genus/species	(Liu et al. 2019)
Kefir grains	V1–V3	Illumina MiSeq	GreenGene	Genus/species	(Dertli and Çon 2017)
Kefir grains	V1-V3	Pyrosequencing	SILVA	Genus/species	(Korsak et al. 2015)
Kefir grains	V1–V3	Illumina MiSeq	NCBI	Genus/species	(Gao and Zhang 2019)
Kefir grains	V1-V2	Illumina MiSeq	RDP	Genus/species	(Wang et al. 2018)
Kefir milk and Kefir grain	V1-V2	Illumina MiSeq	Silva	Genus/species	(Walsh et al. 2016)
Kefir grains	V4	Pyrosequencing	RDP	Family	(Dobson et al. 2011)
Kefir milk	V4–V5	Pyrosequencing	Silva	Genus	(Marsh et al. 2013)
Kefir milk	V1-V3	Pyrosequencing	Greengenes	Genus/species	(Garofalo et al. 2015)
Kefir milk	V3-V4	Illumina MiSeq	QIIME	Genus/species	(Hong et al. 2019)
Kefir milk	V3-V4	Illumina MiSeq	NCBI	Genus/species	(Kim, Kim, and Seo 2020)
Dahi	V4–V5	Illumina MiSeq	Silva	Genus/species	(Shangpliang et al. 2018)
kurut	V1–V3	Pyrosequencing	RDP	Genus	(Liu, Xi, et al. 2015)
Buttermilk	V6–V9	Pyrosequencing	RDP	Family	(Jayashree et al. 2013)
Airag, Khoormog and,Tarag	V1-V2	Pyrosequencing	RDP	Genus	(Oki et al. 2014)
Nunu	16S rRNA gene	Illumina MiSeq	Silva	Genus/species	(Walsh et al. 2017)
Koumiss	16S rRNA gene	PacBio	RDP	Genus/species	(Gesudu et al. 2016)
Koumiss	V3-V4	Illumina MiSeq	Greengenes	Genus/species	(Wurihan et al. 2019)
Fermented cow's milk	V1–V3	Pyrosequencing	RDP	Genus	(Liu, Zheng, et al. 2015)
Kurut	V3-V4	Illumina MiSeq	RDP	Genus	(Jiang et al. 2020)
Fermented mare's milk	V3–V4	Illumina MiSeq	SILVA	Genus	(Jatmiko, Mustafa, and Ardyati 2019)
Suero Costeño	V3	Illumina MiSeq	SILVA	Genus	(Motato et al. 2017)

Grisolia 2017). The choice of the primers for targeting the 16S rDNA region is essential to the success of analysis (Ercolini 2013). Table 2 summarizes the hypervariable regions of the 16S rDNA (namely V1 to V9) and NGS platforms that were used to investigate microbiomes from fermented milks. The hypervariable regions V1-V2-V3 and V4 were the most widely used, allowing identifications down to the species level (Table 2). Other regions covered include V2-V3 (Chakravorty et al. 2007), V1-V2-V3 (Sundquist et al. 2007), V2-V3-V4 (Liu et al. 2008), and V1-V4 (Kim and Bae 2011).

After DNA amplification, oligonucleotides of a known short sequence (called adapters) are connected to the end of each generated 16S rDNA fragment (Bystrykh, de Haan, and Verovskaya 2014). The adapters are complementary and hybridize with synthetic DNA sequences coated on the surface of planar or spherical surfaces. After hybridization, 16S rDNA fragments are amplified and grouped into clusters using different strategies according to the sequencing system to be used, e.g., emulsion or "bridge" PCR (Adessi et al. 2000; Mitra and Church 1999; Williams et al. 2006). The emulsion PCR was the first in vitro clonal amplification technique developed, and it consists of the hybridization of a ssDNA on paramagnetic beads. After hybridization, reagents necessary for PCR are added and the aqueous solution is mixed with oil, capturing the beads in micelles. Each micelle acts then as individual microreactors generating thousands of copies from a single fragment or amplicon (Dressman et al. 2003). This methodology is used in the 454 Roche, Ion Torrent, ABI SOLiD, Complete Genomics, and Polonator G.007 sequencing systems.

On the other hand, the "bridge" PCR amplification is exclusive for Illumina platform, in which is performed in a glass flow cell coated with short synthetic DNA fragments complementary to the adaptors (Glaxo Group Ltd. 1998). The ssDNA fragments are hybridized in the flow cell by the 5′ terminal adaptor, leaving the 3′ termination exposed to allow primer extension. Due to the high density of these complementary sequences, the free 3′-termination of the fragments hybridizes, forming a "bridge" structure during the annealing and extension steps. This cycle is repeated using formamide based denaturation and Bst DNA polymerase, generating "clusters" of clonal amplicons (Cao et al. 2017).

The metagenetic methodology has a few disadvantages, such as biases associated with PCR, overestimation of community diversity or species abundance, and inability to describing biological functions (Xia, Sun, and Chen 2018). As an alternative, shotgun metagenomic sequencing can be used to fulfill lacks and provide a better understanding of the microbiome, especially taxonomic analysis (who is there?), functional analysis (what are they doing?), and comparative analysis (how to compare them?) (Xia, Sun, and Chen 2018). In general, the core steps in preparing DNA by shotgun metagenomic are (*i*) DNA extraction, (*ii*) fragmentation by physical or chemical methods and library preparation, (*iii*) DNA sequencing, (*iv*) quality checking, (*v*) assembly, and (*vi*) binning/annotation. The steps (*i*) to (*iv*) are quite similar to the metagenetic method, except that no specific gene is targeted during PCR amplification. Thus, library construction is performed from random PCR amplification or physical fragmentation, so the entire community DNA is extracted and independently sequenced. This produces a massive number of DNA reads that can be aligned to genomic locations in the sample (Hodkinson and Grice 2015). For instance, it can be sampled from taxonomically informative genome loci (*e.g.*, 16S) or coding sequences, providing insights into the community structure and metagenome. Therefore, the construction of shotgun libraries has the potential to discriminate strains of common species by

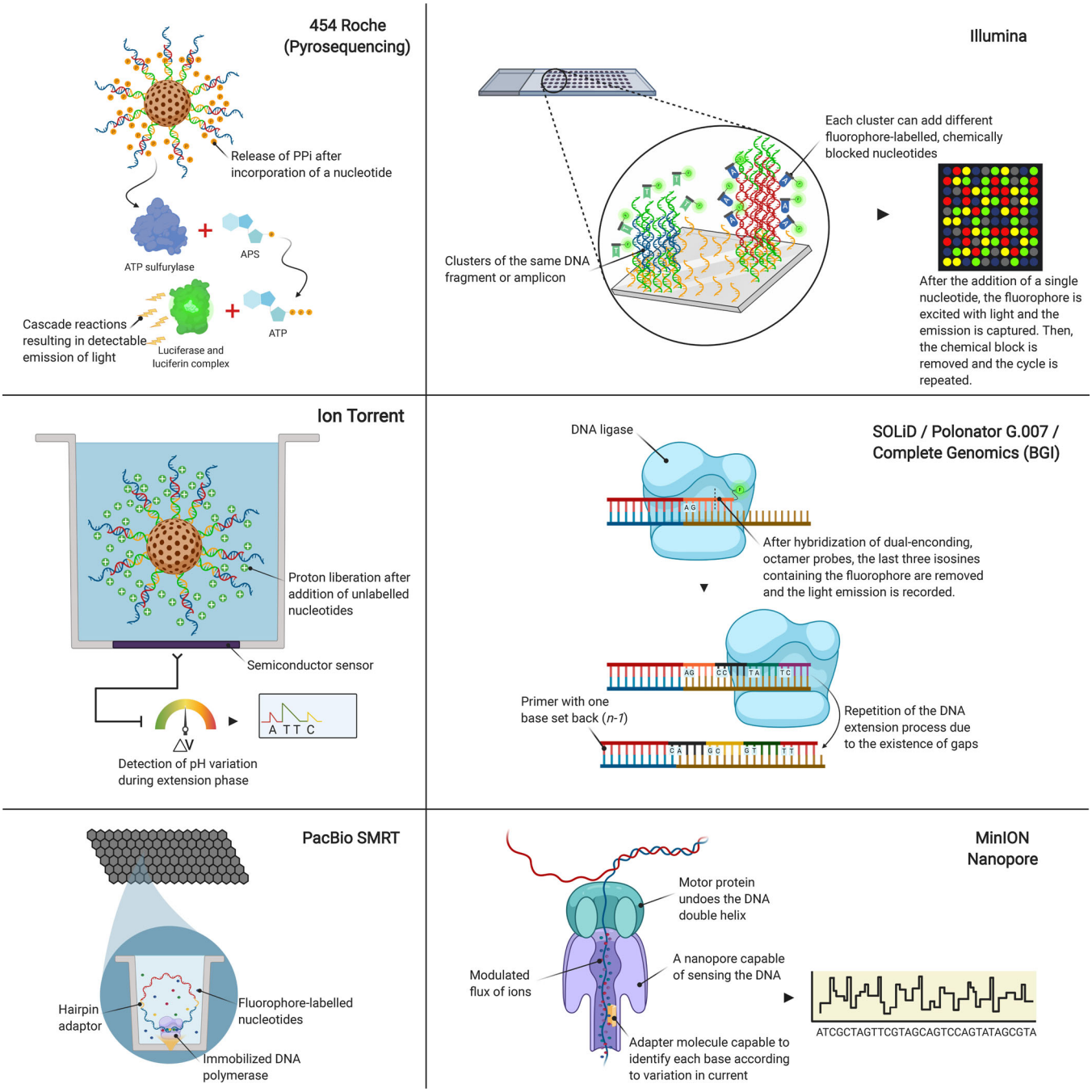

Figure 2. Schematic representation of the different NGS platforms used in microbiome studies.

gene content and the detection of novel microorganisms (Xia, Sun, and Chen 2018). Besides, it offers the possibility to identify genes of interest and to understand the functional pathways that define the microbiome under study. This methodology, however, has a few disadvantages, such as technical challenges in processing huge amounts of data, large and complex outputs that difficult gene tracking, and complications identifying different taxa between communities (Xia, Sun, and Chen 2018).

Sequencing

The sequencing technologies can be categorized according to fragment length read, namely short-read (35–700 bp) and long-read (> 1 kb) (Figure 1). Both metagenetic and shotgun metagenome prepares DNA samples to be compatible with short-read sequencing platforms (*e.g.,* Roche 454, Illumina, ABI SOLiD, Polonator G.007, Complete Genomics, and Ion Torrent). The Roche 454 sequencer was the first NGS platform commercially available. It uses pyrosequencing to identify the nucleotides added during the extension of the fragments. The pyrosequencing operates in a sequencing-by-synthesis methodology mediated by an enzymatic cocktail containing DNA polymerase, ATP sulfurylase, and luciferase (Figure 2). This process consists of three steps: (*i*) a single nucleotide is added and its incorporation in the elongation chain releases inorganic pyrophosphate (PPi) during the condensation reaction; (*ii*) the released PPi is converted into

adenosine triphosphate (ATP); (*iii*) ATP-mediated oxidation of luciferin into oxyluciferin emits light that is captured by a camera and the software records the nucleotide added to the sequence (Harrington et al. 2013). The pyrosequencing of the 16S rRNA gene was a pioneer in revealing microbiomes of fermented milk products, including kefir, buttermilk, *kurut*, *tarag, airag*, and *khoormog* (Sun et al. 2014; Marsh et al. 2013; Jayashree et al. 2013; Oki et al. 2014; Liu, Xi, et al. 2015). However, Roche shut down 454 Life Sciences in 2013 due to high reagents cost and non-competitiveness with the upcoming platforms (Humblot and Guyot 2009).

Illumina became the most current library preparation protocol and sequencing kits available for 16S rRNA amplicon sequencing. This system works using a cyclic reversible termination approach, where the ribose 3′-OH of each base is blocked by a chemically cleavable fluorescent reporter, which prevents elongation (Figure 2). After incorporation of a single base, the reporter is cleaved allowing the identification of the nucleotide and the further extension of the ssDNA template (Cao et al. 2017). This platform was used for microbiome analysis of kefir, *koumiss*¸ *suero costeño*, and *lait caillé* (Groenenboom et al. 2019; Parker et al. 2018; Motato et al. 2017).

Unlike Illumina and Roche 454, the sequencing in SOLiD Polonator G.007 and Complete Genomics are mediated by a DNA ligase instead of DNA polymerase. The sequencing method depends on the hybridization of fluorophore-labeled octamer probes to the ssDNA fragments. These probes are single or dual encoding, which means that only the first and second bases are known and correlate to a specific fluorescent color (Figure 2). The third, fourth, and fifth bases are degenerated in all possible combinations, while the sixth, seventh, and eighth are inosines carrying the fluorophore (Goodwin, McPherson, and McCombie 2016; Ari and Arikan 2016). After the ligation of one probe, the last three nucleotides are removed allowing the incorporation of a new probe and the extension of the DNA strand. Due to the existence of a 3-nucleotide gap, it is necessary the repetition of this process seven times with the addition of universal primers with one base set back (n-1) (Ari and Arikan 2016). Due to the short reading size and low throughput, these platforms have not been used for the analysis of fermented milk microbiomes. Instead, sequence-by-ligation platforms' applicability has been restricted to the identification of evolutionary changes between pathogenic strains and mutation studies (Jarvik et al. 2010; Chin, da Silva, and Hegde 2013).

The Ion Torrent is considered the most versatile and less expensive equipment between the short-read platforms. The core of this technology relies on the quantification of H^+ ions released during the addition of a nucleotide by DNA polymerase (Figure 2). The Ion Torrent was the first platform without an optical sensing detector, which reduced the costs of the runs and the equipment itself (Rothberg et al. 2011). Although this technology offers superior total reads count and length when compared to Illumina platform, it has high error rates related to insertion and deletion, and sequence truncation on both forward and reverse DNA strands, which was associated with the semiconductor sequencing methodology (Salipante et al. 2014). Therefore, few studies on fermented milk microbiomes have been conducted on this platform (de la Fuente et al. 2014; Verce, De Vuyst, and Weckx 2019).

The third-generation sequencing (also known as long-read sequencing) is a class of DNA sequencing methods currently under active development (Figure 1). Pacific Biosciences' (PacBio) single-molecule real-time sequencing (SMRT) and Oxford Nanopore Technologies' (ONT) nanopore sequencing are the two long-read sequencing technologies currently available (Hui 2012; Ameur, Kloosterman, and Hestand 2019). In contrast to short-read sequencing, these platforms work by amplifying long strands of DNA in a single run. The major advance of the PacBio sequencing was the immobilization of the DNA polymerase, instead of the DNA fragment, at the base of each one of the 150,000 zeptoliter wells (Figure 2) (Hui 2012). The immobilized polymerase binds to the hairpin adaptor and an uninterrupted chain elongation is performed through the addition of dNTPs tagged with a fluorescent dye attached to the phosphate group. This technology allows the reduction of background noise, and the throughput range is only limited by the DNA polymerase activity. Until now, only two studies in profiling bacterial community composition of traditional fermented milk (*Koumiss* in Inner Mongolia) were performed using the SMRT sequencing technology (Gesudu et al. 2016; Mo et al. 2019). Finally, in the Nanopore sequencing, a single molecule of DNA can be sequenced without the need for PCR amplification or chemical labeling of the sample. Instead, the circularized DNA is translocated through synthetic or biological nanometer-sized pores when applying a constant electric field (Figure 2). The changes in the ionic current caused by base shifting along the sequence are measured and recorded (Jain et al. 2016). Nanopore sequencing has yet not been used for generating microbiomes of fermented milk. However, a recent study demonstrated the applicability of this methodology in the identification of both Gram + and Gram- - pathogenic bacteria in food matrices through direct metatranscriptome (Yang, Zhang, et al. 2019).

Data analysis

After sequencing, sequence processing and bioinformatics analysis are required to transform the raw data into variant lists that can be used in phylogenetic studies. Sequence processing involves removing chimeras, low-quality sequences, and short reads. It improves accuracy and avoids the overestimation of community taxa (Beiko, Hsiao, & Parkinson, 2018). Pipelines like Quantitative Insights into Microbial Ecology (QIIME) and Morthur allow users to demultiplex files, remove barcodes and adaptors, and perform quality checking. The filtered sequences are clustered using the OTU-based method (or phylotype-based method), providing taxonomic distance between sequences. OTUs are defined as sequences that have great similarity (usually 97% for species) with other sequences. The percentage similarity between OTUs and a referenced database (*e.g.*, SILVA, RDP and

Greengenes, NCBI, and UNITE) allows taxonomy assignment and relative abundances of the microbiome under analysis (Xia, Sun, and Chen 2018).

There is no perfect database choice, since each has its protocols, taxonomic coverage, and particularities. For instance, SILVA, RDP, and Greengenes are commonly used with 16S analysis due to vast archaeal and bacterial data, while UNITE is better used with 18S and ITS analysis due to their high content of fungi data (Beiko, Hsiao, and Parkinson 2018). Finally, it can perform statistical analysis such as alpha/beta diversity, dispersion plots, and boxplots. Buza et al. (2019) developed a full pipeline for 16S analysis in which both Morthur and QIIME are used as platforms, with raw reads and mapping file as input and alpha/beta diversity and phylogenetic trees as outputs, which can give a head start for anyone that just arrived in this field.

Bacterial world diversity in fermented milks

LAB diversity

LAB are largely predominant in milk fermentations comprising 91 species identified by NGS studies (Figure 3). They are divided into two major clades (low G + C content Firmicutes phylum and high G + C content *Bifidobacterium*) occurring in the taxonomic genera *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Enterococcus, Weissella*, and *Oenococcus. Lactobacillus*, which is highly efficient in consuming lactose (Rezvani, Ardestani, and Najafpour 2017), is the most frequent and important genus with 51 species reported. *Lactobacillus helveticus* followed by *Lb. kefiranofaciens, Lb. delbrueckii*, and *Lb. kefiri* are the ubiquity species found in kefir, *koumiss, tarag, buttermilk, dahi, khoormog*, and *kurut* (Figure 3). Genome sequencing of *Lb. helveticus*, *Lb. kefiranofaciens*, and *Lb. delbrueckii* strains revealed that the ongoing reduction of the genome (called "reductive evolution"), together with the acquisition or overexpression of genes related to milk sugar metabolism, reflect their adaptation to the dairy niche (Germond et al. 2003; Callanan et al. 2008; Slattery et al. 2010; Cavanagh, Fitzgerald, and Mcauliffe 2015; Xing et al. 2017). *Lb. helveticus* has a potent proteolytic activity, introducing important lipolysis-derivative aroma compounds for fermented milk (Quigley et al. 2013). *Lb. kefiranofaciens* and *Lb. kefiri* are involved in the mechanism of polysaccharide production, and *Lb. delbrueckii* promotes rapid acidification with desired organoleptic properties (Herve-Jimenez et al. 2009).

Leuconostc and *Streptococcus* are other common genera—with *Leu. mesenteroides* and *S. thermophilus* being the most common species (Figure 3). Milk contains low concentrations of free amino acids and peptides, and nitrogen is a growth-limiting factor for LAB (Christensen et al. 1999; Christiansen et al. 2008; Morishita et al. 1981; Cavanagh, Fitzgerald, and Mcauliffe 2015). However, some species of *Leuconostoc, Lactobacillus* and *Streptococus* exhibit high proteolytic activity supporting their growth in milk (Liu et al. 2010; Sasaki, Bosman, and Tan 1995; Kunji et al. 1996). *S. thermophilus* has important functions for milk fermentation, including rapid acidification through the production of lactic acid, galactose metabolism, proteolytic and urease activities (Iyer et al. 2010). In addition, the production of secondary metabolites (*e.g.,* formate, acetaldehyde or diacetyl) contributes to the development of aroma and texture of fermented milks (Uriot et al. 2017). *Leu. mesenteroides* is often associated with lactic acid and bacteriocins production, assisting the maintenance of fermented milk by inhibiting the development of *Listeria monocytogenes*, *Clostridium botulinum*, *Enterococcus faecalis*, and other pathogenic bacteria (Hechard et al. 1992; Wulijideligen et al. 2012; Arakawa et al. 2016).

NGS studies have enabled the first detection of *Bifidobacterium* in kefir, *koumiss*, *tarag*, *lait caillé*, and *suero costeño* (Figure 3). The cultivation of *Bifidobacterium* from natural habitats is difficult because it is generally overgrown by other LAB or yeasts (Thitaram et al. 2005). In addition, *Bifidobacterium* strains have strict growth requirements, being poorly tolerant to oxygen, refrigeration temperatures, and low pH (González-Sánchez et al. 2010). This underscores the fact that culture-independent analysis is a powerful tool for a better understanding of microbial consortia and that bifidobacteria with unknown taxonomy and physiology may contribute to various extents to such consortia (Gulitz et al. 2013).

Currently, kefir is the most widely studied and with the largest number of LAB species identified (Figure 3). This led to the covering of many less abundant species detected only in kefir, including *Lb. kalixensis, Lb. parafarraginis, Lb. crispatus, Lb. apis, Lb. intestinalis, Lb. gigeriorum, Lb. taiwanensis, Lb. gasseri, Lb. lactis, Lb. psittaci, Lb. reuteri, Lb. rossiae, Lb. thailandensis, Lb. tucceti, Lb. senmaizukei, Lb. sanfranciscensis, Lb. farraginis, Lb. parafarraginis, Lb. rapi, Lb. parakefiri, Lb. sunkii, Lb. parabuchneri, Lb. nagelii, Lb. animalis*, and *Lb. sakei*. On the other hand, fewer LAB were exclusive to other fermentation processes, such as *Lb. crustorum* and *Enterococcus* spp. in *koumiss*, *Lb. bifermentans* and *Lb. curvatus* in *dahi, Lb. acetotolerans, Lb. hamster*, and *Lb. capillatus* in *kurut* (Figure 3). Although present in low relative abundances, their presence indicates a microbial activity specific to geographical region, which can have several implications for community interactions and metabolite formations. Whether a causal influence of these minor LAB groups for milk fermentation exists, it remains unclear.

Fermented products

Kefir

Kefir is produced by adding kefir grains to a quantity of milk at a proportion of 2-5% (w/v) grains-to-milk (Van Wyk 2019). The kefir grains start the fermentation and consist of a symbiotic culture of bacteria and yeast embedded in a polysaccharide matrix called kefiran. During fermentation, LAB convert lactose to lactic acid causing milk proteolysis, and lactose-fermenting yeast and acetic acid bacteria (AAB) produce CO_2, alcohol and acetate, respectively, responsible for the effervescent and acid taste of the final yeast product (Kim et al. 2015; Pogačić et al. 2013; Magalhães et al. 2011; Kesmen and Kacmaz 2011; Taş, Ekinci, and Guzel-Seydim

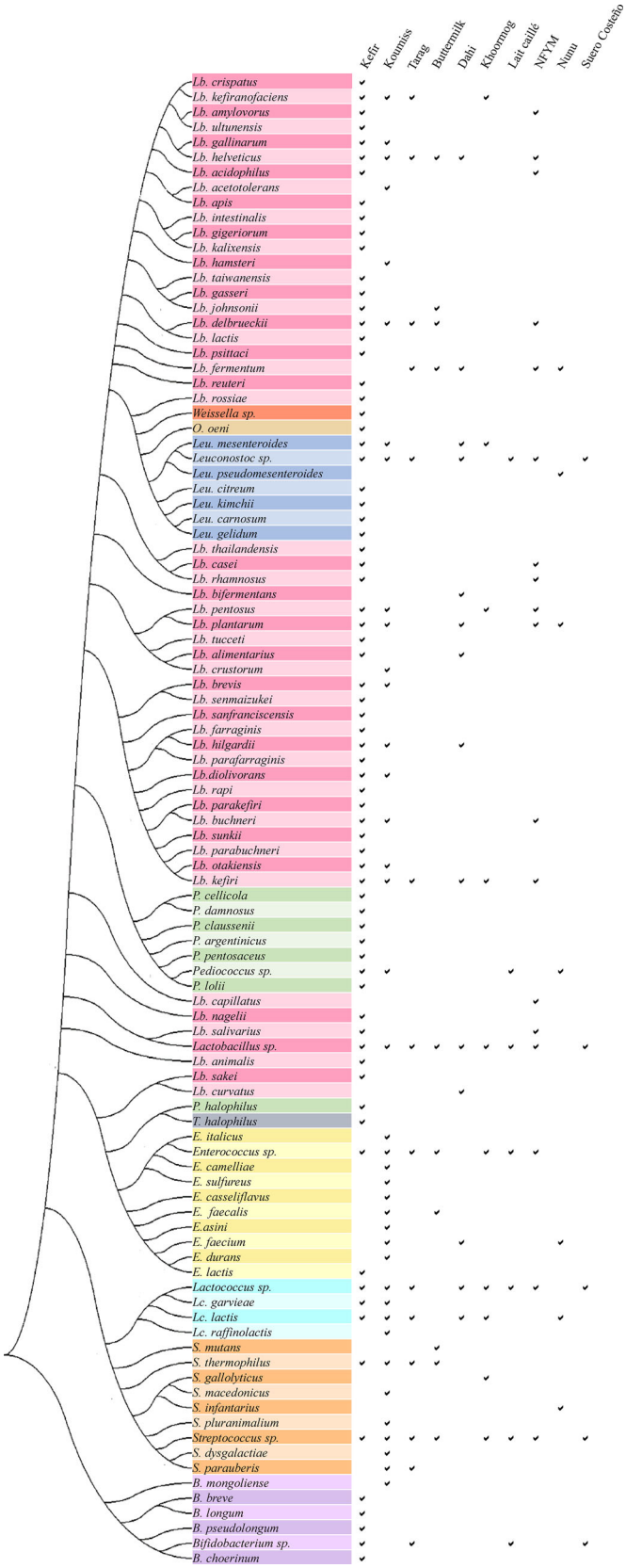

Figure 3. 16S rRNA Neighbor-joining tree showing the phylogenetic proximity of LAB species reported in popular fermented milk products. The 16S rRNA gene sequences were retrieved from GenBank database and aligned with ClustalW. The phylogenetic tree was constructed using MEGA X program. The abbreviation of the LABs genus are as follow: *Lactobacillus* = *Lb.*; *Oenococcus* = *O.*; *Leuconostoc* = *Leu.*; *Pediococcus* = *P.*; *Tetragenococcus* = *T.*; *Enterococcus* = *E.*; *Lactococcus* = *Lc.*; *Streptococcus* = *S.*; *Bifidobacterium* = *B.* (Dobson et al. 2011), (Leite et al. 2012), (Marsh et al. 2013), (Gao et al. 2013), (Jayashree et al. 2013), (Oki et al. 2014), (Nalbantoglu et al. 2014), (Garofalo et al. 2015), (Korsak et al. 2015), (Liu, Xi, et al. 2015), (Liu, Zheng, et al. 2015), (Zamberi et al. 2016), (Walsh et al. 2016), (Zhong et al. 2016), (Gesudu et al. 2016), (Dallas et al. 2016), (Motato et al. 2017), (Walsh et al. 2017), (Yao et al. 2017), (Dertli and Çon 2017), (Parker et al. 2018),(Wang et al. 2018), (Shangpliang et al. 2018), (Gao and Zhang 2019), (Wurihan et al. 2019), (Wenwen Liu et al. 2019), (Hong et al. 2019), (Jiang et al. 2020), (Kim, Kim, and Seo 2020).

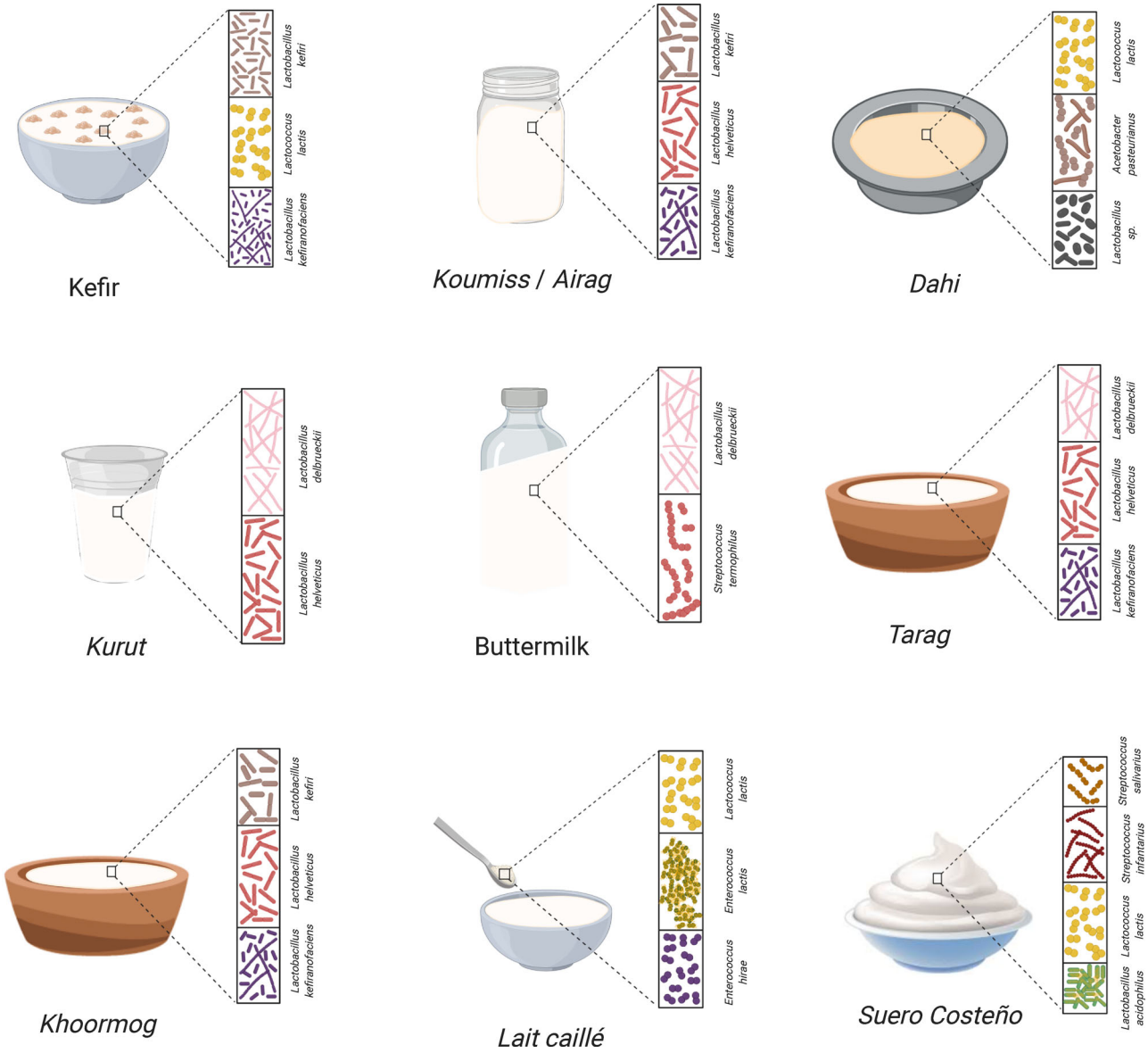

Figure 4. Representation of the dominant bacterial groups of popular fermented milk products. Kefir grains: Dertli & Çon (2017), Gao & Zhang (2019); Garofalo et al. (2015), Hong et al. (2019), Kim, Kim, and Seo (2020); *Koumiss*: Gesudu et al. (2016), Tang et al. (2020), Wang et al. (2012), Wurihan et al. (2019), Yao et al. (2017), Zhong et al. (2016); *Dahi*: Shangpliang et al. (2018); *Kurut*: Zhong et al. (2016) and Jiang et al. (2020); Buttermilk: Jayashree et al. (2013); *Airag* and *Khoormog*: Oki et al. (2014); *Tarag*: Sun et al. (2014); *Lait caillé*: Parker et al. (2018); *Suero costeño*: Walsh et al. (2017) and Motato et al. (2017).

2012; Witthuhn, Schoeman, and Britz 2004; Guzel-Seydim et al. 2005; Grønnevik, Falstad, and Narvhus 2011; Miguel et al. 2010). Other smaller microbial groups generally isolated are *Acinetobacter, Alistipes, Allobaculum, Bacteroides, Brochothrix*, *Clostridium, Enterobacter*, and *Faecalibacterium*. A complete list of all bacterial groups found in kefir and other fermented milk products is shown in the supplementary material Table S2. These are generally more correlated to environmental contamination rather than kefir grain microbiota (Zamberi et al. 2016; Marsh et al. 2013; Walsh et al. 2016; Dertli and Çon 2017).

The bacterial community composition of kefir grains and beverage has been extensively studied by NGS. The dominant species detected by culturing were also identified by NGS technologies (Figure 4). *Lb. kefiranofaciens* was reported as the dominant bacteria in kefir grains from Turkey, Malaysia, France, Ireland, United Kingdom, China, Tibet, USA, Italy, and Belgium (Kim, Kim, and Seo 2020; Garofalo et al. 2015; Korsak et al. 2015; Dallas et al. 2016; Hong et al. 2019; Zamberi et al. 2016; Dertli and Çon 2017; Walsh et al. 2016; Gao and Zhang 2019). All NGS studies reported that *Lb. kefiranofaciens* dominance was accompanied by a rich variety of sub-dominant groups, including *Lb. kefiri*, *Lb. helveticus*, *Lb. parakefiri*, *Lb. crispatus*, *Leu. mesenteroides,* and *Acetobacter orientalis*, except Wang et al. (2018), which found *Lb. kefiranofaciens* as the only dominant species in Tibetan kefir grains cultured in different conditions. The authors reported that *Lb. kefiranofaciens* is more resistant to variations in culture conditions and plays a more important role in the formation and stability of Tibetan kefir grains in comparison to other bacterial species. Although lactose is a suitable carbon source for *Lb. kefiranofaciens* metabolism (Cheirsilp et al. 2018), strong symbiotic association with yeast and particular growth requirements

(*e.g.,* strictly anaerobic) may be limiting factors for the growth of this species in milk (Vardjan et al. 2013; Wang et al. 2008).

Concerning kefir beverage, *Lactococcus lactis* has been detected as the dominant species in different geographical locations (Figure 4), although the number of studies is quite limited compared to kefir grains (Vedamuthu 1994; Gao and Zhang 2019; Korsak et al. 2015). *Lc. lactis* is widely associated with sauerkraut, cheese, yoghurt and like, being an important starter agent in the food industry (Song et al. 2017; Wels et al. 2019). *Lc. lactis* initiates the fermentation by rapidly converting lactose to lactic acid, besides producing volatile metabolites, proteolytic enzymes, and exopolysaccharides (Song et al. 2017). *Lc. lactis* is also responsible for several bio functionalities attributed to regular kefir consumption, including potential probiotic proprieties, conjugated linoleic acid synthesis and antimutagenic and anticarcinogenic effects (Oliveira et al. 2017; Vieira et al. 2017).

Surprisingly, many bacterial genera other than LAB were described as dominant in kefir by NGS studies. In Turkey kefir samples, Dertli and Çon (2017) alerted on food safety when *Enterobacter amnigenus* and *Enterobacter hormaechei* were found as dominant species by Illumina sequencing. The authors showed that these enterobacteria could pass to the kefir grains from the milk, which should be assessed as it can create safety concerns. In Tibet and Belgium, AAB, *Acetobacter orientalis* and *Gluconobacter frateurii*, were, respectively, the dominant species detected by Illumina sequencing system (Gao & Zhang 2019; Korsak et al. 2015). Early microbiological studies considered AAB as contaminants from the handling of kefir grains or improper practices adopted during the preparation of the kefir beverage (Angulo, Lope, and Lema 1993). However, AAB species were constantly reported in kefir from different geographical origins and, today, are considered key microorganisms for kefir fermentation. They are associated with acetic acid production and water-soluble polysaccharides synthesis that increase the viscosity of the kefir beverage. However, some NGS studies using pyrosequencing did not detect any AAB in kefir samples from Ireland, Belgium, and South Africa (Dobson et al. 2011; Korsak et al. 2015).

Plenty of other non-dominant LAB groups, mainly represented by *Lactobacillus* with 44 species, have been detected in kefir by NGS (Figure 3). Many of these minor species represent geographical spread, such as *Lb. ultunensis*, *Lb. rhamnosus*, *Lb. apis*, *Lb. casei, Lb. crispatus Lb. johnsonii*, and *Pediococcus* spp. in Turkey and Malaysia; *S. thermophilus* in Italy, United Kingdom, and France; *Lb. farraginis* in South Korea; and extremely rare *Tetragenococcus* and *Oenococcus* in Tibet and Korea (Nalbantoglu et al. 2014; Dertli and Çon 2017; Garofalo et al. 2015). Finally, microbial groups other than LAB were detected by NGS for the first time in kefir grains, including *Shewanella, Acinetobacter, Pelomonas, Dysgonomonas, Faecalibacterium, Allistipes, Rickenellaceae*, and *Allobaculum* (Gao et al. 2013; Dertli and Çon 2017; Marsh et al. 2013). The identification and understanding of these minor microorganisms contribute to physicochemical assignments of kefir beverage and discovery of new strains with potential probiotic properties (Bengoa et al. 2019; Walsh et al. 2016).

Koumiss (fermented mare's milk)

Koumiss, also known as *airag*, *chige*, *chigo* or *arrag*, is an ancient yeast-lactic fermented product consumed in Mongolia, China, and Russia (Vedamuthu 1994). Traditionally, *koumiss* is prepared with mare's milk by back-sloping process, where a small quantity of the previous *koumiss* is used as starter raw material for the next fermentation batch. Fermentation takes place in wooden casks, containers made of animal skin, urns or porcelain, by 1 to 3 days at ambient temperature (~20 °C). The fermenting mass is beaten or stirred with a wooden stick to ensure mixing evenly and fast fermentation (Yao et al. 2017; Gesudu et al. 2016). The microbiota isolated from *koumiss* consists of LAB, AAB, and yeast, including *Lb. helveticus, Lb. kefiranofaciens, Acetobacter pasteurianus, Kluyveromyces marxianus*, and *Saccharomyces cerevisiae* (Ringø et al. 2014; Bai and Ji 2017).

The first NGS-based metagenomic study on *koumiss* (referenced as *airag*) was performed by Oki et al. (2014) using the pyrosequencing platform. The authors reported the dominance of *Lb. helveticus*, followed by *Lb. kefirofaciens, Lb. kefiri, Lb. parakefiri*, and *Lb. diolivorans,* from 22 *koumiss* samples collected in Mongolia (Figure 4). More recently, Tang et al. (2020), using long-read SMRT sequencing technology (PacBio), confirmed the dominance of *Lb. helveticus* and *Lb. kefirofaciens* in Mongolian *koumis*. However, Tang et al. (2020) also identified a novel dominant bacterial species, *Citrobacter freundii* that had not been reported previously. The authors associated the presence of *Citrobacter freundii* as environmental contamination, since it is widely distributed as an opportunistic pathogen found in soil and human gut (Wang et al. 2000).

NGS also revealed several minor bacterial constituents in *koumiss* uncovered by culturing methods. These included *Lb. casei*, *Lb. farciminis, Lb. parafarraginis, Lb. paraplantarum, Leu. pseudomesenteroides, Leu. Pentosaceus, Enterococcus faecium*, *Acetobacter pasteurianus*, *A. russicus*, *Acidobacteria*, *Tenericutes*, *Verrucomicrobia*, *Escherichia, Clostridium perfingens, Enhydrobacter aerosaccus*, and *Shigella* (Oki et al. 2014; Wurihan et al. 2019; Zhong et al. 2016; Tang et al. 2020). The non-LAB OTUs were regarded as environmental contaminants from soils, animals, and nomads since milk for *koumiss* production are not heat-treated. In addition, the identification of OTUs without species assignment suggested the presence of uncultivable microorganisms (Oki et al. 2014).

Yao et al. (2017) used a modified, single-cell amplification metagenomic method to analyze low-abundant bacteria of *koumiss* samples collected from Mongolia and Inner Mongolia of China. The method involved a serial dilution of samples to a final count of 100 cell, followed by an amplification step to increase the quantity of DNA of diluted samples and Illumina HiSeq 2500 sequencing. With these additional steps, the authors detected *Lb. otakiensis* and *S.*

macedonicus, which has never been isolated in *koumiss* samples. *Lb. otakiensis* has also never been reported in other dairy niches. This procedure proved to be a potential tool for analyzing minority microbial populations, which can be extended for other fermented foods.

Dahi

Dahi is a popular fermented milk beverage produced in India, Bhutan, Bangladesh, Nepal, and Pakistan (Shangpliang et al. 2018; Nahidul-Islam et al. 2018). The fermentation takes place in earthenware, sub-culturing preexistent fermented *dahi* in fresh cow, yak, or buffalo's milk. The fermentation lasts 1–2 days and the finished product has brown color and caramelized flavor characteristics resulted of milk intense heating before fermentation (Tamang et al. 2012; Harun-Ur-Rashid et al. 2007). *Dahi* is a ready-to-drink beverage or it can be used for the preparation of various ethnic fermented products (*e.g., gheu, mohi, chhurpi)* (Shangpliang et al. 2017).

Culture-dependent studies demonstrated that *dahi* fermentation is governed by LAB load from 6.6 to 8.4 log CFU/g (Harun-Ur-Rashid et al. 2007; Shangpliang et al. 2017). However, the dominant species were discrepant. *S. bovis* was reported as the dominant species in Bangladesh (Harun-Ur-Rashid et al. 2007), while *E. faecalis* was isolated in greater numbers from *dahi* samples in India (Shangpliang et al. 2017). In the same way, the application of NGS has reported discrepant dominant species, such as *Lc. lactis* or *A. pasteurianus* dominating in *dahi* samples from India, and *Lactobacillus* sp. in Bangladesh (Figure 4). All this great diversity within the dominant species in *dahi* was attributed to environmental factors, such as the animal origin of the milk, altitude, different technical conditions of product preparation and temperature oscillation (Koirala et al. 2014). In addition, NGS revealed several minor species not detected by cultivation, including *Acinetobacter*, Enterobacteriaceae, *Pseudomonas*, and Micrococcaceae (Nahidul-Islam et al. 2018).

Kurut (natural fermented yak milk)

Kurut is a fermented dairy product in northwestern China that is generally prepared using *qula*—a traditional product made by defatting, acidifying, and air-drying yak milk (Duan et al. 2008; Yang, Cousineau, et al. 2020). Yak (*Bos grunniens*) is a long-haired bovid found throughout the Himalaya region of southern Central Asia, the Tibetan Plateau, Mongolia, and Russia. Its milk is a highly nutritious product, rich in fat, protein, essential minerals, and polyunsaturated fatty acids. Fermentation, flavor, and preservation of the *kurut* are strongly dependent on the milk's natural microbiota. Yak milk usually ferments at 4 to 15 °C for 12 to 36 h (Jiang et al. 2020; Liu, Xi, et al. 2015).

Using the cultivation approach, Wu et al. (2009) detected *Lb. fermentum* as the dominant species in Tibetan *kurut*. NGS studies confirmed *Lactobacillus* as the most dominant genera; however, *Lb. delbrueckii* and *Lb. helveticus* were the most abundant bacterial species (Figure 4; Liu, Xi, et al. 2015). *Lactobacillus* plays a significant role in *kurut* flavor by releasing the volatiles benzaldehyde, 2,3-pentanedione, ethanol, and ethyl acetate (Jiang et al. 2020). Jiang et al. (2020) found a negative correlation between *Lactobacillus* and *Streptococcus* using Illumina MiSeq technology, indicating a competitive relationship in the later stages of fermentation when nutrients are scarce. Using pyrosequencing, Liu, Xi, et al. (2015) explored the microbial community of *kurut* from two Tibetan villages and found significant diversity between microbial composition associated with geographical differences and other external environmental conditions (Gesudu et al. 2016). Among 49 OTU, 42 (*Massilia*, *Propionibacterium*, *Lactococcus*, *Leuconostoc*, and *Enterococcus*) were related to Ningzhong village and 7 (unidentified Firmicutes, *Pantoea*, *Streptococcus*, *Lactobacillus*, unidentified Proteobacteria, *Acinetobacter*, and *Bacteroidetes*) to Geda village.

Buttermilk

Buttermilk is the aqueous phase released during cream churning in the butter-making process. It is rich in protein, lactose, and minerals (Sodini et al. 2006). This precious by-product is traditionally used as a substrate to produce fermented buttermilk in Northern Ethiopia, India, Asian Countries, and USA (Gebreselassie, Abay and Beyene 2016; Jayashree et al. 2013). Fermented buttermilk can be classified as a cultured or a natural beverage. The cultured is manufactured by adding commercial strains (*e.g., Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris*, *Leu. mesenteroides* ssp. *cremoris*, *S. lactis*, *Lc. lactis* ssp. *lactis biovar)* (Gebreselassie, Abay, and Beyene 2016). Naturally fermented buttermilk, in contrast, is prepared by adding previous day's curd as inoculum to cow's milk, fermented overnight at room temperature (~32 °C), and finally churned.

Culture-dependent analyses revealed *Lc. lactis* ssp. *lactis*, *Lb pentosus* and *Lb. plantarum* as the main species of naturally fermented buttermilk from Northern Ethiopia (Gebreselassie, Abay, and Beyene 2016). The study conducted by Jayashree et al. (2013) was the only one to use NGS in naturally fermented buttermilk. Evaluating samples from China by pyrosequencing, the authors found *Lb. delbruecki* as the dominant species, followed by *S. thermophiles*, *Lb. fermentum*, *Lb. johnsonii*, and *Lb. helveticus* (Figure 4). Pyrosequencing of rDNA amplicons also revealed microorganisms that have never been associated with food fermentation before, including *Methylobacterium populi*, *M. radiotolerans*, *Ralstonia solanacearum*, *Synechocystis* sp., and *Thermoanaerobacter* sp.

Tarag

Tarag is a fermented cow's milk produced by backslopping method, consumed in Mongolia and China. Unlike other fermented milk products, *tarag* requires at least 5 days of fermentation to achieve the desired acidity, alcoholic degree, and sensorial characteristics. In most *tarag* samples originated from Mongolia and China, *Lb. helveticus* and *Lb. delbrueckii* ssp. *bulgaricus* were recovered by culturing methods (Yu

et al. 2011; Uchida et al. 2007). These dominant species were late confirmed by the pyrosequencing of the rDNA gene (Sun et al. 2014; Oki et al. 2014). The NGS results also demonstrated that bacterial diversity was stratified by geographic region. For instance, *tarag* samples from Inner Mongolia revealed a high prevalence of *Lb. kefiri, Lb. capillatus*, and *Lb. kefirofaciens*, while samples from China provinces (Sichuan and Gansu) showed the dominance of *Lb. helveticus* and *Lb. delbrueckii* ssp. *bulgaricus* (Figure 4). Finally, several bacterial groups not previously isolated from *tarag* were identified by pyrosequencing, including *Acinetobacter*, *Klebsiella*, *Escherichia*, and *Salmonella* (Sun et al. 2014).

Khoormog

Khoormog is a traditional Mongolian fermented beverage made from raw camel milk. The fermentation is performed spontaneously in a wooden barrel or cow's skin bag (Oki et al. 2014). The microbiome study performed by Oki et al. (2014) was the first microbiological report about *khoormog*. The pyrosequencing of tagged 16S rRNA gene amplicons revealed that the bacteria population was similar to *airag*. Members of the genus *Lactobacillus* were dominant, mainly represented by *Lb. kefiranofaciens*, followed by *Lb. helveticus* and *Lb. kefiri* (Figure 4). Other minor bacteria found included *Lc. lactis, Brevundimonas nasdae*, and *A. pasteurianus*. *Lb. kefiranofaciens* was first isolated from kefir grains in 1988, which was subsequently found in various other fermented milk products (Fujisawa et al. 1988; Sun et al. 2014; Gesudu et al. 2016; Oki et al. 2014). However, this bacterium had never been found as a dominant group in a fermented product other than kefir grains. *Lb. kefiranofaciens* is a strictly anaerobic bacterium known for its auto-aggregation ability. This characteristic confers its protection against stress environmental factors, including temperature and oxygen availability, and may be the reason for its dominance during camel milk fermentation (Trunk, Khalil, and Leo 2018). In addition, *Lb. kefiranofaciens* dominance can be associated with the presence of *Lb. kefiri* during *khoormog* fermentation, supported by the well-known protocooperation between these two species (Wang et al. 2012).

Lait caillé

Lait caillé is an ethnic beverage produced by the Fulani people from sub-Saharan countries, Burkina Faso, and Senegal, by spontaneous fermentation of cow's milk (Bayili et al. 2019). The household production of *lait caillé* is performed as an "imperfect" backslopping method, where the cow's milk is firstly heated in aluminum pots and transferred to familiar clay pots (*lahals*), gourds or calabashes. The fermentation is conducted spontaneously for a period of 1–3 days (Parker et al. 2018; Savadogo et al. 2004). The main species isolated from the *lait caillé* fermentation process include *Enterococcus hirae, E. lactis*, and *Lc. lactis*, as well as subdominant populations of *Lactobacillus, Weissella, Leuconostoc*, and *Pediococcus* (Bayili et al. 2019).

Bacterial community composition of *lait caillé* from different towns and villages in Senegal was investigated by Parker et al. (2018) and Groenenboom et al. (2019) using Illumina technology, which found *Streptococcus* and *Lactobacillus* as the dominant genera. This composition resembles regular yogurt, which is the product of controlled milk fermentation by two species (*Lb. delbrueckii* ssp *bulgaricus* and *S. thermophilus*) of the same two bacterial genera (Groenenboom et al. 2019). However, several other genera were related at relatively high abundances, including *Lactococcus, Weisella, Enterococcus, Leuconostoc, Vagococcus, Pediococcus, Acetobacter, Acinetobacter,* and enterobacteria *Escherichia/Shigella*. In addition, consistent with the uncontrolled nature of *lait caillé* fermentation, over 100 minor bacterial genera were reported, including *Kocuria* and *Bifidobacterium*.

Suero costeño

Suero costeño is a fermented milk product manufactured by rural people of the Colombian Caribbean Coast. It is produced spontaneously with indigenous microorganisms from the fermentation containers (calabash or plastic vessels), the raw cow's milk, and the environmental surroundings, or by backsloping inoculating milk with 30% (v/v) of a precedent successful fermentation. The whey formed during 24 h of fermentation is removed, resulting in a final product with sour cream-like characteristic. The peculiar organoleptic characteristics of *suero costeño* is a result of a combination of factors, including Caribbean warm temperature (~30 C), environmental humidity (greater than 74%), and indigenous microbiota (Motato et al. 2017). The fermentation is mainly conducted by LAB (such as *Lb. plantarum* and *Lb. paracasei* subsp. *paracasei*) and smaller populations of yeast, aerobic mesophilic bacteria, and Enterobacteria (Cueto et al. 2007).

Suero costeño produced under different conditions (recipient, fermentation time, and the existence or not of backslopping) was characterized by Motato et al. (2017) using Illumina MiSeq platform. The study reported the dominance of *Lactobacillus* and *Streptococcus*, and 12 other bacterial genera. Interestingly, a relative high incidence of *Aeromonas* (10%) and the presence of other toxin-producing bacteria (*Escherichia/Shigella*) were found in *suero costeño* produced via backslopping. In the backslopping technique, part of a previous fermentation is recovered, reused, and grown — often over periods of several decades (De Melo Pereira et al. 2020). It is possible to hypothesize that the backslopping process may be contributing to the generation and spread of well-adapted pathogenic bacteria in *suero costeño*. Further investigation is needed to confirm this hypothesis. Finally, the study by Motato et al. (2017) revealed the first report of *Bifidobacterium* and other important genera (e.g., *Lactococcus* and *Leuconostoc*) in *suero costeño*, contributing to a deep knowledge of this peculiar fermentation process (Motato et al. 2017).

Pathogens and food spoilage microorganisms

Increased consumption of fermented milk products has been driven, in part, by the safe status these products confer. The

inhibitory effect on pathogenic and food spoilage microorganisms are due to the various antimicrobial molecules produced by LAB during fermentation, including organic acids, bacteriocins, hydrogen peroxide, carbon dioxide, diacetyl, and ethanol (Reis et al. 2012; Tesfaye, Mehari, and Ashenafi 2011; Magnusson and Schnürer 2001). Therefore, several culture-based studies have been dedicated to elucidating the composition of LAB in natural milk fermentations. However, these antimicrobial factors may not be effective when fermentation is under non- or low-aseptic manipulation conditions. Some crucial factors that affect the fermented milk microbiota composition are the hygienic quality of the milk and the manufacturing process.

High-throughput sequencing also effectively unveiled the presence of a number of unwanted bacteria in traditional milk fermentations. Importantly detrimental bacteria comes from low-quality milk, manly represented by the Pseudomonadaceae family and sub-dominant species of *Acinetobacter*, Enterobacteriaceae, *Sphingomonas*, *Staphylococcus*, and Comamonadaceae (Dogan and Boor 2003; Issa and Tahergorabi 2019). *Pseudomonas* has been shown to be inhibited by hydrogen peroxide, diacetyl, and organic acids produced by LAB, and are rarely part of the milk fermentation microbiota (Reis et al. 2012; Tesfaye, Mehari, and Ashenafi 2011). However, studies using NGS reported that some *Pseudomonas* species, including *P. aeruginosa* and *P. otitidis*, are part of the microbial composition of kefir grains from different origins (Dertli and Çon 2017).

Pseudomonas are known to produce various enzymes (*e.g.,* lipases, proteases, and phospholipases) that lead to odor, flavor, and body defects (Chen, Wei, and Chen 2011). In addition, it may indicate potential health relevance when consumers believe they are ingesting only beneficial microorganisms. Although the incidence of *Pseudomonas* bacteremia from foods is very rare, some studies reported the presence of virulence in *P. aeruginosa* associated with fresh vegetables, water, and meat (Allydice-Francis and Brown 2012; Xu et al. 2019). Recent evidence suggests that virulence factors found in environmental isolates, such as pilin gene, multidrug efflux transport system, porin oprD gene, and hemolytic and proteolytic activities, show no difference with clinical *P. aeruginosa* (Allydice-Francis and Brown 2012). *P. aeruginosa* is considered an opportunistic pathogen, able to cause urinary tract infections, respiratory dermatitis, soft tissue infections, bacteremia, gastrointestinal infections, and a variety of systemic infections (Bentzmann and Plésiat 2011; Lucchetti-Miganeh et al. 2014; Sader et al. 2015; Castaldo et al. 2017). In this sense, great efforts are being explored to prevent contamination by *Pseudomonas* in dairy products (Meesilp and Mesil 2019; Nan et al. 2016; Picoli et al. 2017; Yasmin et al. 2017).

NGS technologies have revealed the presence of members of the *Enterobacteriaceae* family in almost all microbiological studies of natural milk fermentations. *Escherichia, Shigella, Salmonella*, and *Klebsiella* were reported in natural milk fermentations from Northern Senegal, Sumbawa mare's fermented milk (Indonesia), and Tibetan naturally fermented yak milk using Illumina MiSeq platform (Walsh et al. 2017; Jatmiko, Mustafa, and Ardyati 2019; Jiang et al. 2020). Enterobacteriaceae was the dominant family in kefir grains from different regions of Turkey using 16S rRNA gene sequencing on Illumina platform (Wang et al. 2006; Walsh et al. 2016; Dertli and Çon 2017). The presence of these bacterial groups indicates unhygienic conditions and contamination from either fecal material, dairy farm environment or human contact (Martin et al. 2016). Oki et al. (2014) also attributed the presence of these potential pathogen microorganisms by transfer from animals, because the milk for *airag* and *khoormog* are generally not heat-treated. It is important to point out that all of these studies using NGS cannot confirm the presence of viable taxa of Enterobacteria. NGS technologies analyze DNA from pathogens that are present in the sample and do not discriminate viable from non-viable cells (Ursell et al. 2012; Wen et al. 2017). Thus, it is important that food safety-related studies be conducted with plating methods to confirm the presence of viable taxa. Finally, some Enterobacteriaceae family could be not relevant as foodborne pathogens since many of them are plant and human commensal organisms (Jha et al. 2011).

Concluding remarks

The popularization of NGS technology is driving penetration of microbiome research into popular fermented milk products across the globe. The studies produced so far has enormously extended our knowledge on food microbiology and revealed limitations and biases that were previously ignored. While the recent NGS platforms have confirmed the success of culturing approaches for detecting dominant species, they have enabled the discovery of yet uncultured genus- or species-level clades. An important example is the first detection of late-growing species of *Bifidobacterium* and other subdominant populations with potential probiotic activities in kefir, *koumiss, tarag, lait caillé*, and *suero costeño*. The discovery of these new taxa will promote the best opportunities to isolate novel microorganisms with functional proprieties and, ultimately, their use as improved starters.

Pyrosequencing and Illumina platforms have been, by far, the most popular techniques used to study fermented food microbiomes. Coming in, meanwhile, alternative sequencing techniques that can generate long reads, such as Pacific Biosciences' (PacBio) single-molecule real-time sequencing and Oxford Nanopore Technologies' (ONT) sequencing, have yet been underutilized. The introduction of these recent sequencing technologies can increase the length of reads to cover the whole of the 16S rRNA gene and assembly of complete genomes. This will enable accurate taxonomic identification down to the strain level and assist in determining critical microbial variables and better control of food quality and safety. Furthermore, other omics techniques, such as proteomics, transcriptomics, and metabolomics, can be coupled to the current NGS studies to confirm the functions and metabolic capacity of microbiomes of fermented milk products. This multi-omic approach is of pivotal importance for fermented milk products whose microbial composition and interaction have only been

investigated by culture-dependent methods, such as *curd, clabber, doogh, mohi, lassi*, and *shyow*.

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References

Adessi, C., G. Matton, G. Ayala, G. Turcatti, J. J. Mermod, P. Mayer, and E. Kawashima. 2000. Solid phase DNA amplification: Characterisation of primer attachment and amplification mechanisms. *Nucleic Acids Research* 28 (20):E87 doi: 10.1093/nar/28.20.e87.

Akabanda, F., J. Owusu-Kwarteng, K. Tano-Debrah, R. L. K. Glover, D. S. Nielsen, and L. Jespersen. 2013. Taxonomic and molecular characterization of lactic acid bacteria and yeasts in *nunu*, a Ghanaian fermented milk product. *Food Microbiology* 34 (2):277–83. doi: 10.1016/j.fm.2012.09.025.

Al-Awadhi, H., N. Dashti, M. Khanafer, D. Al-Mailem, N. Ali, and S. Radwan. 2013. Bias problems in culture-independent analysis of environmental bacterial communities: A representative study on hydrocarbonoclastic bacteria. *SpringerPlus* 2 (1):369. doi: 10.1186/2193-1801-2-369.

Allydice-Francis, K., and P. D. Brown. 2012. Diversity of antimicrobial resistance and virulence determinants in *Pseudomonas aeruginosa* associated with fresh vegetables. *International Journal of Microbiology* 2012:426241. doi: 10.1155/2012/426241.

Ameur, A., W. P. Kloosterman, and M. S. Hestand. 2019. Single-molecule sequencing: Towards clinical applications. *Trends in biotechnology* 37 (1):72–85. doi: 10.1016/j.tibtech.2018.07.013.

Angulo, L., E. Lope, and C. Lema. 1993. Microflora present in Kefir grains of the galician region (North-West of Spain)." *The Journal of Dairy Research* 60 (2):263–7. doi: 10.1017/S002202990002759X.

Arakawa, K., S. Yoshida, H. Aikawa, C. Hano, T. Bolormaa, S. Burenjargal, and T. Miyamoto. 2016. Production of a bacteriocin-like inhibitory substance by *Leuconostoc mesenteroides* subsp. *dextranicum* 213M0 isolated from Mongolian fermented mare milk, *airag*. *Animal Science Journal = Nihon Chikusan Gakkaiho* 87 (3):449–56. doi: 10.1111/asj.12445.

Ari, S., and M. Arikan. 2016. Next-generation sequencing: Advantages, disadvantages, and future. In *Plant omics: Trends and applications*, ed. K. R. Hakeem, H. Tombuloglu, and G. Tombuloğlu, 110–35. London: Springer. doi: 10.1007/978-3-319-31703-8.

Ariefdjohan, M. W., D. A. Savaiano, and C. H. Nakatsu. 2010. Comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens. *Nutrition Journal* 9 (1):23 doi: 10.1186/1475-2891-9-23.

Arseneau, J.-R., R. Steeves, and M. Laflamme. 2017. Modified low-salt CTAB extraction of high-quality dna from contaminant-rich tissues. *Molecular Ecology Resources* 17 (4):686–93. doi: 10.1111/1755-0998.12616.

Bai, L., and S. Ji. 2017. Isolation and identification of lactic acid bacteria from *koumiss* in Eastern Inner Mongolia of China. *AIP Conference Proceedings* 1794:050005. doi: 10.1063/1.4971951.

Bayili, G. R., P. Johansen, D. S. Nielsen, H. Sawadogo-Lingani, G. A. Ouedraogo, B. Diawara, and L. Jespersen. 2019. Identification of the predominant microbiota during production of *lait caillé*, a spontaneously fermented milk product made in Burkina Faso. *World Journal of Microbiology and Biotechnology* 35 (7):1–13. doi:10.1007/s11274-019-2672-3.

Beiko, R. G., W. Hsiao, and J. Parkinson. 2018. *Microbiome analysis: Methods and protocols. Methods in molecular biology*. Vol. 1849. Hatfield: Human Press. doi: 10.1007/978-1-4939-8728-3_5.

Bengoa, A. A., C. Iraporda, G. L. Garrote, and A. G. Abraham. 2019. Kefir micro-organisms: Their role in grain assembly and health properties of fermented milk. *Journal of Applied Microbiology* 126 (3):686–700. doi: 10.1111/jam.14107.

Bentzmann, S., and P. Plésiat. 2011. The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. *Environmental Microbiology* 13 (7):1655–65. doi:10.1111/j.1462-2920.2011.02469.x.

Buza, T. M., T. Tonui, F. Stomeo, C. Tiambo, R. Katani, M. Schilling, B. Lyimo, P. Gwakisa, I. M. Cattadori, J. Buza, et al. 2019. IMAP: An integrated bioinformatics and visualization pipeline for microbiome data analysis. *BMC Bioinformatics* 20 (1):374 BMC Bioinformatics:doi: 10.1186/s12859-019-2965-4.

Bystrykh, L. V., G. de Haan, and E. Verovskaya. 2014. Barcoded vector libraries and retroviral barcoding of hematopoietic stem cells. In *Hematopoietic stem cell protocols, methods in molecular biology*, ed. K. D. Bunting and C.-K. Qu, 1185, 345–60. New York: Springer Science + Business Media. doi: 10.1007/978-1-4939-1133-2.

Callanan, M., P. Kaleta, J. O'Callaghan, O. O'Sullivan, K. Jordan, O. McAuliffe, A. Sangrador-Vegas, L. Slattery, G. F. Fitzgerald, T. Beresford, et al. 2008. Genome sequence of *Lactobacillus helveticus*, an organism distinguished by selective gene loss and insertion sequence element expansion. *Journal of Bacteriology* 190 (2):727–35. doi: 10.1128/JB.01295-07.

Cao, Y., S. Fanning, S. Proos, K. Jordan, and S. Srikumar. 2017. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. *Frontiers in Microbiology* 8:1829. doi:10.3389/fmicb.2017.01829.

Castaldo, N., F. Givone, M. Peghin, E. Righi, A. Sartor, and M. Bassetti. 2017. Multidrug-resistant *Pseudomonas aeruginosa* skin and soft-tissue infection successfully treated with Ceftolozane/Tazobactam. *Journal of Global Antimicrobial Resistance* 9:100–2. doi: 10.1016/j.jgar.2017.02.012.

Cavanagh, D., G. F. Fitzgerald, and O. Mcauliffe. 2015. From field to fermentation: The origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiology* 47:45–61. doi: 10.1016/j.fm.2014.11.001.

Chakravorty, S., D. Helb, M. Burday, N. Connel, and D. Alland. 2007. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods* 69 (2):330–9. doi:10.1016/j.mimet.2007.02.005.A.

Cheirsilp, B., S. Suksawang, J. Yeesang, and P. Boonsawang. 2018. Co-production of functional exopolysaccharides and lactic acid by *Lactobacillus kefiranofaciens* originated from fermented milk, kefir. *Journal of Food Science and Technology* 55 (1):331–40. doi: 10.1007/s13197-017-2943-7.

Chen, T. R., Q. K. Wei, and Y. J. Chen. 2011. *Pseudomonas* spp. and *Hafnia alvei* growth in UHT milk at cold storage. *Food Control* 22 (5):697–701. doi:10.1016/j.foodcont.2010.10.004.

Chin, E. L. H., C. da Silva, and M. Hegde. 2013. Assessment of clinical analytical sensitivity and specificity of next-generation sequencing for detection of simple and complex mutations. *BMC Genetics* 14:66. doi:10.1186/1471-14-6.

Christensen, J. E., E. G. Dudley, J. A. Pederson, and J. L. Steele. 1999. Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek. International Journal of General and Molecular Microbiology* 76:217–46. doi: 10.1023/A:1002001919720.

Christiansen, J. K., J. E. Hughes, D. L. Welker, B. T. Rodríguez, J. L. Steele, and J. R. Broadbent. 2008. Phenotypic and genotypic analysis of amino acid auxotrophy in *Lactobacillus helveticus* CNRZ 32. *Applied and Environmental Microbiology* 74 (2):416–23. doi: 10.1128/AEM.01174-07.

Cueto, C.,. D. García, F. Garcés, and J. Cruz. 2007. Preliminary studies on the microbiological characterization of lactic acid bacteria in *Suero Costeño*, a Colombian traditional fermented milk product. *Revista Latinoamericana de Microbiologia* 49 (1-2):12–8.

Dallas, D. C., F. Citerne, T. Tian, V. L. M. Silva, K. M. Kalanetra, S. A. Frese, R. C. Robinson, D. A. Mills, and D. Barile. 2016. Peptidomic analysis reveals proteolytic activity of kefir microorganisms on bovine milk proteins. *Food Chemistry* 197 (Pt A):273–84. doi: 10.1016/j.foodchem.2015.10.116.

de la Fuente, G., A. Belanche, S. E. Girwood, E. Pinloche, T. Wilkinson, and C. J. Newbold. 2014. Pros and cons of ion-torrent next generation sequencing versus terminal restriction fragment length polymorphism T-RFLP for studying the rumen bacterial community. *PLoS ONE* 9 (7):e101435. doi: 10.1371/journal.pone.0101435.

Dertli, E., and A. H. Çon. 2017. Microbial diversity of traditional kefir grains and their role on kefir aroma. *LWT - Food Science and Technology* 85:151–7. doi:10.1016/j.lwt.2017.07.017.

Dewan, S., and J. P. Tamang. 2007. Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie Van Leeuwenhoek* 92 (3):343–52. doi: 10.1007/s10482-007-9163-5.

Dobson, A., O. O'Sullivan, P. D. Cotter, P. Ross, and C. Hill. 2011. High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain. *FEMS Microbiology Letters* 320 (1):56–62. doi: 10.1111/j.1574-6968.2011.02290.x.

Dogan, B., and K. J. Boor. 2003. Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. *Applied and Environmental Microbiology* 69 (1):130–8. doi:10.1128/AEM.69.1.130.

Dressman, D.,. H. Yan, G. Traverso, K. W. Kinzler, and B. Vogelstein. 2003. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proceedings of the National Academy of Sciences of the United States of America* 100 (15):8817–22. doi: 10.1073/pnas.1133470100.

Duan, Y., Z. Tan, Y. Wang, Z. Li, Z. Li, G. Qin, Y. Huo, and Y. Cai. 2008. Identification and characterization of lactic acid bacteria isolated from Tibetan Qula cheese. *The Journal of General and Applied Microbiology* 54 (1):51–60. doi:10.1007/s13213-013-0798-3.

Ercolini, D. 2004. PCR-DGGE fingerprinting: Novel strategies for detection of microbes in food. *Journal of Microbiological Methods* 56 (3):297–314. doi: 10.1016/j.mimet.2003.11.006.

Ercolini, D. 2013. High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology* 79 (10):3148–55. doi: 10.1128/AEM.00256-13.

Fujisawa, T., S. Adachi, T. Toba, K. Arihara, and T. Mitsuoka. 1988. *Lactobacillus kefiranofaciens* sp. nov. isolated from kefir grains. *International Journal of Systematic Bacteriology* 38 (1):12–4. doi:10.1099/00207713-38-1-12.

Fusco, V., and G. M. Quero. 2014. Culture-dependent and culture-independent nucleic-acid-based methods used in the microbial safety assessment of milk and dairy products. *Comprehensive Reviews in Food Science and Food Safety* 13 (4):493–537. doi:10.1111/1541-4337.12074.

Gao, J., F. Gu, J. He, J. Xiao, Q. Chen, H. Ruan, and G. He. 2013. Metagenome analysis of bacterial diversity in Tibetan kefir grains. *European Food Research and Technology* 236 (3):549–56. doi:10.1007/s00217-013-1912-2.

Gao, W., and L. Zhang. 2019. Comparative analysis of the microbial community composition between Tibetan kefir grains and milks. *Food Research International (Ottawa, Ont.)* 116:137–44. doi: 10.1016/j.foodres.2018.11.056.

Garofalo, C., A. Osimani, V. Milanović, L. Aquilanti, F. De Filippis, G. Stellato, S. Di Mauro, B. Turchetti, P. Buzzini, D. Ercolini, et al. 2015. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. *Food Microbiology* 49 (1):123–33. doi: 10.1016/j.fm.2015.01.017.

Gatti, M., C. Trivisano, E. Fabrizi, E. Neviani, and F. Gardini. 2004. Biodiversity among *Lactobacillus helveticus* strains isolated from different natural whey starter cultures as revealed by classification trees. *Applied and Environmental Microbiology* 70 (1):182–90. doi:10.1128/AEM.70.1.182-190.2004.

Gebreselassie, N., F. Abay, and F. Beyene. 2016. Biochemical and molecular identification and characterization of lactic acid bacteria and yeasts isolated from Ethiopian naturally fermented buttermilk. *Journal of Food Science and Technology* 53 (1):184–96. doi:10.1007/s13197-015-2049-z.

Germond, J. E., L. Lapierre, M. Delley, B. Mollet, G. E. Felis, and F. Dellaglio. 2003. Evolution of the bacterial species *Lactobacillus delbrueckii*: A partial genomic study with reflections on prokaryotic species concept. *Molecular Biology and Evolution* 20 (1):93–104. doi: 10.1093/molbev/msg012.

Gesudu, Q., Y. Zheng, X. Xi, Q. C. Hou, H. Xu, W. Huang, H. Zhang, B. Menghe, and W. Liu. 2016. Investigating bacterial population structure and dynamics in traditional koumiss from Inner Mongolia using single molecule real-time sequencing. *Journal of Dairy Science* 99 (10):7852–63. doi: 10.3168/jds.2016-11167.

Giraffa, G., and E. Neviani. 2001. DNA-based, culture-independent strategies for evaluating microbial communities in food-associated ecosystems. *International Journal of Food Microbiology* 67 (1-2):19–34. doi:10.1016/S0168-1605(01)00445-7.

Glaxo Group Ltd. 1998. Method of nucleic acid amplification. England.

González-Sánchez, F., A. Azaola, G. F. Gutiérrez-López, and H. Hernández-Sánchez. 2010. Viability of microencapsulated *Bifidobacterium animalis* ssp. *lactis* BB12 in kefir during refrigerated storage. *International Journal of Dairy Technology* 63 (3):431–6. doi: 10.1111/j.1471-0307.2010.00604.x.

Goodwin, S., J. D. McPherson, and W. R. McCombie. 2016. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews. Genetics* 17 (6):333–51. doi: 10.1038/nrg.2016.49.

Granato, D., G. F. Branco, A. G. Cruz, J. A. F. Faria, and N. P. Shah. 2010. Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety* 9 (5):455–70. doi:10.1111/j.1541-4337.2010.00120.x.

Grigoroff, S. 1905. Étude sur une lait fermentée comestible. Le 'kissélo mléko' de Bulgarie [Study on an edible fermented milk. The 'kissélo mléko' from Bulgaria]. *Revue Médicale de La Suisse Romande* 25:714–20.

Groenenboom, A. E., M. E. Parker, A. de Vries, S. de Groot, S. Zobrist, K. Mansen, P. Milani, R. Kort, E. J. Smid, and S. E. Schoustra. 2019. Bacterial community dynamics in *lait caillé*, a traditional product of spontaneous fermentation from Senegal. *PLoS ONE* 14 (5):e0215658 doi:10.4121/uuid.

Grønnevik, H., M. Falstad, and J. A. Narvhus. 2011. Microbiological and chemical properties of Norwegian kefir during storage. *International Dairy Journal* 21 (9):601–6. doi:10.1016/j.idairyj.2011.01.001.

Gulitz, A., J. Stadie, M. A. Ehrmann, W. Ludwig, and R. F. Vogel. 2013. Comparative phylobiomic analysis of the bacterial community of water kefir by 16S rRNA gene amplicon sequencing and ARDRA analysis. *Journal of Applied Microbiology* 114 (4):1082–91. doi: 10.1111/jam.12124.

Guzel-Seydim, Z., J. T. Wyffels, A. C. Seydim, and A. K. Greene. 2005. Turkish kefir and kefir grains: Microbial enumeration and electron microscobic observation. *International Journal of Dairy Technology* 58 (1):25–9. doi:10.1111/j.1471-0307.2005.00177.x.

Harrington, C. T., E. I. Lin, M. T. Olson, and J. R. Eshleman. 2013. Fundamentals of pyrosequencing. *Archives of Pathology & Laboratory Medicine* 137 (9):1296–303. doi: 10.5858/arpa.2012-0463-RA.

Harun-Ur-Rashid, M., K. Togo, M. Ueda, and T. Miyamoto. 2007. Identification and characterization of dominant lactic acid bacteria isolated from traditional fermented milk *dahi* in Bangladesh. *World Journal of Microbiology and Biotechnology* 23 (1):125–33. doi:10.1007/s11274-006-9201-x.

Head, S. R., H. K. Komori, S. A. LaMere, T. Whisenant, F. V. Nieuwerburgh, D. R. Salomon, and P. Ordoukhanian. 2014. Library construction for next-generation sequencing: Overviews and challenges. *BioTechniques* 56 (2):61–77. doi: 10.2144/000114133.

Hechard, Y., B. Derijard, F. Letellier, and Y. Cenatiempo. 1992. Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. *Journal of*

General Microbiology 138 (12):2725–31. doi: 10.1099/00221287-138-12-2725.

Hennig, B. P., L. Velten, I. Racke, C. S. Tu, M. Thoms, V. Rybin, H. Besir, K. Remans, and L. M. Steinmetz. 2018. Large-scale low-cost NGS library preparation using a robust Tn5 purification and tagmentation protocol. *G3. G3 (Bethesda, Md.)* 8 (1):79–89. doi: 10.1534/g3.117.300257.

Herve-Jimenez, L., I. Guillouard, E. Guedon, S. Boudebbouze, P. Hols, V. Monnet, E. Maguin, and F. Rul. 2009. Postgenomic analysis of *Streptococcus thermophilus* cocultivated in milk with *Lactobacillus delbrueckii* subsp. *bulgaricus*: Involvement of nitrogen, purine, and iron metabolism. *Applied and Environmental Microbiology* 75 (7): 2062–73. doi: 10.1128/AEM.01984-08.

Hodkinson, B. P., and E. A. Grice. 2015. Next-generation sequencing: A review of technologies and tools for wound microbiome research. *Advances in Wound Care* 4 (1):50–8. doi: 10.1089/wound.2014.0542.

Hong, J. Y., N. K. Lee, S. H. Yi, S. P. Hong, and H. D. Paik. 2019. Short communication: Physicochemical features and microbial community of milk kefir using a potential probiotic Saccharomyces cerevisiae KU200284. *Journal of Dairy Science* 102 (12):10845–9. doi: 10.3168/jds.2019-16384.

Hugenholtz, P., B. M. Goebel, and N. R. Pace. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* 180 (18):4765–74. doi:10.1128/JB.180.24.6793-6793.1998.

Hui, P. 2012. Next generation sequencing: Chemistry, technology and applications. In *Chemical diagnosis*, ed. N. L. S. Tang and T. Poon, 336, 1–18. Berlin: Springer-Verlag. doi: 10.1007/128.

Humblot, C., and J. P. Guyot. 2009. Pyrosequencing of tagged 16S rRNA gene amplicons for rapid deciphering of the microbiomes of fermented foods such as pearl millet slurries. *Applied and Environmental Microbiology* 75 (13):4354–61. doi: 10.1128/AEM.00451-09.

Hurt, R. A., X. Qiu, L. Wu, Y. Roh, A. V. Palumbo, J. M. Tiedje, and J. Zhou. 2001. Simultaneous recovery of RNA and DNA from soils and sediments. *Applied and Environmental Microbiology* 67 (10): 4495–503. doi:10.1128/AEM.67.10.4495.

Issa, A. T., and R. Tahergorabi. 2019. Milk bacteria and gastrointestinal tract: Microbial composition of milk. In *Dietary Interventions in Gastrointestinal Diseases*, ed. R. R. Watson and V. R. Preedy, 265–75. Boca Raton: Academic Press. doi: 10.1016/B978-0-12-814468-8.00022-3..

Iyer, R., S. K. Tomar, T. U. Maheswari, and R. Singh. 2010. *Streptococcus thermophilus* strains: Multifunctional lactic acid bacteria. *International Dairy Journal* 20 (3):133–41. doi:10.1016/j.idairyj.2009.10.005.

Jain, M., H. E. Olsen, B. Paten, and M. Akeson. 2016. The Oxford Nanopore MinION: Delivery of nanopore sequencing to the genomics community. *Genome Biology* 17 (1):1–11. doi:10.1186/s13059-016-1103-0.

Jarvik, T., C. Smillie, E. A. Groisman, and H. Ochman. 2010. Short-term signatures of evolutionary change in the *Salmonella enterica* serovar *typhimurium* 14028 genome. *Journal of Bacteriology* 192 (2): 560–7. doi: 10.1128/JB.01233-09.

Jatmiko, Y. D., I. Mustafa, and T. Ardyati. 2019. Profile of microbial community of naturally fermented sumbawa mare's milk using next-generation sequencing. *Journal of Biological Researches* 24 (2): 58–62. doi: 10.23869/bphjbr.24.2.20191.

Jayashree, S., M. Pushpanathan, J. Rajendhran, and P. Gunasekaran. 2013. Microbial diversity and phylogeny analysis of buttermilk, a fermented milk product, employing 16S rRNA-based pyrosequencing. *Food Biotechnology* 27 (3):213–21. doi:10.1080/08905436.2013.811084.

Jha, C. K., A. Aeron, B. V. Patel, and K. Dinesh. 2011. Bacteria in agrobiology: Plant growth responses. In *Bacteria in agrobiology: Plant growth responses*, ed. D. K. Maheshwari, 159–82. Berlin: Springer-Verlag. doi: 10.1007/978-3-642-20332-9.

Jiang, Y., N. Li, Q. Wang, Z. Liu, Y. K. Lee, X. Liu, J. Zhao, H. Zhang, and W. Chen. 2020. Microbial diversity and volatile profile of traditional fermented yak milk. *Journal of Dairy Science* 103 (1): 87–97. doi:10.3168/jds.2019-16753.

Keisam, S., W. Romi, G. Ahmed, and K. Jeyaram. 2016. Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods. *Scientific Reports* 6:34155 doi: 10.1038/srep34155.

Kesmen, Z., and N. Kacmaz. 2011. Determination of Lactic microflora of kefir grains and kefir beverage by using culture-dependent and culture-independent methods. J Food Sci 76 (5):M276–M283. doi: 10.1111/j.1750-3841.2011.02191.x.

Kim, D. H., J. W. Chon, H. Kim, H. S. Kim, D. Choi, D. G. Hwang, and K. H. Seo. 2015. Detection and enumeration of lactic acid bacteria, acetic acid bacteria and yeast in kefir grain and milk using quantitative real-time PCR. *Journal of Food Safety* 35 (1):102–7. doi: 10.1111/jfs.12153.

Kim, D. H., D. Jeong, K. Y. Song, and K. H. Seo. 2018. Comparison of traditional and backslopping methods for kefir fermentation based on physicochemical and microbiological characteristics. *LWT - Food Science and Technology* 97:503–7. doi:10.1016/j.lwt.2018.07.023.

Kim, D. H., H. Kim, and K. H. Seo. 2020. Microbial composition of Korean kefir and antimicrobial activity of *Acetobacter fabarum* DH1801. *Journal of Food Safety* 40 (1):e12728. doi:10.1111/jfs.12728.

Kim, K. H., and J. W. Bae. 2011. Amplification methods bias metagenomic libraries of uncultured single-stranded and double-stranded DNA viruses. *Applied and Environmental Microbiology* 77 (21): 7663–8. doi: 10.1128/AEM.00289-11.

Koirala, R., G. Ricci, V. Taverniti, C. Ferrario, R. Malla, S. Shrestha, M. G. Fortina, and S. Guglielmetti. 2014. Isolation and molecular characterization of lactobacilli from traditional fermented *dahi* produced at different altitudes in Nepal. *Dairy Science & Technology* 94 (4):397–408. doi:10.1007/s13594-014-0167-4.

Korsak, N., B. Taminiau, M. Leclercq, C. Nezer, S. Crevecoeur, C. Ferauche, E. Detry, V. Delcenserie, and G. Daube. 2015. Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. *Journal of Dairy Science* 98 (6):3684–9. doi: 10.3168/jds.2014-9065.

Kunji, E. R. S., I. Mierau, A. Hagting, B. Poolman, and W. N. Konings. 1996. The proteolytic systems of lactic acid bacteria. *Applied Microbiology and Biotechnology* 70 (2-4):187–221. doi:10.1007/s00253-006-0427-1.

Lamble, S., E. Batty, M. Attar, D. Buck, R. Bowden, G. Lunter, D. Crook, B. El-Fahmawi, and P. Piazza. 2013. Improved workflows for high throughput library preparation using the transposome-based nextera system. *BMC Biotechnology* 13:104. doi: 10.1186/1472-6750-13-104.

Leite, A. M. O., B. Mayo, C. T. C. C. Rachid, R. S. Peixoto, J. T. Silva, V. M. F. Paschoalin, and S. Delgado. 2012. Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. *Food Microbiology*31 (2):215–21. doi: 10.1016/j.fm.2012.03.011.

Li, H., Y. Wang, T. Zhang, J. Li, Y. Zhou, H. Li, and J. Yu. 2020. Comparison of backslopping and two-stage fermentation methods for *koumiss* powder production based on chemical composition and nutritional properties. *Journal of the Science of Food and Agriculture* 100 (4):1822–6. doi:10.1002/jsfa.10220.

Lienhard, A., and S. Schäffer. 2019. Extracting the invisible: Obtaining high quality DNA is a challenging task in small arthropods. *PeerJ.* 7: e6753. doi:10.7717/peerj.6753.

Liu, M., J. R. Bayjanov, B. Renckens, A. Nauta, and R. J. Siezen. 2010. The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genomics* 11:36 doi: 10.1186/1471-2164-11-36.

Liu, W. J., Sun, Z. H., Y. B. Zhang, C. L. Zhang, Menghebilige, M. Yang, T. S. Sun, Q. H. Bao, W. Chen, and H. P. Zhang. 2012. A survey of the bacterial composition of *kurut* from Tibet using a culture-independent approach. *Journal of Dairy Science* 95 (3):1064–72. doi:10.3168/jds.2010-4119.

Liu, W., X. Xi, Q. Sudu, L. Kwok, Z. Guo, Q. Hou, B. Menhe, T. Sun, and H. Zhang. 2015. High-throughput sequencing reveals microbial community diversity of Tibetan naturally fermented yak milk.

Annals of Microbiology 65 (3):1741–51. doi:10.1007/s13213-014-1013-x.

Liu, W., Y. Zheng, L. Y. Kwok, Z. Sun, J. Zhang, Z. Guo, Q. Hou, B. Menhe, and H. Zhang. 2015. High-throughput sequencing for the detection of the bacterial and fungal diversity in Mongolian naturally fermented cow's milk in Russia. *BMC Microbiology* 15:45. doi:10.1186/s12866-015-0385-9.

Liu, W., M. Zhang, J. Xie, H. Wang, X. Zhao, B. Chen, and H. Suo. 2019. Comparative analyses of microbial community diversities of Tibetan Kefir grains from three geographic regions. *International Journal of Dairy Technology* 72 (4):536–44. doi:10.1111/1471-0307.12616.

Liu, Z., T. Z. Desantis, G. L. Andersen, and R. Knight. 2008. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Research* 36 (18):e120. doi:10.1093/nar/gkn491.

Lucchetti-Miganeh, C., D. Redelberger, G. Chambonnier, F. Rechenmann, S. Elsen, C. Bordi, K. Jeannot, I. Attrée, P. Plésiat, and S. de Bentzmann. 2014. *Pseudomonas aeruginosa* genome evolution in patients and under the hospital environment. *Pathogens (Basel, Switzerland)* 3 (2):309–40. doi:10.3390/pathogens3020309.

Luna, G. M., A. Dell'Anno, and R. Danovaro. 2006. DNA extraction procedure: A critical issue for bacterial diversity assessment in marine sediments. *Environmental Microbiology* 8 (2):308–20. doi:10.1111/j.1462-2920.2005.00896.x.

Magalhães, K. T., G. V. M. Pereira, C. R. Campos, G. Dragone, and R. F. Schwan. 2011. Brazilian kefir: Structure, microbial communities and chemical composition. *Brazilian Journal of Microbiology : [Publication of the Brazilian Society for Microbiology]* 42 (2):693–702. doi:10.1590/S1517-83822011000200034.

Magnusson, J., and J. Schnürer. 2001. *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and Environmental Microbiology* 67 (1):1–5. doi:10.1128/AEM.67.1.1.

Marsh, A. J., O. O'Sullivan, C. Hill, R. P. Ross, and P. D. Cotter. 2013. Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. *PLoS ONE* 8 (7):e69371 doi:10.1371/journal.pone.0069371.

Martin, N. H., A. Trmcic, T. H. Hsieh, K. J. Boor, and M. Wiedmann. 2016. The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology* 7:1549. doi:10.3389/fmicb.2016.01549.

Mayo, B., C. Rachid, A. Alegria, A. Leite, R. Peixoto, and S. Delgado. 2014. Impact of next generation sequencing techniques in food microbiology. Current Genomics 15 (4):293–309. doi:10.2174/1389202915666140616233211.

Meesilp, N., and N. Mesil. 2019. Effect of microbial sanitizers for reducing biofilm formation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on stainless steel by cultivation with UHT milk. *Food Science and Biotechnology* 28 (1):289–96. doi:10.1007/s10068-018-0448-4.

Meybodi, N. M., M. T. Ebdrahimi, and A. M. Mortazavian. 2016. Ethnic fermented foods and beverages of Iran. In *Ethnic fermented foods and alcoholic beverages of Asia*, ed. J. P. Tamang, 309–22. Gangtok: Springer India.

Miguel, M. G. C. P., P. G. Cardoso, L. de Assis Lago, and R. F. Schwan. 2010. Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods. *Food Research International* 43 (5):1523–8. doi:10.1016/j.foodres.2010.04.031.

Mitra, R. D., and G. M. Church. 1999. *In situ* localized amplification and contact replication of many individual DNA molecules. *Nucleic Acids Research* 27 (24):e34. doi:10.1093/nar/27.24.e34.

Mo, L., J. Yu, H. Jin, Q. Hou, C. Yao, D. Ren, X. An, T. Tsogtgerel, and H. Zhang. 2019. Investigating the bacterial microbiota of traditional fermented dairy products using propidium monoazide with single-molecule real-time sequencing. *Journal of Dairy Science* 102 (5):3912–23. doi:10.3168/jds.2018-15756.

Morishita, T., Y. Deguchi, M. Yajima, T. Sakurai, and T. Yura. 1981. Multiple nutritional requirements of lactobacilli: Genetic lesions affecting amino acid biosynthetic pathways. *Journal of Bacteriology* 148 (1):64–71. doi:10.1128/jb.148.1.64-71.1981.

Motato, K. E., C. Milani, M. Ventura, F. E. Valencia, P. Ruas-Madiedo, and S. Delgado. 2017. Bacterial diversity of the Colombian fermented milk "Suero Costeño" assessed by culturing and high-throughput sequencing and DGGE analysis of 16S rRNA gene amplicons . *Food Microbiology* 68:129–36. doi:10.1016/j.fm.2017.07.011.

Nahidul-Islam, S. M., T. Kuda, H. Takahashi, and B. Kimura. 2018. Bacterial and fungal microbiota in traditional Bangladeshi fermented milk products analysed by culture-dependent and culture-independent methods. *Food Research International (Ottawa, Ont.)* 111:431–7. doi:10.1016/j.foodres.2018.05.048.

Nalbantoglu, U., A. Cakar, H. Dogan, N. Abaci, D. Ustek, K. Sayood, and H. Can. 2014. Metagenomic analysis of the microbial community in kefir grains. *Food Microbiology* 41:42–51. doi:10.1016/j.fm.2014.01.014.

Nan, L., G. Ren, D. Wang, and K. Yang. 2016. Antibacterial performance of Cu-bearing stainless steel against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in whole milk. *Journal of Materials Science and Technology* 32 (5):445–51. doi:10.1016/j.jmst.2016.01.002.

Oberman, H., and Z. Libudzisz. 1998. Fermented Milks. In *Microbiology of fermented foods*, edited by Brian J. B. Wood, 308–50. Weinheim: Blackie Academic & Professional. doi:10.1016/B978-0-12-384947-2.00726-1.

Oki, K., J. Dugersuren, S. Demberel, and K. Watanabe. 2014. Pyrosequencing analysis of the microbial diversity of *airag, khoormog* and *tarag*, traditional fermented dairy products of Mongolia. *Bioscience of Microbiota, Food and Health* 33 (2):53–64. doi:10.12938/bmfh.33.53..

Oliveira, L. C., T. D. L. Saraiva, W. M. Silva, U. P. Pereira, B. C. Campos, L. J. Benevides, F. S. Rocha, H. C. P. Figueiredo, V. Azevedo, and S. C. Soares. 2017. Analyses of the probiotic property and stress resistance-related genes of *Lactococcus lactis* subsp. *lactis* NCDO 2118 through comparative genomics and *in vitro* assays. *PLoS ONE* 12 (4):e0175116. doi:10.1371/journal.pone.0175116.

Owusu-Kwarteng, J., F. Akabanda, P. Johansen, L. Jespersen, and D. S. Nielsen. 2017. *Nunu,* a West African fermented yogurt-like milk product. In *Yogurt in health and disease prevention*, ed. N. P. Shah, 275–83. Kidlington: Academic Press. doi:10.1016/B978-0-12-805134-4.00015-8..

Parker, M., S. Zobrist, C. Donahue, C. Edick, K. Mansen, M. Hassan Zade Nadjari, M. Heerikhuisen, W. Sybesma, D. Molenaar, A. M. Diallo, et al. 2018. Naturally fermented milk from Northern Senegal: Bacterial community composition and probiotic enrichment with *Lactobacillus rhamnosus*. *Frontiers in Microbiology* 9:2218. doi:10.3389/fmicb.2018.02218.

Pereira, G. V. M., D. P. Carvalho Neto, A. C. O. Junqueira, S. G. Karp, L. A. J. Letti, A. I. Magalhães Júnior, and C. R. Soccol. 2020. A review of selection criteria for starter culture development in the food fermentation industry. *Food Reviews International* 36 (2):135–67. doi:10.1080/87559129.2019.1630636.

Picoli, T., Peter, C. M. J. L. Zani, S. B. Waller, M. G. Lopes, K. N. Boesche, G. D. ´Á. Vargas, S. O. Hübner, and G. Fischer. 2017. Melittin and Its potential in the destruction and inhibition of the biofilm formation by *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* isolated from bovine milk. *Microbial Pathogenesis* 112:57–62. doi:10.1016/j.micpath.2017.09.046..

Pogačić, T., S. Šinko, Š. Zamberlin, and D. Samaržija. 2013. Microbiota of kefir grains. *Mljekarstvo* 63 (1):3–14.

Quigley, L., O. O'Sullivan, T. P. Beresford, R. Paul Ross, G. F. Fitzgerald, and P. D. Cotter. 2012. A Comparison of methods used to extract bacterial DNA from raw milk and raw milk cheese. *Journal of Applied Microbiology* 113 (1):96–105. doi:10.1111/j.1365-2672.2012.05294.x.

Quigley, L., O. O'Sullivan, C. Stanton, T. P. Beresford, R. P. Ross, G. F. Fitzgerald, and P. D. Cotter. 2013. The complex microbiota of raw milk. *FEMS Microbiology Reviews* 37 (5):664–98. doi:10.1111/1574-6976.12030.

Ray, B., and A. Bhunia. 2007. *Fundamental food microbiology*. 4th ed. Boca Raton: CRC Press.

Reis, J. A., A. T. Paula, S. N. Casarotti, and A. L. B. Penna. 2012. Lactic acid bacteria antimicrobial compounds: Characteristics and applications. *Food Engineering Reviews* 4 (2):124–40. doi:10.1007/s12393-012-9051-2.

Rezvani, F., F. Ardestani, and G. Najafpour. 2017. Growth kinetic models of five species of *lactobacilli* and lactose consumption in batch submerged culture. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology]* 48 (2):251–8. doi:10.1016/j.bjm.2016.12.007.

Ringø, E., R. Andersen, S. Sperstad, Z. Zhou, P. Ren, E. M. Breines, E. Hareide, G. J. Yttergård, K. Opsal, H. M. Johansen, et al. 2014. Bacterial community of *koumiss* from Mongolia investigated by culture and culture-independent methods. *Food Biotechnology* 28 (4):333–53. doi:10.1080/08905436.2014.964253.

Rothberg, J. M., W. Hinz, T. M. Rearick, J. Schultz, W. Mileski, M. Davey, J. H. Leamon, K. Johnson, M. J. Milgrew, M. Edwards, et al. 2011. An integrated semiconductor device enabling non-optical genome sequencing. *Nature* 475 (7356):348–52. doi:10.1038/nature10242.

Sader, H. S., M. Castanheira, R. E. Mendes, R. K. Flamm, D. J. Farrell, and R. N. Jones. 2015. Ceftazidime-avibactam activity against multidrug-resistant *Pseudomonas aeruginosa* isolated in U.S. Medical Centers in 2012 and 2013. *Antimicrobial Agents and Chemotherapy* 59 (6):3656–9. doi:10.1128/AAC.05024-14.

Salipante, S. J., T. Kawashima, C. Rosenthal, D. R. Hoogestraat, L. A. Cummings, D. J. Sengupta, T. T. Harkins, B. T. Cookson, and N. G. Hoffman. 2014. Performance comparison of Illumina and Ion Torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. *Applied and Environmental Microbiology* 80 (24):7583–91. doi:10.1128/AEM.02206-14.

Sasaki, M., B. W. Bosman, and P. S. T. Tan. 1995. Comparison of proteolytic activities in various lactobacilli. *The Journal of Dairy Research* 62 (4):601–10. doi:10.1017/S0022029900031332.

Savadogo, A., C. A. T. Ouattara, P. W. Savadogo, A. S. Ouattara, N. Barro, and A. S. Traore. 2004. Microorganisms involved in fulani traditional fermented milk in Burkina Faso. *Pakistan Journal of Nutrition* 3 (2):134–9.

Shangpliang, H. N. J., R. Rai, S. Keisam, K. Jeyaram, and J. P. Tamang. 2018. Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Scientific Reports* 8 (1):1532. doi:10.1038/s41598-018-19524-6..

Shangpliang, H. N. J., S. Sharma, R. Rai, and J. P. Tamang. 2017. Some technological properties of lactic acid bacteria isolated from *dahi* and *datshi*, naturally fermented milk products of Bhutan. *Frontiers in Microbiology* 8:116. doi:10.3389/fmicb.2017.00116.

Shrivastava, N., and L. Ananthanarayan. 2015. Use of the backslopping method for accelerated and nutritionally enriched idli fermentation. *Journal of the Science of Food and Agriculture* 95 (10):2081–7. doi:10.1002/jsfa.6923.

Silva, D. B. S., K. M. P. de Oliveira, and A. Grisolia. 2017. Molecular methods developed for the identification and characterization of *Candida* species. *International Journal of Genetic Science* 4 (1):1–6. doi:10.15226/2377-4274/4/1/00114.

Slattery, L., J. O'Callaghan, G. F. Fitzgerald, T. Beresford, and R. P. Ross. 2010. Invited review: *Lactobacillus helveticus*-a thermophilic dairy starter related to gut bacteria. *Journal of Dairy Science* 93 (10):4435–54. doi:10.3168/jds.2010-3327.

Sodini, I., P. Morin, A. Olabi, and R. Jiménez-Flores. 2006. Compositional and functional properties of buttermilk: A comparison between sweet, sour, and whey buttermilk. *Journal of Dairy Science* 89 (2):525–36. doi:10.3168/jds.S0022-0302(06)72115-4.

Song, A. A. L., Lionel, L. A. In, S. H. E. Lim, and R. A. Rahim. 2017. A review on *Lactococcus lactis*: From food to factory. *Microbial Cell Factories* 16 (1):55. doi:10.1186/s12934-017-0669-x..

Sun, Z., W. Liu, Q. Bao, J. Zhang, Q. Hou, L. Kwok, T. Sun, and H. Zhang. 2014. Investigation of bacterial and fungal diversity in *tarag* using high-throughput sequencing. *Journal of Dairy Science* 97 (10):6085–96. doi:10.3168/jds.2014-8360.

Sundquist, A., S. Bigdeli, R. Jalili, M. L. Druzin, S. Waller, K. M. Pullen, Y. Y. El-Sayed, M. M. Taslimi, S. Batzoglou, and M. Ronaghi. 2007. Bacterial flora-typing with targeted, chip-based pyrosequencing. *BMC Microbiology* 7 (1):108. doi:10.1186/1471-2180-7-108.

Tamang, J. P., N. Tamang, S. Thapa, S. Dewan, B. Tamang, H. Yonzan, A. K. Rai, R. Chettri, J. Chakrabarty, and N. Kharel. 2012. Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. *Indian Journal of Traditional Knowledge* 11 (1):7–25.

Tang, H., H. Ma, Q. Hou, W. Li, H. Xu, W. Liu, Z. Sun, H. Haobisi, and B. Menghe. 2020. Profiling of *koumiss* microbiota and organic acids and their effects on *koumiss* taste. *BMC Microbiology* 20 (1):85. doi:10.1186/s12866-020-01773-z.

Taş, T. K., F. Y. Ekinci, and Z. B. Guzel-Seydim. 2012. Identification of microbial flora in kefir grains produced in Turkey using PCR. *International Journal of Dairy Technology* 65 (1):126–31. doi:10.1111/j.1471-0307.2011.00733.x.

Tesfaye, A., T. Mehari, and M. Ashenafi. 2011. Inhibition of some food borne pathogens by pure and mixed LAB cultures during fermentation and storage of *ergo*, a traditional Ethiopian fermented milk. *Journal of Agricultural and Biological Science* 6 (4):13–9.

Thitaram, S. N., C. H. Chung, D. F. Day, A. Hinton, J. S. Bailey, and G. R. Siragusa. 2005. Isomaltooligosaccharide increases fecal *Bifidobacterium* population in young broiler chickens. *Poultry Science* 84 (7):998–1003. doi:10.1093/ps/84.7.998.

Trunk, T.,. H. S. Khalil, and J. C. Leo. 2018. Bacterial Autoaggregation. *AIMS Microbiology* 4 (1):140–64. doi:10.3934/microbiol.2018.1.140.

Uchida, K., M. Hirata, H. Motoshima, T. Urashima, and I. Arai. 2007. Microbiota of '*airag*', '*tarag*' and other kinds of fermented dairy products from nomad in Mongolia. *Animal Science Journal* 78 (6):650–8. doi:10.1111/j.1740-0929.2007.00486.x..

Uriot, O., S. Denis, M. Junjua, Y. Roussel, A. Dary-Mourot, and S. Blanquet-Diot. 2017. *Streptococcus thermophilus*: From yogurt starter to a new promising probiotic candidate? *Journal of Functional Foods* 37:74–89. doi:10.1016/j.jff.2017.07.038.

Ursell, L. K., J. L. Metcalf, L. W. Parfrey, and R. Knight. 2012. Defining the human microbiome. *Nutrition Reviews* 70 (Suppl. 1):S38–S44. doi:10.1111/j.1753-4887.2012.00493.x.

Van Wyk, J. 2019. Kefir: The champagne of fermented beverages. In *Fermented beverages*, ed. A. M. Grumezescu and A. M. Holban, 473–527. New York: Woodhead Publishing. doi:10.1016/b978-0-12-815271-3.00012-9.

Vardjan, T., P. Mohar Lorbeg, I. Rogelj, and A. Č. Majhenič. 2013. Characterization and stability of lactobacilli and yeast microbiota in kefir grains. *Journal of Dairy Science* 96 (5):2729–36. doi:10.3168/jds.2012-5829.

Vedamuthu, E. R. 1994. The dairy *Leuconostoc*: Use in dairy products. *Journal of Dairy Science* 77 (9):2725–37. doi:10.3168/jds.S0022-0302(94)77215-5.

Verce, M., L. De Vuyst, and S. Weckx. 2019. Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel *Oenococcus* species. *Frontiers in Microbiology* 10:479 doi:10.3389/fmicb.2019.00479.

Vieira, C. P., C. C. Cabral, B. R. C. da Costa Lima, V. M. F. Paschoalin, K. C. Leandro, and C. A. Conte-Junior. 2017. *Lactococcus lactis* ssp. *cremoris* MRS47, a potential probiotic strain isolated from kefir grains, increases cis-9, trans-11-CLA and PUFA contents in fermented milk. *Journal of Functional Foods* 31:172–8. Elsevier Ltd: doi:10.1016/j.jff.2017.01.047.

Walsh, A. M., F. Crispie, K. Daari, O. O'Sullivan, J. C. Martin, C. T. Arthur, M. J. Claesson, K. P. Scott, and P. D. Cotter. 2017. Strain-level metagenomic analysis of the fermented dairy beverage *Nunu*. *Applied and Environmental Microbiology* 83 (16):e01144–17. doi:10.1128/AEM.01144-17.

Walsh, A. M., F. Crispie, K. Kilcawley, O. O'Sullivan, M. G. O'Sullivan, M. J. Claesson, and P. D. Cotter. 2016. Microbial succession and flavor production in the fermented dairy beverage kefir. *Applied and*

Environmental Science 1 (5):e00052–16. doi:10.1128/mSystems.00052-16.Editor.

Wang, J. T., S. C. Chang, Y. C. Chen, and K. T. Luh. 2000. Comparison of antimicrobial suceptibility of *Citrobacter Freundii* in two different time periods. *Journal of Microbiology, Immunology and Infection* 33 (4):258–62.

Wang, S. Y., K. N. Chen, Y. M. Lo, M. L. Chiang, H. C. Chen, J. R. Liu, and M. J. Chen. 2012. Investigation of microorganisms involved in biosynthesis of the kefir grain. *Food Microbiology* 32 (2):274–85. doi:10.1016/j.fm.2012.07.001.

Wang, X., J. Xiao, Y. Jia, Y. Pan, and Y. Wang. 2018. *Lactobacillus kefiranofaciens*, the sole dominant and stable bacterial species, exhibits distinct morphotypes upon colonization in Tibetan kefir grains. *Heliyon* 4 (6):e00649. doi:10.1016/j.heliyon.2018.e00649.

Wang, Y., Z. Ahmed, W. Feng, C. Li, and S. Song. 2008. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. *International Journal of Biological Macromolecules* 43 (3):283–8. doi:10.1016/j.ijbiomac.2008.06.011.

Wang, Y.-Y., H.-R. Li, S.-F. Jia, Z.-J. Wu, and B.-H. Guo. 2006. Analysis of bacterial diversity of kefir grains by denaturing gradient gel electrophoresis and 16S rDNA sequencing. *Wei Sheng wu Xue Bao = Acta Microbiologica Sinica* 46 (2):310–3.

Wels, M., R. Siezen, S. van Hijum, W. J. Kelly, and H. Bachmann. 2019. Comparative genome analysis of *Lactococcus lactis* indicates niche adaptation and resolves genotype/phenotype disparity. *Frontiers in Microbiology* 10:4 doi:10.3389/fmicb.2019.00004.

Wen, C., L. Wu, Y. Qin, J. D. Van Nostrand, D. Ning, B. Sun, K. Xue, F. Liu, Y. Deng, Y. Liang, et al. 2017. Evaluation of the reproducibility of amplicon sequencing with Illumina MiSeq platform. *PLoS One* 12 (4):e0176716. doi:10.1371/journal.pone.0176716.

Williams, R., S. G. Peisajovich, O. J. Miller, S. Magdassi, D. S. Tawfik, and A. D. Griffiths. 2006. Amplification of complex gene libraries by emulsion PCR. *Nature Methods* 3 (7):545–50. doi:10.1038/nmeth896.

Witthuhn, R. C., T. Schoeman, and T. J. Britz. 2004. Isolation and characterization of the microbial population of different South African kefir grains. *International Journal of Dairy Technology* 57 (1):33–7. doi:10.1111/j.1471-0307.2004.00126.x..

Wu, X. H., Z. Luo, L. Yu, F. Z. Ren, B. Z. Han, and M. J. R. Nout. 2009. A survey on composition and microbiota of fresh and fermented yak milk at different Tibetan altitudes. *Dairy Science and Technology* 89 (2):201–9. doi:10.1051/dst/2009007.

Wulijideligen, T. A., K. Hara, K. Arakawa, H. Nakano, and T. Miyamoto. 2012. Production of bacteriocin by *Leuconostoc mesenteroides* 406 isolated from Mongolian fermented mare's milk, *airag*. *Animal Science Journal* 83 (10):704–11. doi:10.1111/j.1740-0929.2012.01010.x.

Wurihan, L. B., Hasigaowa, X. Bao, Y. Dai, and S. Jia. 2019. Bacterial community succession and metabolite changes during the fermentation of *koumiss*, a traditional Mongolian fermented beverage. *International Dairy Journal* 98:1–8. doi:10.1016/j.idairyj.2019.06.013.

Xia, Y., J. Sun, and D.-G. Chen. 2018. *Statistical analysis of microbiome data with R*. Singapore: Springer Nature.

Xing, Z., W. Geng, C. Li, Y. Sun, and Y. Wang. 2017. Comparative genomics of *Lactobacillus kefiranofaciens* ZW3 and related members of *Lactobacillus*. spp reveal adaptations to dairy and gut environments. *Scientific Reports* 7 (1):12827. doi:10.1038/s41598-017-12916-0.

Xu, Z., J. Xie, T. Soteyome, B. M. Peters, M. E. Shirtliff, J. Liu, and J. M. Harro. 2019. Polymicrobial interaction and biofilms between *Staphylococcus aureus* and *Pseudomonas aeruginosa*: An underestimated concern in food safety. *Current Opinion in Food Science* 26:57–64. doi:10.1016/j.cofs.2019.03.006.

Yang, C., Y. Zhang, F. Hou, J. P. Millner, Z. Wang, and S. Chang. 2019. Grazing activity increases decomposition of yak dung and litter in an alpine meadow on the Qinghai-Tibet plateau. *Plant and Soil* 444 (1-2):239–50. doi:10.1007/s11104-019-04272-x.

Yang, M., A. Cousineau, X. Liu, Y. Luo, D. Sun, S. Li, T. Gu, L. Sun, H. Dillow, J. Lepine, et al. 2020. Direct metatranscriptome RNA-Seq and multiplex RT-PCR amplicon sequencing on Nanopore MinION - Promising Strategies for Multiplex Identification of Viable Pathogens in Food. *Frontiers in Microbiology* 11:514. doi:10.1101/700674.

Yao, G., J. Yu, Q. Hou, W. Hui, W. Liu, L. Y. Kwok, B. Menghe, T. Sun, H. Zhang, and W. Zhang. 2017. A perspective study of *koumiss* microbiome by metagenomics analysis based on single-cell amplification technique. *Frontiers in Microbiology* 8:165. doi:10.3389/fmicb.2017.00165.

Yasmin, N., S. Hameed, R. Javed, S. Ahmed, and M. Imran. 2017. Inactivation of foodborne pathogens on food packaging and in cow milk by exposure to a Nd:YAG laser. *Canadian Journal of Physics* 95 (7):662–9. doi:10.1139/cjp-2016-0676.

Yu, J., W. H. Wang, B. L. G. Menghe, M. T. Jiri, H. M. Wang, W. J. Liu, Q. H. Bao, Q. Lu, J. C. Zhang, F. Wang, et al. 2011. Diversity of lactic acid bacteria associated with traditional fermented dairy products in Mongolia. *Journal of Dairy Science* 94 (7):3229–41. doi:10.3168/jds.2010-3727.

Zamberi, N. R., N. E. Mohamad, S. K. Yeap, H. Ky, B. K. Beh, W. C. Liew, S. W. Tan, W. Y. Ho, S. Y. Boo, Y. H. Chua, et al. 2016. 16S metagenomic microbial composition analysis of kefir grain using MEGAN and BaseSpace. *Food Biotechnology* 30 (3):219–30. doi:10.1080/08905436.2016.1200987.

Zhong, Z.,. Q. Hou, L. Kwok, Z. Yu, Y. Zheng, Z. Sun, B. Menghe, and H. Zhang. 2016. Bacterial microbiota compositions of naturally fermented milk are shaped by both geographic origin and sample type. *Journal of Dairy Science* 99 (10):7832–41. doi:10.3168/jds.2015-10825.