UNIVERSIDADE FEDERAL DO PARANÁ

BRUNA LEAL MASKE

EFFICIENCY OF SPONTANEOUS LACTIC ACID FERMENTATION TO IMPROVE THE QUALITY OF RAW MILK CONTAINING HIGH LEVELS OF *Pseudomonas* CONTAMINATION: AN ALERT FOR SANITARY MEASURES

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Dissertação apresentada ao curso de Pós-Graduação em Engenharia de Bioprocessos e Biotecnologia, Setor de Tecnologia, Universidade Federal do Paraná, como requisito parcial à obtenção do título de mestre em Engenharia de Bioprocessos e Biotecnologia.

Orientador: Prof. Dr. Gilberto Vinícius de Melo Pereira

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Tenho felicidade o bastante para fazê-la doce,

dificuldades para fazê-la forte,

tristeza para fazê-la humana e esperança suficiente para fazê-la feliz.

As pessoas mais felizes não têm as melhores coisas, elas sabem fazer o melhor das oportunidades que aparecem em seus caminhos”

Clarice Lispector

RESUMO

Produtos lácteos fermentados têm sido um componente vital na dieta de grupos étnicos de todo o mundo desde tempos remotos. Nos dias atuais, a popularidade destes produtos tem aumentado devido às suas propriedades funcionais e ao aumento do tempo de vida de prateleira. Estes processos são realizados em sistemas abertos e baseiam-se na microbiota indígena presente na matéria-prima ou do inóculo originado pelo processo chamado *backslooping*. No primeiro capítulo deste trabalho, foi construída uma revisão crítica sobre o surgimento de tecnologias de sequenciamento de nova geração (SNG) e os impactos na caracterização microbiológica de produtos lácteos fermentados tradicionais. Os produtos abrangidos nesta revisão incluíram kefir, leitelho, *koumiss, dahi, kurut, airag, tarag, khoormog, lait caillé* e *suero costeño*. Grupos dominantes detectados por SNG, principalmente bactérias do ácido lático, também foram identificados por técnicas anteriores de caracterização microbiológica. No entanto, as plataformas de SNG revelaram a diversidade bacteriana total, incluindo microrganismos não cultiváveis, populações sub-dominantes e espécies de crescimento tardio. Os conhecimentos adquiridos são vitais para melhorar a monitorização, manipulação e segurança destes alimentos fermentados. Na segunda fase deste trabalho, foi avaliado o desempenho da fermentação espontânea de iogurte do tipo Mar Cáspio para melhorar a qualidade de leite pasteurizado contendo altos níveis de contaminação por *Pseudomonas*, com foco na segurança microbiológica e estabilidade do produto final. A diversidade bacteriana do leite pasteurizado, processo de fermentação e após 60 dias de armazenamento foi analisada por SNG e a presença de células viáveis foi confirmada através de cultivos em meios seletivos. Além disso, os metabólitos produzidos durante o processo fermentativo foram analisados por cromatografia líquida de alta performance e cromatografia gasosa com espectrometria de massa. Inicialmente, leites pasteurizados contendo altas contagens de *Pseudomonas* foram selecionados para a fermentação. Durante o processo fermentativo, sequências relacionadas com *Pseudomonas*, e em menor grau para Enterobacteriaceae, permaneceram constantes até o fim do processo. A abordagem dependente de cultivo confirmou a presença de *Pseudomonas* viáveis durante a fermentação. Acido lático foi o principal metabólito produzido (5.93 g/L em 24 horas de fermentação) e, entre os compostos voláteis encontrados, ácido benzóico e ácido hexanóico apresentaram aumentos significativos durante a fermentação. 2-nonanona foi também observado em concentrações significativas, o qual é considerado um biomarcador volátil de *P. aeruginosa* e espécies correlacionadas. Os resultados demonstraram que fermentações naturais podem frequentemente não inibir o desenvolvimento de agentes patogênicos e deteriorantes de origem alimentar. Finalmente, a terceira etapa deste estudo avaliou diferentes cepas de bactérias ácido-láticas (obtidas a partir do leite fermentado no estilo do mar Cáspio, mantido a 4ºC durante 60 dias) contra *Pseudomonas aeruginosa*. Isolados dos gêneros *Leuconostoc* e *Lactobacillus* apresentaram halos de inibição significativos, indicando que o produto é uma fonte promissora para isolamento de bactérias do ácido lático com atividade antimicrobiana.

Palavras-chave: Leites fermentados; sequenciamento de nova geração; diversidade microbiana; microrganismos de origem alimentar; bactérias lácticas; segurança alimentar; probiótico.

ABSTRACT

Traditional fermented milk products have been a vital component in the daily diet of ethnic groups all around the world since ancient times. Today, the popularity and availability of these products have been increased due to their functional properties and prolonged shelf-life. The fermentation process is performed in open systems and it is based on the indigenous microbiota present in the raw material or on the inoculum, originated from backslooping process. In the first chapter of this work, a critical review on the application of NGS for microbiome analysis of traditional fermented milk products worldwide was constructed. Fermented milk products covered in this review include kefir, buttermilk, *koumiss, dahi, kurut, airag, tarag, khoormog, lait caillé*, and *suero costeño*. In general, dominant species detected by culturing were also identified by NGS. However, NGS studies have revealed a more complex bacterial diversity, comprising uncultivable microorganisms, sub-dominant populations, and late-growing species. The knowledge that has been gained is vital in improving the monitoring, manipulation, and safety of these traditional fermented foods. In the second stage of this work, the performance of Caspian Sea-style spontaneous milk fermentation to improve the quality of pasteurized milk containing high levels of *Pseudomonas* contamination was evaluated, with a focus on microbiological safety and stability of the final product. Bacterial diversity of pasteurized milk, fermentation process, and after 60 days of storage was analyzed by NGS and the presence of viable taxa was confirmed by culturing on selective media. Microbial-derived metabolites were also analyzed by high–performance liquid chromatography and gas chromatography–mass spectrometry. Initially, pasteurized milks containing high *Pseudomonas* counts were selected for fermentation. Sequences related to *Pseudomonas*, and to a lesser extent to Enterobacteriaceae, remained constant throughout the fermentation process and the culture-dependent approach confirmed the presence of viable *Pseudomonas* through fermentation. Lactic acid (5.93 g/L in 24 h) was the major end-metabolite produced. Among the volatile compounds found, benzoic and hexanoic acids showed significant increase during fermentation in addition to 2-nonanone, a volatile biomarker of *P. aeruginosa* and related species. The results demonstrated that natural milk fermentation may often not inhibit the development of foodborne pathogens and food spoilage microorganisms. Finally, the third stage of this work evaluated lactic acid bacteria strains (isolated from Caspian Sea-style sample kept at 4ºC for 60 days) against *Pseudomonas aeruginosa*. *Leuconostoc* and *Lactobacillus* isolates showed a significant inhibition halo, suggesting that the product is a promising source for isolation of lactic acid bacteria with antimicrobial activity.

Keywords: Fermented milks; next generation sequencing; microbial diversity; food-borne microorganisms; lactic acid bacteria; food safety; probiotic.

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SUMMARY

1. INTRODUCTION

Fermentation practice was an important step for prehistoric farmers to extend the lifetime of milk (Carrer et al., 2016). These products were produced spontaneously by the action of indigenous microorganisms present in the raw milk of different animals or from the environment. The spontaneous fermentations were then replaced by the backslopping technique, which involves inoculating milk in a small amount of previous successful fermentation (Shrivastava and Ananthanarayan 2015). This procedure naturally selects well adapted microorganisms, reducing the fermentation time and increasing the quality of the product. To date, milks produced by natural fermentation are part of the diet of several cultures, including caspian yogurt, kefir, *koumiss, dahi*, 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; Motato et al. 2017; Uchida et al. 2007). The popularity and availability of these products has increased due to their functional properties, being a vital component not just in traditional ethnic groups but also in modern life (Panesar, 2011; Grandos Conde et al., 2013; Singh and Shah, 2017; Granato et al., 2010).

Fermented milks have a rich microbiota, composed predominantly of lactic acid bacteria (LAB). Although many of these microorganisms are beneficial, these traditional products are highly susceptible to contamination with deteriorating and pathogenic bacteria due to the conduction of fermentation in open systems and the poor microbiological quality of the raw material (Capozzi et al., 2017). *Pseudomonas* spp., for example, is commonly found in milk and, because of its high metabolic versatility, is a relevant contaminant. Besides causing defects in texture, odor and taste, this group harbors members with pathogenic potential (Scatamburlo et al., 2015; Chen et al., 2011). Microbiological characterization and control are, therefore, of extreme necessity for the safety in the consumption of these products.

However, for a long time, microbiological characterization of fermented milks was performed using culture-dependent methods, mainly based on morphological and biochemical characteristics, accessing only dominant groups. Therefore, contaminating microrganisms present at low levels were not detected. The advancement of molecular technologies enabled the use of the ribosomal RNA region (rDNA amplicons) for

microbiota characterization (Ray and Bhunia 2007; De Melo Pereira et al. 2019). The improvement of studies resulted in independent cultivation methodologies to overcome limitations of previous methods. Today, high-throughput sequencing (HTS) technologies are available in the era of next generation sequencing (NGS), which has revolutionized the way of investigating the microbial diversity of traditional fermented milks. Roche's 454 pyrosequencing platforms, Life Sciences' SOLiD/Ion Torrent PGM, and Illumina's Genome Analyzer/HiSeq 2000/MiSeq permited the discovery of several non-cultivable microorganisms, sub-dominant populations, and late-growing species in several fermented milks (Humblot and Guyot, 2009; Serafini et al., 2014; Dertli and Çon, 2017). To date, Caspian Sea yoghurt has not yet been analyzed by NGS.

This study aimed to evaluate the microbial safety of the Caspian Sea yogurt inoculum circulated in Brazil and its efficiency to improve the quality of pasteurized milk containing high levels of *Pseudomonas* used as substrate, by characterizing the fermentation dynamic process using dependent and independent cultivation methodologies.

2. OBJECTIVES

2.1 Main objective

The present study aimed to evaluate the efficiency of Caspian Sea yogurt inoculum circulated in Brazil to improve the quality of pasteurized milk containing high levels of *Pseudomonas* used as substrate, and characterize the diversity, composition and dynamics of the fermentation process.

2.2 Secondary objectives

- Build a literature review addressing the importance of next generation sequencing in the elucidation of traditional fermented milks microbiota;
- Characterize the microbiota of Caspian Sea style fermented milk during fermentation process, substrate (pasteurized milk) and fermented milk after 60 days of storage in low temperatures through Illumina Miseq platform;
- Confirm the presence of viable cells by culture-dependent method in selected medium;
- Determine substrate consumption, production of organic acids and volatile compounds during Caspian Sea style milk fermentation;
- Evaluate the antimicrobial activity of presumptive strains of lactic acid bacteria isolated from Caspian Sea style fermented milk stored for 60 days at low temperatures.

CHAPTER ONE (LITERATURE REVIEW) – AN UPDATE 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.

1.1 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, mohi, chhurpi, 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; 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. 2019). 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 fastidious microorganisms may be unable to grow *in vitro* by the difficulty in simulating the natural habitat conditions (Gatti et al. 2008). 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 A1.1). 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).

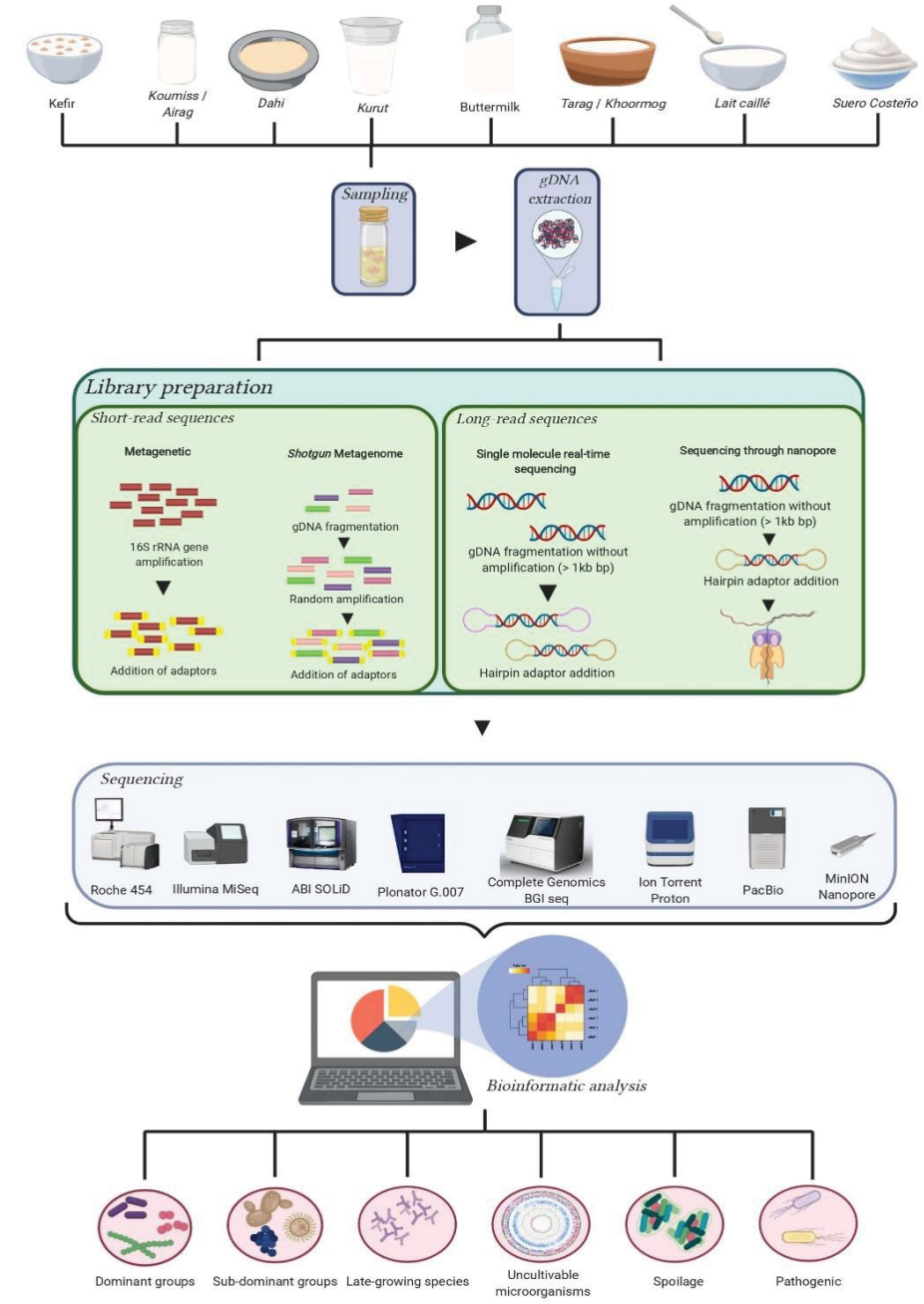
The emergence of next-generation DNA sequencing (NGS) methodology, and the first application of the pyrosequencing platform in kefir samples from Ireland (Dobson et al., 2011), produced exceedingly high numbers of DNA sequences and allowed an in-depth characterization of the microbial constituents of 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 400–600 operational taxonomic units (OTUs), represented by Proteobacteria, Bacteroides, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, Deinococcus-Thermus, TM7, and Spirochaetes (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.

1.2 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 1.1 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.

FIGURE 1.1 - SCHEMATIC WORKFLOW FOR THE IMPLEMENTATION OF NGS-BASED STUDIES IN FERMENTED MILK MICROBIOME ANALYSIS.



1.2.1 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 ºC (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).

1.2.2 DNA extraction

Following sampling, a variety of DNA extraction methods has been utilized to isolate DNA from fermented milk samples (TABLE 1.1); 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 1.1), 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 1.1). 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; 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 2016).

TABLE 1.1 LIST OF DNA EXTRACTION METHODS USED IN NGS FERMENTED MILK STUDIES.

Cell lysis principles	Lysis agents	Sample	Reference
Chemical Physical Enzymatic	50 mM EDTA, 0.1 M NaCl, 10 mM Tris-HCI, 25 mM sucrose, 20% SDS Three freeze-thawing cycles 30 mg/mL lysozyme, 5000 µL/mL mutanolysin, 10 mg/mL proteinase K, 10 mg/mL RNAase	Kefir grains	(Nalbantoglu et al. 2014)
Chemical Physical	CTAB, 1.4 M NaCl, ß-mercaptoethanol Heating at 60 ºC	Kefir grains	(Zamberi et al. 2016)
Chemical Enzymatic	20 mM Tris-HCl, 2 mM sodium EDTA, 1.2% Triton-100 50 U lyticase, 20 mg/mL lysozyme, 20 mg/mL proteinase K	Kefir grains	(X. Wang et al. 2018)
Physical Chemical	Heating at 90 ºC Genematrix food-extract DNA purification kit (Eurx, Poland)	Kefir grain	(Dertli and Çon 2017)
Chemical	DNeasy Blood & Tissue Kits Print	Kefir grain	(Korsak et al. 2015)
Chemical Physical Enzymatic	Power Food® Microbial DNA Isolation Kit (Mo Bio, USA) Qiagen TissueLyser II 100 U/mL mutanolysin, 50 mg/mL, lysozyme, 250 mg/mL proteinase K	Kefir milk	(Marsh et al. 2013)
Automated	NucliSENS easyMAG system (BioMérieux, France)	Kefir milk	(D. H. Kim, Kim, and Seo 2020)
Chemical Physical	PowerSoil DNA isolation kit (Mo Bio)	Kefir milk	(Hong et al. 2019)
Chemical	Power Food® Microbial DNA Isolation Kit (Mo Bio)	Kefir grains and milk	(W. Gao and Zhang 2019)
Enzymatic Physical	50 mg/mL lysozyme, 100 U/mL mutanolysin, proteinase K PowerBead tube on the TissueLyser II	Kefir grains and milk	(Walsh et al. 2016)

Enzymatic Chemical	50 U lysozyme, 25 U mutanolysin, 20 U lyticase, 25 mg/mL proteinase-K 50 mM Tris, 1 mM EDTA, 8.7% sucrose	*Dahi*	(Shangpliang et al. 2018)
Chemical Mechanical	10% SDS, 5 M NaCl, CTAB/NaCl glass beads (Mini-Beadbeater-8)	*Kurut*	(Wenjun Liu, Zheng, et al. 2015)
Physical	FastDNA® Spin Kit for Soil (MP Biomedicals, USA)	*Kurut*	(Jiang et al. 2020)
Chemical Enzymatic	Tris-EDTA, 10% SDS 20 mg/mL lysozyme, 20 mg/mL proteinase K	Buttermilk	(Jayashree et al. 2013)
Chemical Mechanical	100 mM Tris–HCl, 40 mM EDTA, benzyl chloride, 10% SDS glass beads (FastPrep FP120)	*Airag, Khoormog* and, *Tarag*	(Oki et al. 2014)
Chemical Physical	0.5 M EDTA five freeze-thawing cycles	*Nunu*	(Walsh et al. 2017)
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	(Wenjun Liu, Zheng, et al. 2015)b
Physical Chemical	Frozen in liquid nitrogen, thawed in a water bath at 60°C 10% SDS, 10 mg /mL proteinase K	*Kurut*	(W. J. Liu et al. 2012)
Not mentioned	Qiagen DNA Stool Mini Kit*	*Suero costeño*	(Motato et al. 2017)

* - Out-of-circulation extraction kit

1.2.3 Library preparation

Library construction prepares DNA into a form that is compatible with the sequencing system to be used (FIGURE 1.1). 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 non-specific 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 (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 Grisolia 2016). The choice of the primers for targeting the 16S rDNA region is essential to the success of analysis (Ercolini 2013). TABLE 1.2 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 1.2). 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 (K. H. Kim and Bae 2011).

TABLE 1.2 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	(Wenwen 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	(W. Gao and Zhang 2019)
Kefir grains	V1-V2	Illumina MiSeq	RDP	Genus/species	(X. 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	(D. H. Kim, Kim, and Seo 2020)
Dahi	V4–V5	Illumina MiSeq	Silva	Genus/species	(Shangpliang et al. 2018)
Kurut	V1–V3	Pyrosequencing	RDP	Genus	(Wenjun 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	(Wenjun 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)

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 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 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).

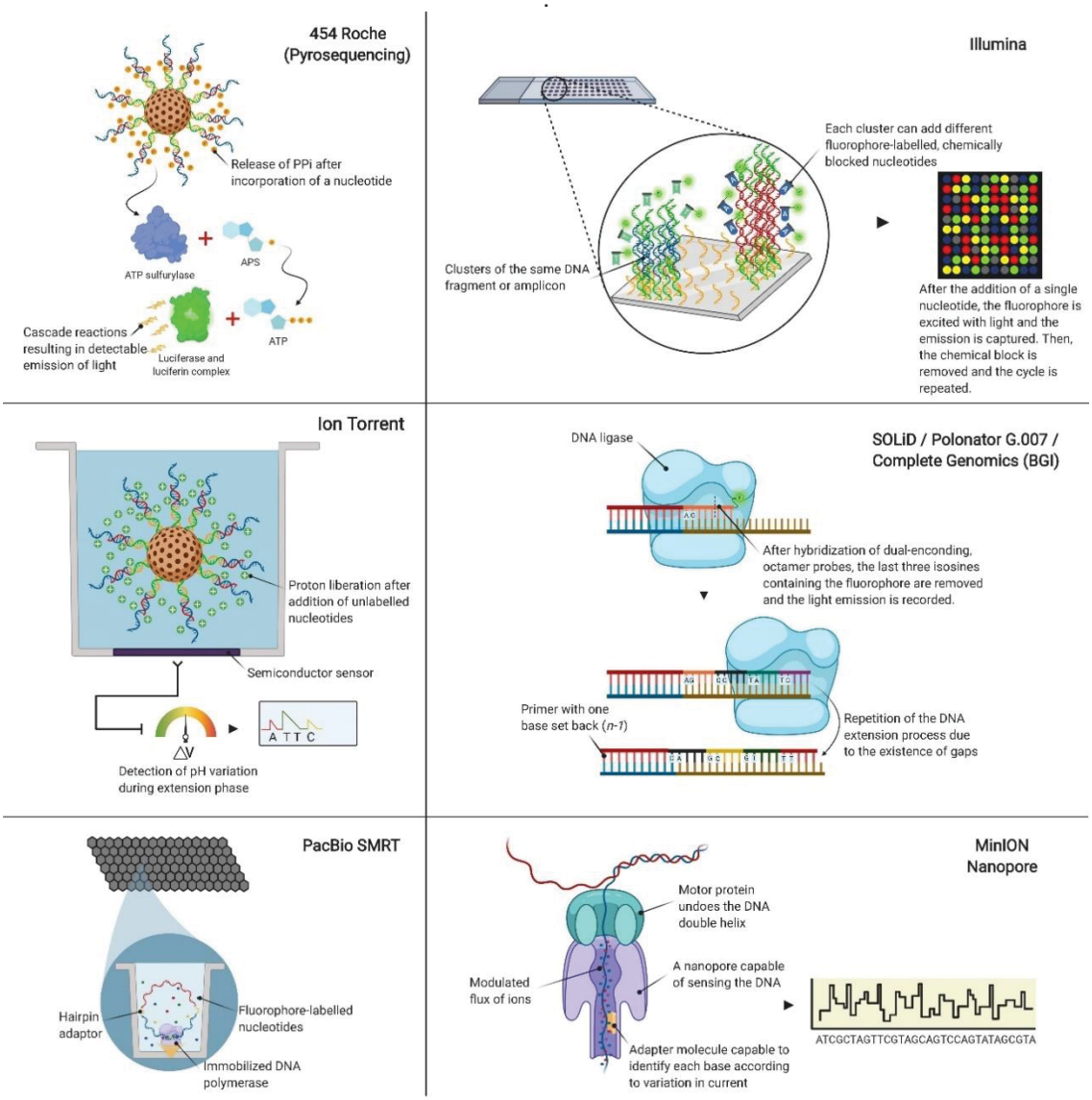
1.2.4 Sequencing

The sequencing technologies can be categorized according to fragment length read, namely short-read (35 ~700 bp) and long-read (> 1 kb) (FIGURE 1.1). 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 1.2). 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 1.2). 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).

FIGURE 1.2 SCHEMATIC REPRESENTATION OF THE DIFFERENT NGS PLATFORMS USED IN MICROBIOME STUDIES.



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 1.2). 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 1.2). 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 1.1). 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 1.2). 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 1.2). 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).

1.2.5 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).

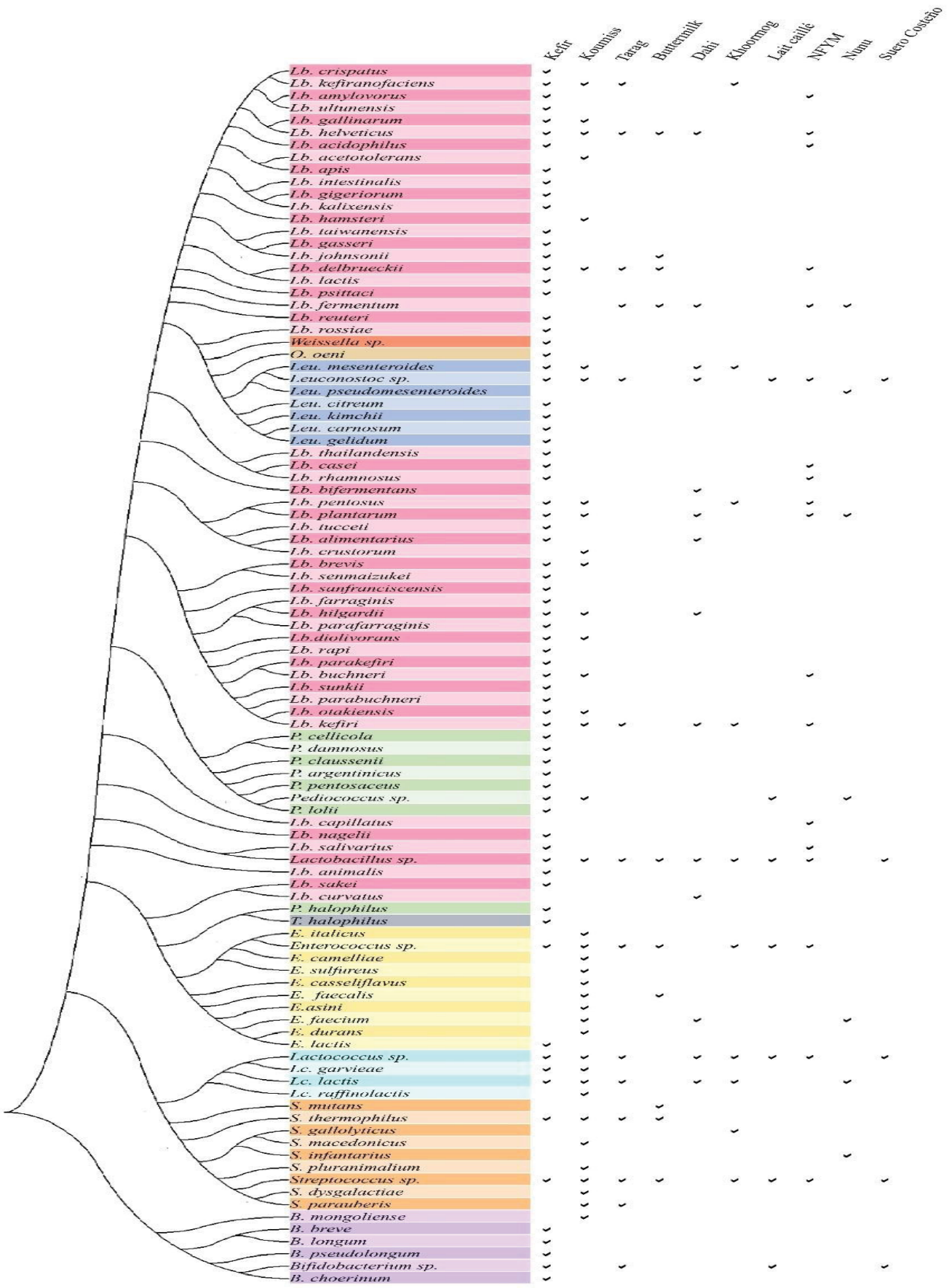
There is no perfect database choice, since each has its protocols, taxonomic coverage, and particularities. For instance, SILVA, RDP, and Greengenes are commonly used with 16S analysis due to vast archaeal and bacterial data, while UNITE is better used with 18S and ITS analysis due to their high content 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.

1.3 BACTERIAL WORLD DIVERSITY IN FERMENTED MILKS

1.3.1 LAB diversity

LAB are largely predominant in milk fermentations comprising 91 species identified by NGS studies (FIGURE 1.3). 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 (Beermann and Hartung, 2016), 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 1.3). 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).

FIGURE 1.3 - 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).


Kefir
Kouniss
Taraq
Buttermilk
Dahi
Khoormog
Lait caillé
NFYM
Nunu
Suero Costeño
Lb. crispatus
Lb. kefiranofaciens
Lb. amylovorus
Lb. ultunensis
Lb. gallinarum
Lb. helveticus
Lb. acidophilus
Lb. acetotolerans
Lb. apis
Lb. intestinalis
Lb. gigeriorum
Lb. kalixensis
Lb. hamsteri
Lb. taiwanensis
Lb. gasseri
Lb. johnsonii
Lb. delbrueckii
Lb. lactis
Lb. psittaci
Lb. fermentum
Lb. reuteri
Lb. rossiae
Weissella sp.
O. oeni
Leu. mesenteroides
Leuconostoc sp.
Leu. pseudomesenteroides
Leu. citreum
Leu. kimchii
Leu. carnosum
Leu. gelidum
Lb. thailandensis
Lb. casei
Lb. rhamnosus
Lb. bifermentans
Lb. pentosus
Lb. plantarum
Lb. tucceti
Lb. alimentarius
Lb. crustorum
Lb. brevis
Lb. senmaizukei
Lb. sanfranciscensis
Lb. farraginis
Lb. hilgardii
Lb. parafarraginis
Lb.diolivorans
Lb. rapi
Lb. parakefiri
Lb. buchneri
Lb. sunkii
Lb. parabuchneri
Lb. otakiensis
Lb. kefiri
P. cellicola
P. dammosus
P. claussenii
P. argentinicus
P. pentosaceus
Pediococcus sp.
P. lolii
Lb. capillatus
Lb. nagelii
Lb. salivarius
Lactobacillus sp.
Lb. animalis
Lb. sakei
Lb. curvatus
P. halophilus
T. halophilus
E. italicus
Enterococcus sp.
E. camelliae
E. sulfureus
E. casseliflavus
E. faecalis
E.asini
E. faecium
E. durans
E. lactis
Lactococcus sp.
Lc. garvieae
Lc. lactis
Lc. raffinolactis
S. mutans
S. thermophilus
S. gallolyticus
S. macedonicus
S. infantarius
S. pluranimalium
Streptococcus sp.
S. dysgalactiae
S. parauberis
B. mongoliense
B. breve
B. longum
B. pseudolongum
Bifidobacterium sp.
B. choerinum

Leuconostc and *Streptococcus* are other common genera — with *Leu. mesenteroides* and *S. thermophilus* being the most common species (FIGURE 1.3). 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 s*uero costeño* (FIGURE 1.3). 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 1.3). 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 1.3). 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.

1.4 FERMENTED PRODUCTS

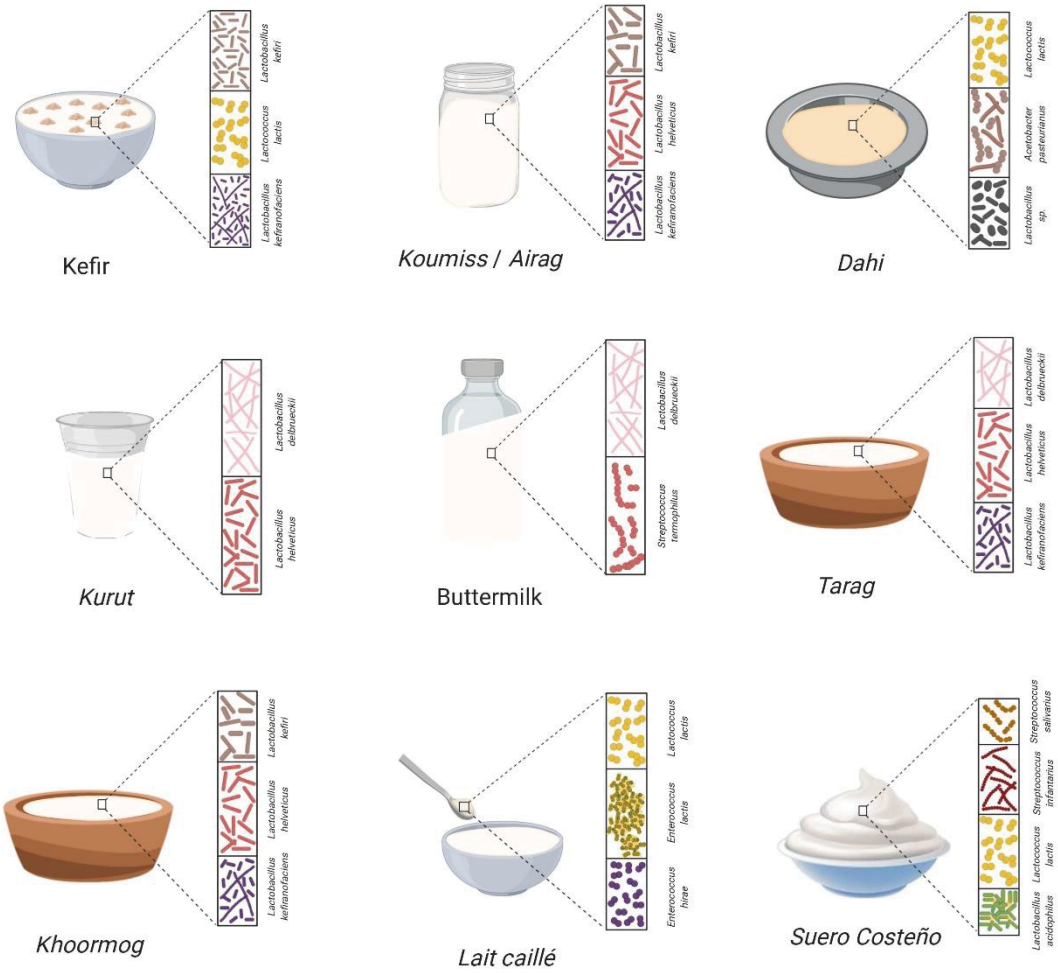
1.4.1 Kefir

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; Leite et al., 2013; Pogačić et al. 2013; Magalhães et al. 2011; Kesmen and Kacmaz 2011; Taş, Ekinci, and Guzel-Seydim 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 A1.2. 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 1.4). *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).

FIGURE 1.4 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).



Concerning kefir beverage, *Lactococcus lactis* has been detected as the dominant species in different geographical locations (FIGURE 1.4), 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 increases the viscosity of the kefir beverage (Irigoyen et al., 2005). 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 1.3). 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).

1.4.2 *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 backsloping 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 1.4). 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.

1.4.3 *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 pre-existent fermented *dahi* in fresh cow, yak, or buffalo's milk. The fermentation lasts 1 to 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 1.4). 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).

1.4.4 *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, Alyssa, et al. 2019). 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 1.4; 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.

1.4.5 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 ºC), 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 1.4). 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.

1.4.6 *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 1.4). Finally, several bacterial groups not previously isolated from *tarag* were identified by pyrosequencing, including *Acinetobacter*, *Klebsiella*, *Escherichia*, and *Salmonella* (Sun et al. 2014).

1.4.7 *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 1.4). 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).

1.4.8 *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 to 3 days (Savadogo et al. 2004; Parker et al. 2018). 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*.

1.4.9 *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 ºC), 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., 2017).

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. 2019). 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).

1.5 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 et al., 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 haemolytic 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; Martínez et al., 2013). 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).

1.6 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 sub-dominant 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.

CHAPTER TWO (RESEARCH RESULTS) – PRESENCE AND PERSISTENCE OF *Pseudomonas* spp. DURING CASPIAN SEA STYLE SPONTANEOUS MILK FERMENTATION HIGHLIGHTS THE IMPORTANCE OF SAFETY AND REGULATORY CONCERNS FOR TRADITIONAL AND ETHNIC FOODS

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ABSTRACT

The aim of this study was to evaluate the performance of Caspian Sea-style spontaneous milk fermentation to improve the quality of pasteurized milk containing high levels of *Pseudomonas* contamination, with a focus on microbiological safety and stability of the final product. Bacterial diversity of pasteurized milk, fermentation process, and after 60 days of storage was analyzed by Illumina-based sequencing, and presence of viable taxa was confirmed by culturing on selective media. Low quality pasteurized milk harbored mainly Gram-negative bacteria, markedly dominated by *Pseudomonas*. Following fermentation, lactic acid bacteria rapidly became dominant with maximum population of 10.15 log CFU/mL at 18 h, represented mainly by *Lactococcus*. However, sequences related to *Pseudomonas*, and to a lesser extent for enterobacteria, remained constant throughout the fermentation process. The culture-dependent approach confirmed the presence of viable *Pseudomonas*, with a final population of 5.60 log CFU/mL. Biochemical transformations were further analyzed, indicating lactic acid as the main end-metabolite produced (maximum concentration of 5.93 g/L at 24 h). In addition, the increase of 2-nonanone can be correlated as a volatile biomarker of *P. aeruginosa* and related species. Altogether, the results demonstrated that natural milk fermentation may often not inhibit the development of pathogens and food spoilage microorganisms.

Keywords: Fermented milk; food-borne microorganisms; lactic acid fermentation; food safety; probiotic.

2.1 INTRODUCTION

The emergence of dairying was a critical step in early agriculture, with considerable importance in the human diet (Panesar, 2011). As a rich nutritional source for microbial growth, prehistoric farmers used lactic acid fermentation to prolong the shelf life of milk (Carrer et al., 2016). The finding of abundant milk residues in pottery vessels from seventh- millennium sites from north-western Anatolia provided the earliest evidence of milk processing (Salque et al., 2013). Until now, fermented dairy 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).

The popularity and the availability of fermented dairy products (*e.g.,* kefir, *koumiss*, curd, *lassi*, *laben*, and *Suero costeño*) have been increased throughout the world due to their functional properties and prolonged shelf-life, given by the dynamics of the microbial community living there (Grandos Conde et al., 2013; Panesar, 2011; Singh & Shah, 2017). The fermentation is based on the indigenous microbiota present in the raw material or using part of a successful fermentation as back-slopping in order to ensure the dominance of the original microbiota (Capozzi et al., 2012; Pereira et al., 2019; Capozzi et al., 2020). Caspian Sea-style spontaneously fermented milk is widespread as a traditional product. It is usually produced by natural fermentation (12-24 h) of raw cow's milk at ambient temperature (approximately 25 ºC) (Kiryu et al., 2009). The finished product has a highly viscous consistency with a pleasant acid taste, due to the presence of *Lactococcus lactis* ssp. *cremoris* and, to a lesser extent, *Leuconostoc* sp., *Gluconobacter* sp., and *Acetobacter orientalis* (Ishida et al., 2005; Uchida et al., 2009). In Brazil, a similar fermented milk is produced by different families (private households) that believe the "mother inoculum" is originated from Caucasus region, so-called Caspian Sea-style fermented milk. However, there are no studies of this traditional product circulated in Brazil.

Although natural fermented milk products harbored various beneficial microorganisms, they are susceptible to contamination due to the conduct of fermentation in open systems and poor microbiological quality of the raw material (Fernández et al., 2015; Capozzi et al., 2017). *Pseudomonas* spp. is a relevant contaminant for fermented milk products (del Olmo et al., 2018; Reichler et al., 2018; Scatamburlo et al., 2015). Due to their high metabolic versatility, *Pseudomonas* are able to survive in different environments, such as food, soil, water, and air (Scatamburlo et

al., 2015). In raw milk, *Pseudomonas* are the dominant group due to their proteolytic activity (Ercolini et al., 2009). In addition, *Pseudomonas* have also been found in pasteurized milks due to post-pasteurization contamination or processing environment (Gennari & Dragotto, 1992; Reichler et al., 2018). Reichler et al. (2018) identified the contaminating bacteria of pasteurized milk samples from 10 facilities across the northeastern United States. The study revealed that 76.5% of the spoiled samples were contaminated with *Pseudomonas* spp., and 8 out of 10 facilities showed repeated isolation of one or more *Pseudomonas* strains, suggesting a difficulty on controlling this microorganism in the supplying industries. Thus, the use of low-quality milk can affect the bacterial ecosystem through fermentation processes and compromise both quality and health- assurance of finalized products (Leitner et al., 2008; Motato et al., 2017).

The emergence of high-throughput sequencing (HTS) technologies has revolutionized the way to investigate microbial diversity of traditional fermentations. These recent platforms have enabled the discovery of several uncultivable microorganisms, sub-dominant populations, and late-growing species, overlooked by culture-based approaches. In the field of food microbial ecology, different HTS platforms have been used for community analysis, including 454 pyrosequencing from Roche, SOLiD/Ion Torrent PGM from Life Sciences, and Genome Analyzer/HiSeq 2000/MiSeq from Illumina (Dertli & Çon, 2017; Humblot & Guyot, 2009; Serafini et al., 2014). These platforms have been used to accurately detect, identify, and characterize foodborne pathogens without any culturing step (Jagadeesan et al., 2019; Leonard et al., 2015).

The aim of this study was to evaluate the performance of Caspian-style milk spontaneous fermentation using low quality milk as a raw material, with focus on microbiological safety and stability of the final product. Due to the high incidence and persistence of *Pseudomonas* found in this study, we applied culture-dependent methods to confirm the presence of viable taxa. In addition, biochemical transformation dynamics (sugar consumption and end-metabolite generation) were examined for better insight into microbial activity during the process.

2.2 MATERIAL AND METHODS

2.2.1 Fermentation and sampling

The sample of domestic fermented milk was obtained from a private household that traditionally produces Caspian Sea-style spontaneously fermented milk through back-slopping in Curitiba city, Paraná State, Brazil. 50 mL of the "mother" culture was inoculated into 450 mL pasteurized whole cow's milk and renewed daily in the same proportion (10 % vol/vol) at 25 ºC for 7 days. This was performed for microbial stabilization before experimental fermentation.

100 mL of resulting fermented milk ("mother" culture) was transferred in triplicate into 2-L Erlenmeyer flasks, containing 900 mL of industrially pasteurized milk of low microbiological quality, and incubated under static condition at 25 ºC for 24 h. The low- quality milk, purchased at a local Curitiba market, was selected after quantification of *Pseudomonas,* a common contaminant in pasteurized milk*,* on *Pseudomonas* F agar (PFA; Thermo Fisher Oxoid). The milk samples that reached plate counts above 20,000 CFU/mL were selected for the fermentation assay (Alles et al., 2018).

Samples (20 mL) of fermenting milk in triplicate were collected at intervals of 6 hours (0, 6, 12, 18, and 24 h) to perform microbiological and metabolite target analysis. At each sampling point, the pH was measured using a digital pH meter (LUCA-210 model, Requipal, Curitiba, PR, Brazil). The resulting fermented product was stored at 4 ºC for 60 days (storage stability) and submitted to both culture-dependent and-independent microbiological analyses.

2.2.2 Total DNA extraction and high-throughput sequencing

Samples of pasteurized milk, fermentation times, and storage stability were withdrawn to perform total genomic DNA extraction and metagenetic analysis. The extraction protocol was performed according Junqueira et al. (2019), with slight modifications. The cell pellets, obtained after centrifugation of each sample at 12,000 ×*g* for 1 min, were resuspended in 500 µL Tris-EDTA (pH 8.0), vortexed with 10 µL of lysozyme solution at 20 mg/mL (Sigma Aldrich, San Louis, MO, USA), and incubated at 30 °C for 60 min. Then, 50 µL of sodium dodecyl sulfate (10% w/v in distilled, deionized water) and 10 µL of proteinase K solution (20 mg/mL in deionized water; Sigma Aldrich) were added to the lysis solution, followed by incubation at 60 ºC during 60 min. 150 µL of phenol-chloroform (25:24; Sigma Aldrich) was added, homogenized

by inversion, and centrifuged at 12,000 ×*g* for 5 min. The supernatant was collected, and the DNA was precipitated with 3x (v/v) absolute ethanol. Pellets were washed with 80% ethanol, dried, and resuspended in ultrapure water. Extracted DNA quality was checked on a 0.8% (w/v) agarose gel and quantified with the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Twenty ng of the extracted DNA, containing complementary adaptors for Illumina platform, was amplified using degenerated primers for the hypervariable V4 region of 16S (515F and 806R) rRNA gene (Caporaso et al., 2012). Bar-coded amplicons were generated by PCR following conditions described by Junqueira et al. (2019). Samples were sequenced in the MiSeq platform using the 500 V2 kit, following standard Illumina protocols. Resulting sequences in FASTQ files were deposited in the NCBI Sequence Read Archive (SRA) repository with accession BioProject ID PRJNA592162.

2.2.3 Bioinformatic analyses

After sequencing, chimeric sequences detection, removal of noises from pre-cluster, and taxonomic attribution were performed using standard parameters of QIIME software package, version 1.9.0. Applying the UCLUST method (Edgar, 2010), sequences presenting identity above 97% were considered the same operational taxonomic units (OTUs**)** according to the SILVA database (Quast et al., 2013).

2.2.4 Microbial counts

Aliquots of 1 mL of each sample (pasteurized milk, fermentation times, and storage stability) were vortexed with 9 mL of 0.1% saline-peptone water (10^{-1} solution) and diluted serially. Total aerobic bacteria (TAB) were enumerated on Nutrient Agar medium (NA; Thermo Fisher Oxoid, Waltham, MA, USA), lactic acid bacteria (LAB) on De Man, Rogosa, and Sharpe Agar (MRS, Thermo Fisher Oxoid), and *Pseudomonas* on *Pseudomonas* F agar (PFA; Thermo Fisher Oxoid); all media containing 0.1% (w/v) nystatin (Sigma Aldrich, San Louis, MO, USA) for fungal growth inhibition. NA and MRS plates were incubated at 30 ºC for 24 h, and PFA plates were incubated at 37 ºC for 48 h. Subsequently, the numbers of cell- forming units (CFU) were recorded.

2.2.5 Substrates and metabolites

Lactose consumption and organic acids production were determined at intervals of 6 hours (0, 6, 12, 18, and 24 h) by high-performance liquid chromatography (HPLC) according to Junqueira et al. (2019), with slight modifications. Aliquots of 2 mL were centrifuged at 6000×*g* for 15 min and filtered through a 0.22 µm pore size hydrophilic Polyethersulfone (PES) membrane (Millipore Corp., Burlington, MA, USA). 100 µL of filtered samples were injected into the HPLC system, equipped with an Aminex HPX 87 H column (300 x 7.8 mm; Bio-Rad, Richmond, CA, USA), and a refractive index (RI) detector (HPG1362A; Hewlett- Packard Company, Palo Alto, CA, USA). The column was eluted in an isocratic mode with a mobile phase of 5 mM H_2SO_4 at 60 ºC, and a flow rate of 0.6 mL/min.

The extraction of volatile compounds was performed using a headspace (HS) vial coupled to a SPME fiber (CAR/PDMS df75 µm partially crosslinked, Supelco., Saint Louis, MO, USA). For each determination, 2 mL of sample was stored in a 20 mL HS vial, in triplicate. The SPME fiber was exposed for 30 min at 60 ºC. The compounds were thermally desorbed into the GC injection system gas phase (GC-MS TQ Series 8040 and 2010 Plus GC- MS; Shimadzu, Tokyo, Japan) at 260 ºC. The column oven temperature was maintained at 60 ºC for 10 min, followed by two heating ramps of 4 and 10 ºC/min until reaching the temperatures of 100 and 200 ºC, respectively. The compounds were separated on a column 95% PDMS/5% PHENYL (30 m x 0.25 mm x 0.25 mm film thickness). The GC was equipped with an HP 5972 mass selective detector (Hewlett Packard, Palo Alto, CA, USA). The compounds were identified by comparison to the mass spectra from library databases (Nist'98 and Wiley7n). For quantification, standard solutions of ethanol were prepared in different concentration levels (1, 10, 20, 50, 100 and 1000 µmol L^{-1}) and used to construct a calibration curve. The volatile compound concentrations were expressed as µmol L^{-1} of headspace, as ethanol equivalent.

2.2.6 Statistical analysis

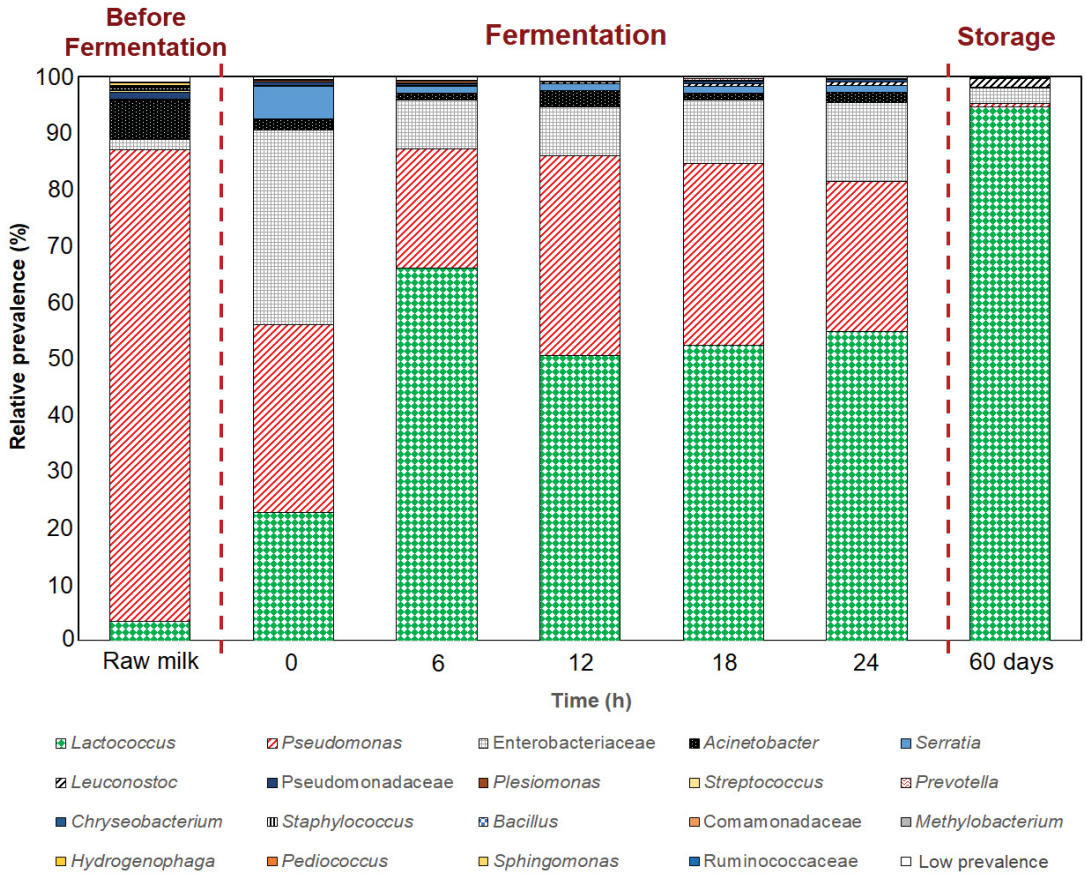
The data obtained of microbial count and target metabolites were analyzed by post-hoc comparison of means by Duncan's test. Statistical analyses were performed using the SAS program (Statistical Analysis System Cary, NC, USA). The level of significance was established in a two-sided *p*-value <0.05.

2.3 RESULTS AND DISCUSSION

2.3.1 Microbiological analysis

A total of 651,747 Illumina paired-end reads (average of 93,107 reads/sample) were obtained and clustered into 215 OTUs at 97% sequence similarity. Rarefaction curve analysis showed a trend to level-off at the genus level, indicating that the majority of bacterial communities were covered (Annexes FIGURE A2.1). Sequences were classified, using QIIME and SILVA database, to the lowest possible taxonomic rank (*i.e.,* genus level), and the results are represented in FIGURE 2.1. Selected low-quality pasteurized milk harbored mainly Gram-negative bacteria, markedly dominated by *Pseudomonas* (83.74%), and subpopulations of *Acinetobacter*, Enterobacteriaceae, *Sphingomona*s, *Staphylococcus,* and Comamonadaceae, while *Lactococcus* (3.40%) was the only Gram-positive bacteria found. Viable cell count of pasteurized milk on *Pseudomonas*-specific culture medium was 6.40 log CFU/mL, while LAB and TAB were not detected (FIGURE 2.2A). The milk used in this study was within the shelf life and stored under ideal conditions. Thus, it is possible to assume that failures occurred during the pasteurization process (Elmoslemany et al., 2010; Vidal et al., 2017; Martin et al., 2018; Russo et al., 2020). Recalls of pasteurized milk contaminated with spoilage bacteria are relatively frequent (Kumaresan & Villi, 2008; Quigley et al., 2013; Samet-Bali et al., 2013; Walsh et al., 2016;).

FIGURE 2.1 - RELATIVE BACTERIAL ABUNDANCE AND DYNAMICS DURING CASPIAN SEA-STYLE SPONTANEOUS MILK FERMENTATION PRODUCED WITH LOW QUALITY PASTEURIZED MILK. LOW PREVALENCE: BACTERIAL GROUPS WITH RELATIVE PREVALENCE ≤0.1%. THE COMPLETE LIST OF BACTERIA PRESENT AT A LOW PREVALENCE IS SHOWN IN THE SUPPLEMENTAL MATERIAL (TABLE A2.1).

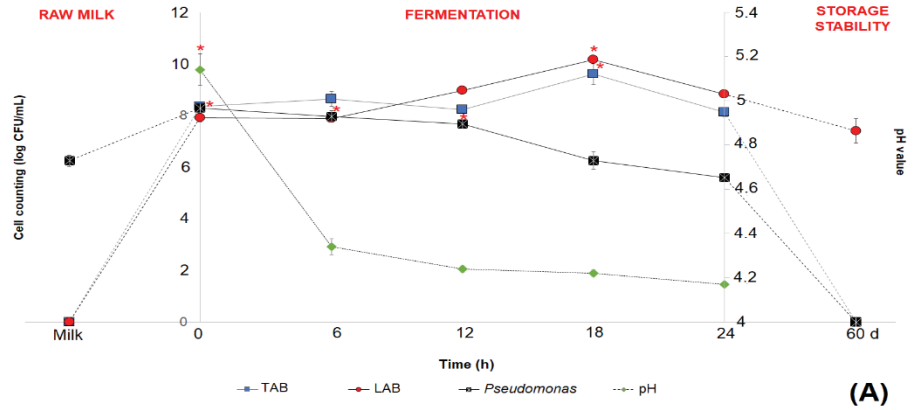


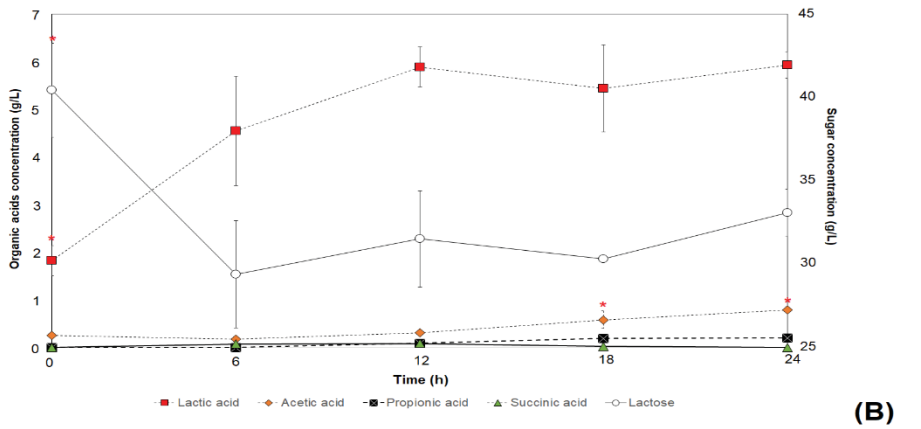
Following fermentation, the number of LAB was always higher, showing the maximum value of 10.15 log CFU/mL at 18 h (FIGURE 2.2A). Similarly, TAB increased through the fermentation process, reaching 9.60 log CFU/mL at 18 h. Illumina-based amplicon sequencing showed that LAB population was mainly represented by *Lactococcus*, reaching 66% among the common OTUs at 6h (FIGURE 2.1). Previous studies, using culture-dependent approaches, reported the dominance of *Lactococcus lactis* ssp. *cremoris* in Caspian Sea yogurt circulated in Japan, accompanied by species of *Leuconostoc*, *Lactobacillus*, *Gluconobacter* and *Acetobacter* (Kiryu et al., 2009; Uchida et al., 2009). *Lactococcus* dominance genus was confirmed in this study using Illumina-based amplicon sequencing. *Lactococcus* species have also been found as part of LAB members of other naturally fermented dairy products (Elortondo et al., 1998; Ferchichi et al., 2001; Fortina et al., 2003; López-Díaz et al., 2000). This microbial group has several important implications for fermentative process, including (i) milk acidification and casein proteolysis, (ii) metabolism of

amino acids and fatty acids for flavor development, and (iii) action against food-borne pathogens and spoilage bacteria (El-Ghaish et al., 2011; Matamoros et al., 2009).

In addition, a more complex bacterial diversity, uncovered by the previous traditional cultivation studies, was revealed. These includes *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Plesiomonas*, *Bacillus*, *Sphingomonas*, *Acinetobacter*, Comamonadaceae, Ruminococcaceae, *Serratia, Prevotella, Staphylococcus, Chryseobacterium, Hydrogenophaga, Methylobacterium* (FIGURE 2.1), and other 143 minor bacterial groups with relative prevalence ≤0.1% (Annexes TABLE A2.1). These findings indicate the need for the use of next-generation sequencing technologies for an in-deep knowledge of the microbial ecology of natural milk fermentation. 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.

FIGURE 2.2 - VIABLE CELL COUNT AND BIOCHEMICAL CHANGES DURING CASPIAN SEA-STYLE SPONTANEOUS MILK FERMENTATION PRODUCED WITH LOW QUALITY PASTEURIZED MILK. (A) ENUMERATION OF LACTIC ACID BACTERIA (LAB), TOTAL AEROBIC BACTERIA (TAB) AND *Pseudomonas*, AND pH MONITORING. (B) COURSE OF SUGAR CONSUMPTION AND ORGANIC ACIDS PRODUCTION. *ASTERISK* = SIGNIFICANTLY HIGHER FROM ONE ANOTHER IN A TWO-SIDED *P* VALUE < 0.05 ACCORDING TO DUNCAN'S TEST.





Even though *Pseudomonas* load decreased by more than 99% after 24h of fermentation (8.29 to 5.60 log CFU/mL; FIGURE 2.2A), the significant final population can compromise the quality of the fermented product since *Pseudomonas* are known to produce various enzymes (*e.g.,* lipases, proteases, and phospholipases) that lead to odor, flavor, and body defects (Chen et al., 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 & 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 haemolytic and proteolytic activities, show no difference with clinical *P. aeruginosa* (Allydice-Francis & 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 & Mesil, 2019; Nan et al., 2016; Picoli et al., 2017; Yasmin et al., 2017).

Sequences related to Enterobacteriaceae remained constant throughout the fermentation (FIGURE 2.1). The presence of Enterobacteriaceae indicates poor hygiene during the manufacturing process, and high numbers of enterobacteria have been linked to the accumulation of undesirable compounds with implications to flavor and texture defects in dairy products (Linares et al., 2012; Morales et al., 2003). Several studies showed the presence of enterobacteria in kefir grains (Dertli & Çon, 2017; Walsh et al., 2016; Wang et al., 2006) and in cheese samples (Saxer et al., 2013). Dertli and Çon (2017) showed that *Enterobacter* species can pass to the kefir grains from the milk, which should be assessed as they might create safety concerns. However, further studies using enterobacteria-selective culture media should be performed to confirm the presence of viable taxa through the Caspian Sea-style spontaneous milk fermentation. In addition, some Enterobacteriaceae genera could be not relevant as food-borne pathogens since many of them are plant commensal organisms (Jha et al., 2011).

There was a substantial discrepancy between viable cell count and Illumina-based amplicon sequencing; for example, *Pseudomonas* spp. accounted for more than 20% of the 16S rRNA gene sequences after 24h (FIGURE 2.1), while culture-dependent analysis demonstrated that these organisms account for less than 1% of viable bacterial cells (FIGURE 2.2A). This discrepancy is frequently observed in DNA-dependent analyses after contaminants that were present in the raw material are killed while their DNA is still amplified (Mayo et al., 2014). This overestimation of β-diversity in culture-independent analysis is recurrently observed in several studies (Martínez et al., 2013; Ursell et al., 2012; Wen et al., 2017).

Interestingly, after the storage process at 4 ºC for 60 days, LAB was present at 7.41 log CFU/mL and *Lactococcus* represented 90% of total OTUs by sequencing (FIGURE 2.1 and 2.2). On the other hand, *Pseudomonas* was no longer detected by plating and represented less than 5% of total OTUs (FIGURE 2.1 and 2.2), indicating amplification of reminiscent dead cells (Mayo et al., 2014). The metabolites formed during the fermentation process promotes the survivability of LAB, maintaining their viability over storage time. It is widely known that refrigerated storage is a key point in LAB dominance, increasing shelf life of fermented beverages (Łopusiewicz et al., 2019). Even so, the sampled fermented product should still represent a health concern since Caspian Sea-style spontaneously fermented milk consumption is usually performed within a few days after refrigeration.

2.3.2 Substrates and metabolites

Changes in non-volatiles (lactose and organic acids) and volatile compounds (carboxylic acids, aldehydes, and ketones) were monitored during the course of the fermentation (FIGURE 2.2B and TABLE 2.1, respectively). The initial lactose content (40.43 g/L) was rapidly reduced to 29.39 g/L within 6 h and remained constant until the end of the process. Lactose is the main carbohydrate in milk, with an average concentration of around 5% (w/v) (Barros et al., 2019). Fermentation reduces lactose in dairy products, helping to prevent symptoms in lactose-intolerant individuals (Savaiano, 2014). In the present study, the highest population of LAB was represented by the genus *Lactococcus,* which uses lactose by active transportation into the cytoplasm *via* phosphotransferase (PTS) system and hydrolyzing it into glucose and galactose (Mayo

et al., 2010). Both monosaccharides enter glycolysis at the level of glucose-6P or metabolized *via* the Leloir pathway (Kandler, 1983; Mayo et al., 2010).

TABLE 2.1 CONCENTRATION OF VOLATILE AROMA COMPOUNDS (AREA *105) FORMED DURING FERMENTATION OF CASPIAN SEA-STYLE SPONTANEOUSLY FERMENTED MILK CIRCULATED IN BRAZIL.

Compounds	**Aroma and taste description**	**Milk fermentation (h)**				
		0	6	12	18	24
GC						
Carboxylic acids (6)						
Hexanoic acid	Sour, fatty, sweaty, cheesy	53.89 ± 1.86^{a}	34.19 ± 3.70^{b}	55.29 ± 12.27^{a}	65.81 ± 2.95^{ac}	71.11 ± 1.23^{c}
Heptanoic acid	Cheesy, waxy, fermented pineapple	1.29 ± 0.11^{ab}	0.69 ± 0.19^{ab}	0.96 ± 0.39^{ab}	1.59 ± 0.16^{a}	1.58 ± 0.29^{a}
n-Decanoic acid	Rancid, sour, fatty, citrus	35.25 ± 11.56^{a}	31.51 ± 7.26^{a}	30.01 ± 6.84^{a}	44.73 ± 0.29^{a}	48.03 ± 14.83^{a}
Benzoic acid	Balsamic	ND	2.06 ± 0.34^{a}	14.81 ± 3.15^{b}	20.31 ± 3.23^{b}	21.00 ± 2.53^{b}
Octanoic acid	Fatty, oily, cheesy	68.10 ± 3.66^{a}	62.83 ± 9.76^{a}	69.31 ± 17.14^{a}	96.61 ± 1.12^{b}	97.98 ± 12.41^{b}
Nonanoic acid	Cheesy, dairy	0.54 ± 0.01^{a}	0.53 ± 0.01^{a}	0.72 ± 0.18^{a}	0.74 ± 0.00^{a}	0.81 ± 0.22^{a}
Aldehydes (3)						
Nonanal	Green lemon peel like nuance, citrus, melon rindy	ND	ND	ND	ND	0.39 ± 0.35
Decanal	Sweet, fatty, citrus, orange peel	ND	ND	0.44 ± 0.04^{a}	ND	0.30 ± 0.17^{a}
Benzaldehyde	Almond, fruity, powdery, nutty	ND	ND	1.05 ± 0.47	ND	ND
Ketones (3)						
2-Heptanone	Cheesy, fruity, ketonic	ND	0.36 ± 0.01^{a}	0.86 ± 0.61^{a}	ND	0.43 ± 0.20^{a}
2-Nonanone	Cheesy, fruity	ND	ND	0.31 ± 0.24^{a}	0.58 ± 0.03^{a}	0.35 ± 0.01^{a}
δ-dodelactone	Sweet, creamy, coconut, milky,	ND	0.20 ± 0.09^{a}	0.42 ± 0.04^{a}	0.21 ± 0.09^{a}	0.47 ± 0.05^{a}

ND, not detected; GC, gas chromatography

Means of triplicate in each row bearing the same letters are not significantly different ($p > 0.05$) from one another using Duncan's Test (mean± standard variation).

Lactic acid is the primary end-product observed, showing a continuous increase with maximum concentration of 5.93 g/L at 24 h. The accentuated production of lactic acid is in agreement with the strong dominance of LAB found in the present study (FIGURE 2.1 and 2.2), resulting in pH decrease from 5.10 to 4.10 at the end of fermentation. Lactic acid is the major fermentation product of various bacterial families related to dairy products. This compound is responsible for the milk acidification and partial casein coagulation, resulting in the formation of desirable sensory notes and rheological modifications. In addition, lactic acid is the main antimicrobial metabolite produced by LAB, which is responsible for the inhibition of various pathogens and food-borne microorganisms (Chahad et al., 2012; Gálvez et al., 2010; Ito et al., 2003; Nakai & Siebert, 2004). Specifically, Nakai and Siebert (2004) showed that *P. aeruginosa* was extremely sensitive to lactic acid, having the lowest MIC (minimum inhibitory concentration) among six different bacteria analyzed. Other factors that also contribute to antagonist action of LAB include the production of hydrogen peroxide, bacteriocins, and antibiotic-like substances (Arqués et al., 2015). Other minor organic acids produced during the fermentative process can be associated with the growth of sub-dominant bacteria reported by the 16S rRNA gene high-throughput sequencing, including succinic acid produced by *Leuconostoc* or *Acinetobacter*, propionic acid from hexose metabolism of *Enterobacter* species, and acetic acid by both acetic acid bacteria and heterofermentative LAB (Andriani et al., 2019; de Souza et al., 2019; Kang et al., 2012).

Volatiles compound metabolites were, for the first time, measured during Caspian-sea milk fermentation. Twelve volatile compounds were detected by GC/MS during fermentation, including six carboxylic acids, three aldehydes, and three ketones (TABLE 2.1). Amongst the carboxylic acids class, benzoic, hexanoic, and octanoic acids showed a significant increase through the fermentation. Hexanoic and octanoic acids are mainly related to *Lactococcus* metabolism of lipids and have been associated with cheesy aroma in fermented milk beverages (Azizan et al., 2012; Ziadi et al., 2008). In addition, benzoic acid was detected after 6 h and showed a steady increase, reaching a maximum peak at 24 h. This organic acid is commonly produced by species of *Lactococcus, Lactobacillus,* and *Streptococcus* through the conversion of hippuric acid, a natural component of milk. Benzoic acid has an inhibitory effect against spoilage microorganisms, such as yeast, mold, *Listeria innocua, Listeria ivanovii, P. aeruginosa,*

and *Oenococcus oeni* (Garmiene et al., 2010; Horníčková et al., 2014; Nakai & Siebert, 2004).

Ketones were the second class of volatile aroma reported. Both 2-heptanone and δ- dodelactone were produced after 6 h of fermentation, referring to LAB activity of unsaturated fatty acids hydrolysis (Azizan et al., 2012; Wanikawa et al., 2002). These volatile compounds are commonly reported in the literature for conferring a cheesy-like aroma in fermented milk beverages (Braun, 2019; Walsh et al., 2016). On the other hand, 2-nonanone was produced after 18 h, time in which *Pseudomonas* count showed a significant increase (FIGURE 2.1). This molecule has been recurrently used as a volatile biomarker for rapid detection of *Pseudomonas aeruginosa* and related species in hospital environments (Savelev et al., 2011; Zechman, 1985). In this sense, this volatile can also be used for monitoring of quality control in dairy production facilities. Finally, as a minority group, the aldehydes nonanal, decanal, and benzaldehyde were produced at specific fermentation times, being mainly associated with lipid oxidation by LAB (Gänzle et al., 2007).

2.4 CONCLUSIONS

The results of this study demonstrated that Caspian Sea-style spontaneous milk fermentation is not an efficient tool to overcome poor microbiological quality of the milk used as raw material. Although Caspian Sea-style fermented milk showed a high load of LAB and lactic acid content, the presence and persistence of *Pseudomonas* and enterobacteria through fermentation indicate a potential health risk in the final fermented product. Because of the increased focus on consumption of naturally fermented dairy products, this is a major health concern, since the spread of pathogenic organisms can be facilitated among unsuspecting individuals. The use of poor-quality pasteurized milk is also recurrent in other natural milk fermentation (e.g., kefir, *koumiss*, curd, *lassi*, *laben*, and *Suero costeño*) and further studies should be expanded to cover the safety status of these traditional foods. In addition, for a better understanding of *Pseudomonas* control using natural milk fermentation, it is crucial to evaluate different *Pseudomonas* inoculum concentration and more time during storage. The establishment of programs emphasizing hygienic manufacturing procedures can have a major effect on improving the microbiological quality of traditional and ethnic foods circulated in Brazil.

CHAPTER THREE (COMPLEMENTARY RESEARCH RESULTS) – ANTIMICROBIAL POTENTIAL OF PRESUNCTIVE LACTIC ACID BACTERIA ISOLATED FROM CASPIAN SEA STYLE FERMENTED MILK STORED AT LOW TEMPERATURES

3.1. INTRODUCTION

Traditional fermented milks are part of several culture's diet around the world, and also plays a key role in the routine of modern life in many countries (Gavrilova et al., 2019). Lactic acid bacteria (LAB) are widely distributed in the indigenous microbiota of naturally fermented dairy products, promoting digestibility and nutritional quality to the final product (Gavrilova et al., 2019; Rhee et al., 2011). The group includes the genera *Lactobacillus*, *Lactococcus, Streptococcus, Leuconostoc*, *Pediococcus,* among others (Sharma, 2019). The practice of fermentation of traditional milk-based products is performed in open systems under uncontrolled hygiene conditions and is, therefore, susceptible to contamination (Biratu & Seifu, 2016; Parry-Hanson Kunadu et al., 2019). These foods often contain pathogenic bacteria that must be eliminated during fermentation (Aliyu et al., 2020; De Buyser et al., 2001; Keba et al., 2020). Presence of LAB can reduce the risk of pathogenic microorganism's occurrence, as they excrete organic acids, hydrogen peroxide and bacteriocins during fermentation, acting as bio preservative agents and increasing the safety and lifetime of the final product (Özogul & Hamed, 2018; Gao et al., 2019).

Pseudomonas spp. are deteriorating and pathogenic Gram-negative bacteria (FAO 1992). The genus consists of microorganisms capable of surviving at low temperatures and are classified as psychrotrophic. They often contaminate milk and its derivatives, multiplying during storage at low temperatures (Doyle et al., 2017). The group includes *Pseudomonas aeruginosa*, which is an opportunistic pathogen that causes fatal infections, mainly affecting immunocompromised people (Fijan, 2015). *P. aeruginosa* is one of the pathogens most associated with contamination in the food industry (Bellil et al., 2018). Among other six bacteria, *P. aerugionosa* showed high sensitivity to lactic acid, having the lowest minimum inhibitory concentration (MIC) (Nakai & Siebert 2004). The isolation of LAB strains able to survive at low temperatures with antimicrobial properties is essential on traditional fermented milks

manufacture processes and in the food industry (Liu et al., 2017; Messens & De Vuyst, 2002).

Recently, the presence and persistence of considerable amounts of *Pseudomonas* sp. has been found during 24 hours of spontaneous fermentation of Caspian-style milk using the next generation Illumina sequencing platform. Surprisingly, after 60 days of storage at low temperatures, there was a large increase in LAB and a drastic decrease in the *Pseudomonas* genus. In order to exploit the antimicrobial capacity of indigenous Caspian Sea style LAB isolated at low temperatures against *P. aeruginosa*, colonies from this sample were isolated, purified, identified and tested against this pathogen. Acidification capacity was also evaluated.

3.2 MATERIAL AND METHODS

3.2.1 Sampling and isolation of presumptive lactic acid bacteria

Caspian Sea style fermented milk was stored at 4 ºC for 60 days after the main fermentation described by Maske et al (2020). At the end of this period, 1 mL aliquots of fermented milk were vortexed with 9 mL of 0.1% peptone water (10^{-1} solution) and diluted in series in triplicate. Then, 100 µL of each dilution was inoculated using the spread plate technique on the surface of De Man, Rogosa, and Sharpe Agar (MRS, Thermo Fisher Oxoid), selective for lactic acid bacteria (LAB), containing 0.1% (w/v) Nistatin (Sigma Aldrich, San Louis, MO, USA) for fungal growth inhibition. The plates were incubated at 30 ºC for 24 h. 16 colonies were selected by morphology and were purified on MRS agar added on MRS broth containing 10% (v/v) glycerol and stored at -80 ºC..

3.2.2 Pathogen

The ATCC 27853 strain of *P. aeruginosa* used as an indicator was obtained at Laboratório de Microbiologia in Centro Politécnico (UFPR), Curitiba-PR, Brazil. It was maintained by subculture in Brain Heart Broth Infusion (BHI) (Himedia) and incubation at 40 ºC for 18 h. Stock cultures were prepared in BHI broth containing 10% (v/v) glycerol and stored at -80 ºC.

3.2.3 Identification of presumptive isolates of lactic acid bacteria

The 16 selected isolates were identified by analysis of the 16S rRNA gene partial sequence. Genomic DNA was extracted from presumptive LAB isolates, previously cultured in 10 mL of MRS broth at 30°C for 24 hours, using the phenol/chloroform method adapted from the standard method by Neumann et al., (1992). Universal primers 27F (5'-AGAGTTGATCCTGCTCAG-3') and 1492R (5′ CGGCTACCTTGTACGACTT-3′) were used to amplify the 16S rRNA gene region (Lane et al., 1985) by PCR in a Veriti thermal cycler (Applied Biosystems, Paisley, United Kingdom). Amplifications were performed at a final volume of 25 µL containing 5 µL of 5x GoTaq® reaction buffer supplied with $MgCl_2$ (7.5 mM) (Promega, Madison, WI, USA), 0.55 µL of dNTP Mix (10 mM) (Invitrogen, Carlsbad, CA, USA), 0.5 µL of 27F and 1492R (10 mM) primers and 0.2 µL of GoTaq® DNA Polymerase (5U/µL) (Promega). Amplicons were generated by PCR under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 15 s, ringing at 55°C for 45 s, extension at 72°C for 90 s, and final extension at 72°C for 6 min. The PCR products were sequenced by automated capillary electrophoresis in an ABI 3730xl DNA analyzer (Applied Biosystems, Paisley, United Kingdom). The sequences obtained were analyzed and aligned using the BioEdit 7.7 sequence alignment editor and compared with the GenBank database. Homology research to determine the closest known species of ribosomal DNA partial sequences was performed using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) at the National Center for Biotechnology Information (NCBI) (Alstchul et al., 1990).

3.2.4 In vitro determination of antimicrobial activity

The antimicrobial activity of the 16 presumptive isolates of lactic acid bacteria was investigated by the agar plug diffusion assay against *P. aeruginosa* ATCC 27853 according to Balouiri et al. (2016), with modifications. Presumptive isolates of LAB and *P. aeruginosa* were reactivated in MRS broth at 30 °C and BHI broth at 40 °C, respectively, in the proportion of 1% v/v and incubated overnight. The media were centrifuged (10,000g for 15 minutes) and the supernatant was discarded. Then, the pellets were resuspended with their respective autoclaved culture media to adjust the turbidity scale. The media were adjusted to 0.5 Mac Fareland scale, corresponding to approximately 1.5 x 10^8 colony forming units (CFU)/mL. A sterile swab was used to inoculate the bacteria on agar medium (MRS agar for presumptive LAB and BHI agar

for *P. aeruginosa* ATCC) using the plate spreading technique. After drying at room temperature, three 6 mm agar cylinders were aseptically cut from the presumptive LAB plates and deposited on the surface of the BHI medium containing *P. aeruginosa* ATCC 27853 indicator microorganisms. The plates were incubated at 30°C for 24 hours. Antimicrobial activity was detected by the appearance of the inhibition zone around the agar plug. The diameters of the halos were measured using the ImageJ® program.

3.2.5 LAB acidification capacity on ultrahigh temperature (UHT) skim milk

After the antimicrobial assay, nine LAB strains were selected for acid production capacity test. Milk cultures were prepared in a medium composed of 100% UHT skim milk (Naturalle) according to Raveschot et al. (2020). Strains were inoculated to 30 mL of UHT skim milk, reaching an initial cellular OD600 of 0.3, and grown at 37 °C for 48 h statically. Concomitantly, a non-inoculated UHT skim milk was incubated in the same conditions and served as negative control. After incubation, the pH value of culture media was determined using a pH meter (Requipal, Curitiba, Brazil). Experiments were performed in triplicate and the results were expressed as the mean plus standard error.

3.2.6 Statistical analysis

The results obtained from the triplicate measurement of inhibition halos and pH values were expressed as mean and standard deviation. Statistical analysis of the data was performed in the STATISTICA 7 StatSoft software (STATSOFT, 2007), with a level of statistical significance determined as $p<0.05$. The data were submitted to ANOVA and the differences between treatments versus control were evaluated by a Tukey's HSD.

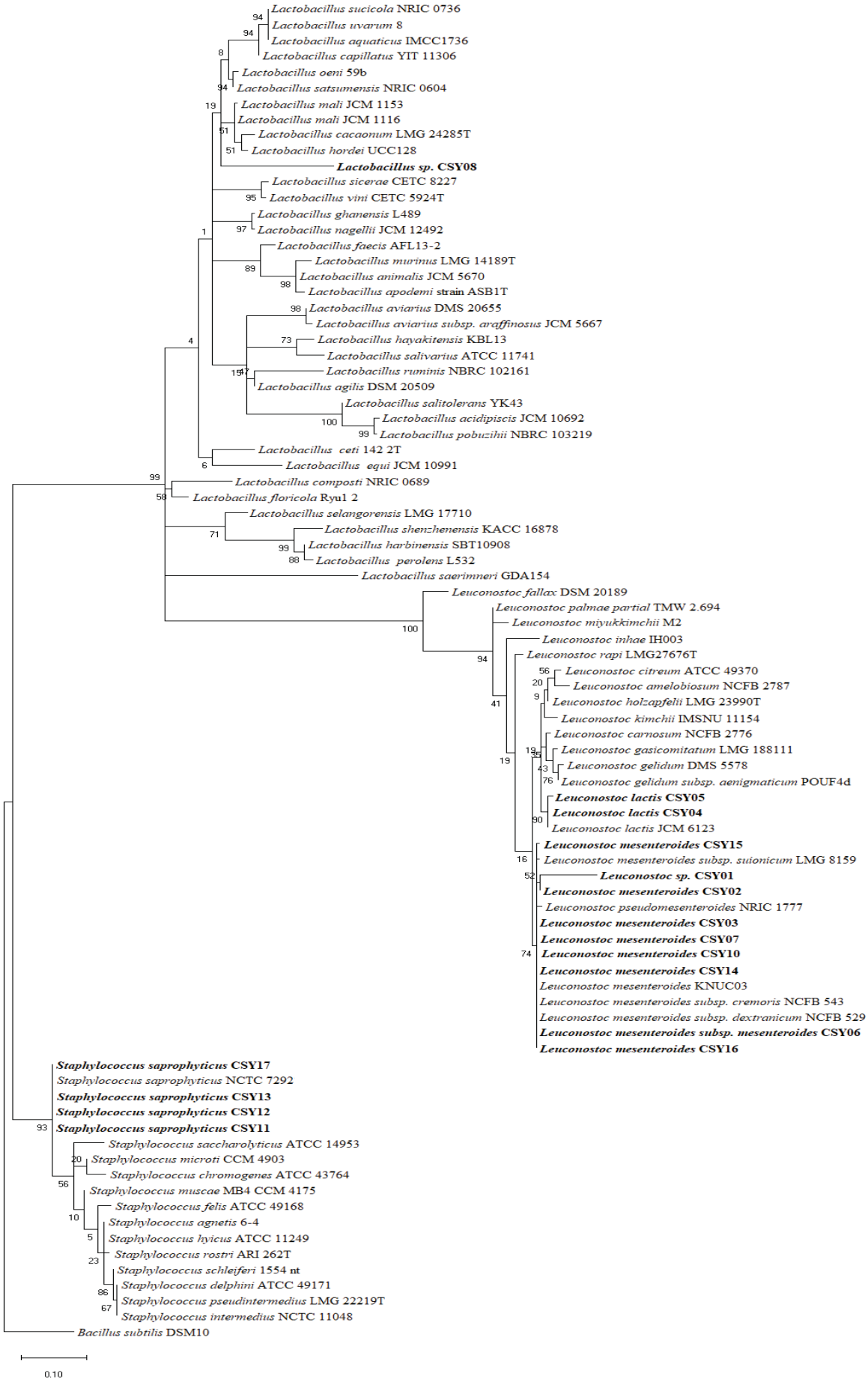
3.3 RESULTS AND DISCUSSION

Illumina Miseq sequencing of the viability test sample (Caspian Sea-style fermented milk sample stored at 4°C refrigerated for 60 days) performed by Maske et al (2020) revealed a decrease in *Pseudomonas* load compared to the alarming concentration and persistence in 24 hours of fermentation process. Additionally, there was a significant increase in the lactic acid bacteria population level. In order to explore the antagonisctic activity of LAB toward *P. aeruginosa*, a pathogenic species of the

genus *Pseudomonas*, presumptive colonies were randomly selected from the viability test sample, identified and evaluated.

The 16 isolates had their 16S rRNA partial gene sequenced and taxonomic strain characterization was performed by comparing the sequences of each isolate with those reported in the NCBI Reference. Considering species and subspecies with 97 to 100% similarity, the isolates were identified as *Leuconostoc mesenteroides* subsp. *mesenteroides* (n=1), *Leu. mesenteroides* (n=7), *Staphylococcus saprophyticus* (n=4), *Leu. lactis* (n=2), *Leuconostoc* sp. (n=1) and *Lactobacillus* sp. (n=1). Three distinct clusters were formed according to neighbor-joining method (*Lactobacillus*, *Leuconostoc* and *Staphylococcus*) (FIGURE 3.1).

FIGURE 3.1 - MAXIMUM-LIKELIHOOD TREE BASED ON 16S rRNA GENE SEQUENCES SHOWING THE PHYLOGENETIC RELATIONSHIPS OF PRESUMPTIVE LAB ISOLATED FROM CASPIAN SEA-LIKE FERMENTED MILK FERMENTATION. BOOTSTRAP VALUES (%) BASED ON 1000 REPLICATIONS ARE SHOWN AT BRANCH POINTS. THE SUBSTITUTION MODEL USED WAS KIMURA 2-PARAMETER MODEL. BAR = 0.05% SEQUENCE DIVERGENCE.


Lactobacillus sucicola NRIC 0736
Lactobacillus uvarum 8
Lactobacillus aquaticus IMCC1736
Lactobacillus capillatus YIT 11306
Lactobacillus oeni 59b
Lactobacillus satsumensis NRIC 0604
Lactobacillus mali JCM 1153
Lactobacillus mali JCM 1116
Lactobacillus cacaonum LMG 24285T
Lactobacillus hordei UCC128
Lactobacillus sp. CSY08
Lactobacillus sicerae CETC 8227
Lactobacillus vini CETC 5924T
Lactobacillus ghanensis L489
Lactobacillus nagelii JCM 12492
Lactobacillus faecis AFL13-2
Lactobacillus murinus LMG 14189T
Lactobacillus animalis JCM 5670
Lactobacillus apodemi strain ASB1T
Lactobacillus aviarius DMS 20655
Lactobacillus aviarius subsp. araffinosus JCM 5667
Lactobacillus hayakitensis KBL13
Lactobacillus salivarius ATCC 11741
Lactobacillus ruminis NBRC 102161
Lactobacillus agilis DSM 20509
Lactobacillus salitolerans YK43
Lactobacillus acidipiscis JCM 10692
Lactobacillus pobuzihii NBRC 103219
Lactobacillus ceti 142 2T
Lactobacillus equi JCM 10991
Lactobacillus composti NRIC 0689
Lactobacillus floricola Ryu1 2
Lactobacillus selangorensis LMG 17710
Lactobacillus shenzhenensis KACC 16878
Lactobacillus harbinensis SBT10908
Lactobacillus perolens L532
Lactobacillus saerimneri GDA154
Leuconostoc fallax DSM 20189
Leuconostoc palmae partial TMW 2.694
Leuconostoc miyukkimchii M2
Leuconostoc inhae IH003
Leuconostoc rapi LMG27676T
Leuconostoc citreum ATCC 49370
Leuconostoc amelobiosum NCFB 2787
Leuconostoc holzapfelii LMG 23990T
Leuconostoc kimchii IMSNU 11154
Leuconostoc carnosum NCFB 2776
Leuconostoc gasicomitatum LMG 188111
Leuconostoc gelidum DMS 5578
Leuconostoc gelidum subsp. aemigmaticum POUF4d
Leuconostoc lactis CSY05
Leuconostoc lactis CSY04
Leuconostoc lactis JCM 6123
Leuconostoc mesenteroides CSY15
Leuconostoc mesenteroides subsp. suionicum LMG 8159
Leuconostoc sp. CSY01
Leuconostoc mesenteroides CSY02
Leuconostoc pseudomesenteroides NRIC 1777
Leuconostoc mesenteroides CSY03
Leuconostoc mesenteroides CSY07
Leuconostoc mesenteroides CSY10
Leuconostoc mesenteroides CSY14
Leuconostoc mesenteroides KNUC03
Leuconostoc mesenteroides subsp. cremoris NCFB 543
Leuconostoc mesenteroides subsp. dextranicum NCFB 529
Leuconostoc mesenteroides subsp. mesenteroides CSY06
Leuconostoc mesenteroides CSY16
Staphylococcus saprophyticus CSY17
Staphylococcus saprophyticus NCTC 7292
Staphylococcus saprophyticus CSY13
Staphylococcus saprophyticus CSY12
Staphylococcus saprophyticus CSY11
Staphylococcus saccharolyticus ATCC 14953
Staphylococcus microti CCM 4903
Staphylococcus chromogenes ATCC 43764
Staphylococcus muscae MB4 CCM 4175
Staphylococcus felis ATCC 49168
Staphylococcus agnetis 6-4
Staphylococcus hyicus ATCC 11249
Staphylococcus rostri ARI 262T
Staphylococcus schleiferi 1554 nt
Staphylococcus delphini ATCC 49171
Staphylococcus pseudintermedius LMG 22219T
Staphylococcus intermedius NCTC 11048
Bacillus subtilis DSM10
0.10

Although MRS medium is selective for lactic acid bacteria, other groups of bacteria may also be isolated eventually (Yang et al 2018). *Staphylococcus saprophyticus* can be found in fermented foods (Fiorentini et al., 2009) and its presence can cause urinary tract infections (Zhang & Zhao, 2018). To avoid isolation of non-LAB groups, prior to the identification of the strains, a biochemical evaluation can be performed, selecting Gram-positive, catalase-negative and nitrate-negative bacilli (Sáez et al., 2018), and also by supplementing the agar medium with $CaCO_3$ to indicate lactic acid production (Viesser et al., 2020).

The antimicrobial activity of the isolates against *P. aeruginosa* ATCC 27853 through agar plug diffusion method is shown in TABLE 3.1. The inhibition zones were measured, and the halo diameter was expressed in mm. *P. aeruginosa* was more sensitive to the isolate CSY08, corresponding to *Lactobacillus* sp., with a halo of 22.22d ± 0.35 mm. *Leuconostoc* sp. and *Leuconostoc mesenteroides* were the most abundant LAB isolates and also showed significant antimicrobial activity.

TABLE 3.1 - SPECIES IDENTIFIED THROUGH THE PARTIAL SEQUENCE OF 16S AND THE AVERAGE VALUES OF HALOS' DIAMETERS RESULTING FROM THE ANTIMICROBIAL TEST EXPRESSED IN mm AGAINST *Pseudomonas aeruginosa* INDICATOR ATCC 27853.

Isolates	Inhibition halo diameter (mm)
Leuconostoc sp. CSY01	8,09±0,06^{abc}
Leu. mesenteroides CSY02	9,83±0,61^{abc}
Leu. mesenteroides CSY03	n.d.
Leu. lactis CSY04	10,91±0,13^{abc}
Leu. lactis CSY05	3,05±0,07^{a}
Leu. mesenteroides subsp. *mesenteroides* CSY06	n.d.
Leu. mesenteroides CSY07	2,21±0,05^{a}
Lactobacillus sp. CSY08	22,22±0,35^{d}
Leu. mesenteroides CSY010	15,86±0,06^{bd}
Staphylococcus saprophyticus CSY011	3,41±0,03^{ac}
S. saprophyticus CSY012	n.d.
S. saprophyticus CSY013	n.d.
Leu. mesenteroides CSY014	n.d.
Leu. mesenteroides CSY015	11,83±0,43^{bc}
Leu. mesenteroides CSY016	17,70±0,28^{bd}
S. saprophyticus CSY017	n.d.

*The diameter of each agar plug was not considered. Values in triplicate on each line with the same letters are not significantly different ($p>0.05$) from each other using the Tukey test (mean value±standard deviation). ND = Not detected.

Lactobacillus is the largest genus within the LAB group, widely used in the manufacture of fermented dairy products. They are generally regarded as safe (GRAS) and have great potential to be used in starter cultures and as probiotics (De Angelis & Gobbetti, 2016). Innumerous studies have tested the supernatants of *Lactobacillus* sp.

cultures isolated from milk-based fermented foods against *P. aeruginosa*, finding a wide spectrum of halos diameters, ranging from 2 to 18.6 mm (Adesina et al., 2016; Haghshenas et al., 2016; Poornachandra Rao et al., 2015; Karami et al. 2017; Hashemi et al. 2017; Sharma, 2019). Among them, the highest value of 18.6 mm was found by Hashemi et al. (2017), who tested the supernatant of *L. plantarum* culture isolated from *sarshir*, a milk-based fermented product, through the well diffusion method. They attributed the antimicrobial activity to the presence of bacteriocin compounds and the generation of organic acids. Nevertheless, the isolate CSY08 corresponding to *Lactobacillus* sp. showed the largest halo size within the milk-based fermented products in the literature. Recently, Evurani et al. (2019) reported a halo diameter of 25 mm by the small spot technique, achieved through mixed culture of strains of *L. brevis* and *L. casei*, isolated from Kunun-Zak, a traditional non-alcoholic fermented drink widely consumed in Nigeria. The authors suggested, therefore, that a combination of two strains could have a superior result.

Leuconostoc also belongs to the LAB group and is commonly used in starter cultures. The isolated species identified as *Leu. mesenteroides*, *Leu. mesenteroides* subsp. *mesenteroides* and *Leu. lactis* have already been isolated from several other fermented foods such as vegetables and fermented dairy products, where they produce aromatic components and cause changes in their textures (Chen et al., 2012; Duthoit et al., 2005; Firmesse et al., 2008; Nieto-Arribas et al., 2010). Theheir presence has already been reported to have an effect on pathogen growth control (Cotter & Beresford, 2017; Korkeala & Johanna Björkroth, 1997; Samelis & Georgiadou, 2000). The reported diameters of *Leuconostoc* ranged from 2.21 to 17.70 mm against *P. aeruginosa*, being in agreement with previous studies that isolated *Leuconostoc* strains from the fermented food.(Ahmaed, 2019; Bellil et al., 2018; Coulibaly et al., 2017; Morandi et al., 2013; Olaniyi et al., 2019). Bellil et al. (2018) evaluated, by the agar well diffusion method, the antimicrobial activity of *Leu. mesenteroides* isolated from fermented dromedary milk, reaching an inhibition zone of 15 to 20 mm in diameter. The tested supernatant was treated with catalase to avoid antimicrobial effect of hydrogen peroxide and the antimicrobial potential was inhibited by protease, indicating that bacteriocin was the main agent against *P. aeruginosa*. The isolates CSY10 and CSY16 of *Leu. mesenteroides* reached halos of 15.86 and 17.70 respectively and did not differ

statistically from the result for *Lactobacillus* sp. CSY08, also showing a promising result for antimicrobial activity against foodborne pathogens.

The acidification capacity of selected strains (CSY strains 01, 02, 04, 05, 07, 08, 10, 15 and 16) was measured by pH determination after 48h of culture (TABLE 3.2). The initial pH value of skim milk was 6.68 and, after 48h incubation, reached 6.59±0.005 (control). *Lactobacillus* and *Leuconostoc* displayed similar acidification pattern. *Leu. lactis* strains led to a pH decrease of 2 pH units, reaching a mean of 4,71±0,15 , whereas the pH of milk inoculated with *Lactobacillus* sp. and *Leu. mesenteroides* decreased by a maximum of 1 pH unit. *Leu. lactis* strains were the most efficient for acidification capacity and, as expected, were the only that led to milk protein coagulation due to casein precipitation at pH lower than 4.6 (Raak, Rohm, & Jaros, 2017).

TABLE 3.2 – ACIDIFICATION CAPACITY TEST.

Isolates	**pH**
Skim milk	$6,68 \pm 0,00^{a}$
Control	$6,59 \pm 0,05^{ab}$
Leuconostoc sp. CSY01	$5,42 \pm 0,20^{def}$
Leu. mesenteroides CSY02	$5,19 \pm 0,07^{efg}$
Leu. lactis CSY04	$4,68 \pm 0,10^{g}$
Leu. lactis CSY05	$4,74 \pm 0,21^{g}$
Leu. mesenteroides CSY07	$5,14 \pm 0,31^{fg}$
Lactobacillus sp. CSY08	$5,57 \pm 0,08^{def}$
Leu. mesenteroides CSY010	$6,31 \pm 0,03^{abc}$
Leu. mesenteroides CSY015	$5,99 \pm 0,58^{bcd}$
Leu. mesenteroides CSY016	$5,83 \pm 0,04^{cde}$

*Values in triplicate on each line with the same letters are not significantly different ($p>0.05$) from each other using the Tukey test (mean value±standard deviation).

3.4 CONCLUDING REMARKS

This is the first experiment to date that reports the antimicrobial activity of lactic acid bacteria isolated from Caspian Sea style spontaneously fermented milk. This product has proven to be a promising source for isolation of LAB with action against *P. aeruginosa* growth. Further tests should be carried out with the supernatants of the strains to unravel to which component(s) the antimicrobial activity is attributed.

GENERAL CONCLUSIONS

In summary, the popularization of next generation sequencing technology allowed a successful characterization of popular fermented milk products microbiota around the globe. Besides confirming dominant species identified previously through culture-dependent methods, recent NGS platforms enabled the discovery of yet uncultured genera/species and sub-dominant populations for the first time. The discovery of these new taxa enhances the chance to isolate potential microorganisms with functional proprieties and facilitates food quality and safety control. Illumina Miseq platform successfully accessed the microbiota of Caspian Sea style fermented milk, showing that it is not an efficient process to overcome the poor microbiological quality of milk used as substrate. Although the final product exhibited significant number of LAB and lactic acid concentration, the presence and persistence of *Pseudomonas* through fermentation indicated a potential health risk in the final fermented product. Results indicate that food safety authorities in Brazil need to carry out more rigorous surveillance of ethnic fermented dairy products. In addition, presumptive LAB strains isolated from Caspian Sea style fermented milk stored for 60 days at low temperatures showed significant antimicrobial activity against *P. aeruginosa*, a pathogenic member of *Pseudomonas*, often associated with food contamination. Thus, the present study allowed the use of NGS platform to monitor food security of traditional fermented milks and technological advance of discovery of new functional strains.

REFERENCES

ADESINA, I., OJOKOH, A., & AROTUPIN, D. (2016). Screening, Identification and Antibiotic Susceptibility Pattern of Bacteriocin-producing Lactic Acid Bacteria Isolated from Selected Traditionally Fermented Products. **British Microbiology Research** Journal, 11(1), 1–9. https://doi.org/10.9734/bmrj/2016/21427

ADESSI, C., G. MATTON, G. AYALA, G. TURCATTI, J.-J. MERMOD, P. MAYER, AND E. KAWASHIMA 2000. Solid phase DNA amplification: Characterization of primer attachment and amplification mechanisms. **Nucleic Acids Research** 28(20):e87.

AHMAED, A. S. (2019). 2019 Antimicrobial activity of *Leuconostoc mesenteroides* biofilm against different microorganisms. 13(1).

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** 34:277–83. 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.

ALIYU, Y., ABDULLAHI, I., C. W OF M. 2020. Occurrence and antibiotic susceptibility of methicillin-resistant Staphylococcus aureus in fresh milk and milk products in Nasarawa State, **North-Central Nigeria. Academicjournals**.Org, 12(June), 32–41. https://doi.org/10.5897/JMA2020.0424

ALLES, A. A., WIEDMANN, M., & MARTIN, N. H. (2018). Rapid detection and characterization of postpasteurization contaminants in pasteurized fluid milk. **Journal of Dairy Science**, 101(9), 7746–7756. https://doi.org/10.3168/jds.2017-14216

ALLYDICE-FRANCIS, K., AND BROWN, P. D. (2012). Diversity of antimicrobial resistance and virulence determinants in *Pseudomonas aeruginosa* associated with fresh vegetables. **Int. J. Microbiol**. 2012. doi:10.1155/2012/426241.

AL-MALKEY, M. K., ISMEEAL, C., ABO, J., MOHAMMED, S. W., & NAYYEF, H. J. (2017). Antimicrobial effect of probiotic *Lactobacillus spp*. on *Pseudomonas aeruginosa*. **Journal of Contemporary Medical Sciences**, 3(10), 218–223. https://doi.org/10.22317/jcms.06201704

AMEUR, A., W. P. KLOOSTERMAN, AND M. S. HESTAND. 2019. Single-molecule sequencing: Towards clinical applications. **Trends in Biotechnology** 37:72–85. doi:10.1016/j.tibtech.2018.07.013.

ANDRIANI, A., NURYANA, I., RAHMANI, N., HARTATI, S., LISDIYANTI, P., AND YOPI (2019). The potency of cassava starch (var. kristal merah and var. revita) for bio-succinic acid production using indigenous lactic acid bacteria (*Leuconostoc sp*). IOP Conf. Ser. **Earth Environ. Sci**. 251, 012039. doi:10.1088/1755-1315/251/1/012039.

ANGULO, L., E. LOPE, AND C. LEMA. 1993. Microflora present in kefir grains of the Galician region (North-West of Spain). **Journal of Dairy Research** 60(2): 263–67. 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** 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.

ARQUÉS, J. L., RODRÍGUEZ, E., LANGA, S., LANDETE, J. M., AND MEDINA, M. (2015). Antimicrobial activity of lactic acid bacteria in dairy products and gut: Effect on pathogens**. Biomed Res. Int**. 2015, 584183. doi:10.1155/2015/584183.

ARSENEAU, J.-R., R. STEEVES, AND M. LAFLAMME. 2016. Modified low-salt CTAB extraction of high-quality DNA from contaminant-rich tissues. **Molecular Ecology Resources** 17(4):686–93.

AZIZAN, K. A., BAHARUM, S. N., AND NOOR, N. M. (2012). Metabolic profiling of *Lactococcus lactis* under different culture conditions. **Molecules** 17, 8022–8036. doi:10.3390/molecules17078022.

BALOUIRI, M., SADIKI, M., & IBNSOUDA, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. **Journal of Pharmaceutical Analysis**, 6(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

BARROS, R. F., CUTRIM, C. S., COSTA, M. P. DA, CONTE JUNIOR, C. A., AND CORTEZ, M. A. S. (2019). Lactose hydrolysis and organic acids production in yogurt prepared with different onset temperatures of enzymatic action and fermentation. **Ciência Anim. Bras**. 20, e-43549. doi:10.1590/1809-6891v20e-43549.

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):100. doi:10.1007/s11274-019-2672-3.

BEIKO, R. G., W. HSIAO, AND J. PARKINSON, EDS. 2018. Microbiome Analysis: Methods and Protocols. **Methods in Molecular Biology**. vol. 1849. Hatfield: Human Press. doi:10.1007/978-1-4939-8728-3_5.

BELLIL, Y., BENMECHERNENE, Z., CHAHROUR BELLIL, W., & KIHAL, M. (2018). Antibacterial and antibiofilm activity of the bacteriocin-producing strain *Leuconostoc mesenteroides* CHBY46 isolated from Algerian dromedary milk against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. 120 **South Asian J Exp Biol**, 8(4), 2230–9799. http://www.sajeb.org

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.

BIRATU, K., & SEIFU, E. (2016). Chemical composition and microbiological quality of Dhanaan: Traditional fermented camel milk produced in eastern Ethiopia. **International Food Research Journal**, 23(5), 2223–2228.

BRAUN, M. (2019). **Flavoured milk product**. 20.

BUZA, T. M., T. TONUI, F. STOMEO, C. TIAMBO, R. KATANI, M. SCHILLING, B. LYIMO, ET AL. 2019. IMAP: An integrated bioinformatics and visualization pipeline for microbiome data analysis. BMC **Bioinformatics** 20(1):374. 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**, eds. K. D. Bunting and C.-K. Qu, vol. 1185, 345–360. 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, 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** 8:1289. doi:10.3389/fmicb.2017.01829.

CAPORASO, J. G., LAUBER, C. L., WALTERS, W. A., BERG-LYONS, D., HUNTLEY, J., FIERER, N., ET AL. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **ISME** J. 6, 1621–1624. doi:10.1038/ismej.2012.8.

CAPOZZI, V., FRAGASSO, M., & RUSSO, P. (2020). Microbiological safety and the management of microbial resources in artisanal foods and beverages: The need for a transdisciplinary assessment to conciliate actual trends and risks avoidance. **Microorganisms**, 8(2), 1–9. https://doi.org/10.3390/microorganisms8020306

CAPOZZI, V., FRAGASSO, M., ROMANIELLO, R., BERBEGAL, C., RUSSO, P., AND SPANO, G. (2017). Spontaneous food fermentations and potential risks for human health. Fermentation 3, 1–19. doi:10.3390/fermentation3040049.

CAPOZZI, V., RUSSO, P., & SPANO, G. (2012). Microbial information regimen in EU geographical indications. **World Patent Information**, 34(3), 229–231. https://doi.org/10.1016/j.wpi.2012.04.001

CARRER, F., COLONESE, A. C., LUCQUIN, A., GUEDES, E. P., THOMPSON, A., WALSH, K., ET AL. (2016). Chemical analysis of pottery demonstrates prehistoric origin for high-altitude alpine dairying. **PLoS One** 11, 1–11. doi:10.1371/journal.pone.0151442.

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** 9:100–102. 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.

CHAHAD, O. B., EL BOUR, M., CALO-MATA, P., BOUDABOUS, A., AND BARROS-VELÀZQUEZ, J. (2012). Discovery of novel biopreservation agents with inhibitory effects on growth of food-borne pathogens and their application to seafood products. **Res. Microbiol**. 163, 44–54. doi:10.1016/j.resmic.2011.08.005.

CHAKRAVORTY, S., D. HELB, M. BURDAY, N. CONNEL, AND D. ALLAND. 2007. 16s rDNA based identification of bacteria in the organophosphates treated agricultural soil. **Journal of Microbiological Methods** 69(2):330–39. doi:10.1016/j.mimet.2007.02.005.A.

CHAVES-LÓPEZ, C., A. SERIO, C. D. GRANDE-TOVAR, R. CUERVO-MULET, J. DELGADO-OSPINA, AND A. PAPARELLA. 2014. Traditional fermented foods and beverages from a microbiological and nutritional perspective: The Colombian heritage. **Comprehensive Reviews in Food Science and Food Safety** 13(5): 1031–48. doi:10.1111/1541-4337.12098.

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

CHEN, Y. SHENG, WU, H. CHUNG, LO, H. YIN, LIN, W. CHEN, HSU, W. HSUAN, LIN, C. WEN, LIN, P. YA, & YANAGIDA, F. (2012). Isolation and characterisation of lactic acid bacteria from Jiang-gua (fermented cucumbers), a traditional fermented food in Taiwan. **Journal of the Science of Food and Agriculture**, 92(10), 2069–2075. https://doi.org/10.1002/jsfa.5583

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** 14:6. doi:10.1186/1471-2156-14-6.

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.

COTTER, P. D., & BERESFORD, T. P. (2017). Microbiome Changes During Ripening. In Cheese: **Chemistry, Physics and Microbiology**: Fourth Edition (Fourth Edi, Vol. 1). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-417012-4.00015-6

COULIBALY, K. E., COULIBALY, K.-J., THIERRY, L., AKPA, E. E., GOUALIE, G. B., NIAMKE, S. L., & DOSSO, M. (2017). Probiotic profiling of *Leuconostoc* species isolated from a traditional fermented cassava product. **African Journal of Microbiology Research**, 11(10), 408–413. https://doi.org/10.5897/ajmr2017.8457

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** 197:273–84. doi:10.1016/j.foodchem.2015.10.116.

DE ANGELIS, M., & GOBBETTI, M. (2016). *Lactobacillus SPP.*: General Characteristics. Reference Module in **Food Science**, September. https://doi.org/10.1016/b978-0-08-100596-5.00851-9

DE BUYSER, M. L., DUFOUR, B., MAIRE, M., & LAFARGE, V. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. **International Journal of Food Microbiology**, 67(1–2), 1–17. https://doi.org/10.1016/S0168-1605(01)00443-3

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.

DE MELO PEREIRA, G. V., D. P. CARVALHO NETO, A. C. O. JUNQUEIRA, S. G. KARP, L. A. J. LETTI, A. I. MAGALHÃES JÚNIOR, AND C. R. SOCCOL. 2019. 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.

DE SOUZA, I. T., MOREIRA, F. S., FERREIRA, J. S., CARDOSO, V. L., AND BATISTA, F. R. X. (2019). Technological advances in hydrogen production by Enterobacter bacteria upon substrate, luminosity and anaerobic conditions. **Int. J. Hydrogen Energy** 44, 16190–16198. doi:10.1016/j.ijhydene.2019.05.036.

DEL OLMO, A., CALZADA, J., AND NUÑEZ, M. (2018). The blue discoloration of fresh cheeses: A worldwide defect associated to specific contamination by *Pseudomonas fluorescens*. **Food Control** 86, 359–366. doi:10.1016/j.foodcont.2017.12.001.

DERTLI, E., AND A. H. ÇON. 2017. Microbial diversity of traditional kefir grains and their role on kefir aroma. LWT - **Food Science and Technology** 85:151–57. 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):130–38. doi:10.1128/AEM.69.1.130.

DOYLE, C. J., O'TOOLE, P. W., & COTTER, P. D. (2017). Metagenome-based surveillance and diagnostic approaches to studying the microbial ecology of food production and processing environments. **Environmental Microbiology**, 19(11), 4382–4391. https://doi.org/10.1111/1462-2920.13859

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 tomato pomace. **Journal of General and Applied Microbiology** 54:51–60. doi:10.1007/s13213-013-0798-3.

DUTHOIT, F., TESSIER, L., & MONTEL, M. C. (2005). Diversity, dynamics and activity of bacterial populations in "Registered Designation of Origin" Salers cheese by single-strand conformation polymorphism analysis of 16S rRNA genes. **Journal of Applied Microbiology**, 98(5), 1198–1208. https://doi.org/10.1111/j.1365-2672.2005.02575.x

EDGAR, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. **Bioinformatics** 26, 2460–2461. doi:10.1093/bioinformatics/btq461.

EL-GHAISH, S., AHMADOVA, A., HADJI-SFAXI, I., EL MECHERFI, K. E., BAZUKYAN, I., CHOISET, Y., ET AL. (2011). Potential use of lactic acid bacteria for reduction of allergenicity and for longer conservation of fermented foods. **Trends Food Sci. Technol**. 22, 509–516. doi:10.1016/j.tifs.2011.05.003.

ELMOSLEMANY, A. M., KEEFE, G. P., DOHOO, I. R., WICHTEL, J. J., STRYHN, H., AND DINGWELL, R. T. (2010). The association between bulk tank milk analysis for raw milk quality and on-farm management practices. **Prev. Vet. Med**. 95, 32–40. doi:10.1016/j.prevetmed.2010.03.007.

ELORTONDO, F. J. P., ECHOBARRIA, P. A., ALBISU, M., AND BARCINA, Y. (1998). Indigenous lactic acid bacteria in Idiazabal ewes' milk cheese. **Int. Dairy J**. 8, 725–732. doi:10.1016/S0958-6946(98)00113-7.

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.

ERCOLINI, D., RUSSO, F., FERROCINO, I., AND VILLANI, F. (2009). Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. **Food Microbiol**. 26, 228–231. doi:10.1016/j.fm.2008.09.005.

EVURANI, S. A., GODWIN, A., CHARITY, N. U., CHINELO, C. E., & OKAFOR, G. (2019). Antimicrobial Activity of Lactic Acid Bacteria from Kunun-Zaki on Selected Clinical Pathogens. **South Asian Journal of Research in Microbiology**, 3(4), 1–8. https://doi.org/10.9734/sajrm/2019/v3i430091

FERCHICHI, M., FRÈRE, J., MABROUK, K., AND MANAI, M. (2001). Lactococcin MMFII, a novel class IIa bacteriocin produced by Lactococcus lactis MMFII, isolated from a Tunisian dairy product. **FEMS Microbiol**. Lett. 205, 49–55. doi:10.1016/S0378-1097(01)00435-9.

FIJAN, S. (2015). Influence of the Growth of Pseudomonas aeruginosa in Milk Fermented by Multispecies Probiotics and Kefir Microbiota. **Journal of Probiotics & Health**, 04(01). https://doi.org/10.4172/2329-8901.1000136

FIORENTINI, Â. M., SAWITZKI, M. C., BERTOL, T. M., ANGONESI BROD, F. C., PELISSER, M. R., MAISONNAVE ARISI, A. C., & SANT'ANNA, E. S. (2009). Phenotypic and molecular characterization of *Staphylococcus xylosus*: Technological potential for use in fermented sausage. **Brazilian Archives of Biology and Technology**, 52(3), 737–746. https://doi.org/10.1590/S1516-89132009000300026

FIRMESSE, O., ALVARO, E., MOGENET, A., BRESSON, J. L., LEMÉE, R., LE RUYET, P., BONHOMME, C., LAMBERT, D., ANDRIEUX, C., DORÉ, J., CORTHIER, G., FURET, J. P., & RIGOTTIER-GOIS, L. (2008). Fate and effects of Camembert cheese micro-organisms in the human colonic microbiota of healthy volunteers after regular Camembert consumption. **International Journal of Food** Microbiology, 125(2), 176–181. https://doi.org/10.1016/j.ijfoodmicro.2008.03.044

FORTINA, M. G., RICCI, G., ACQUATI, A., ZEPPA, G., GANDINI, A., AND MANACHINI, P. L. (2003). Genetic characterization of some lactic acid bacteria occurring in an artisanal protected denomination origin (PDO) Italian cheese, the Toma piemontese. **Food Microbiol**. 20, 397–404. doi:10.1016/S0740-0020(02)00149-1.

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): 12–14. 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.

GÁLVEZ, A., ABRIOUEL, H., BENOMAR, N., AND LUCAS, R. (2010). Microbial antagonists to food-borne pathogens and biocontrol. **Curr. Opin. Biotechnol**. 21, 142–148. doi:10.1016/j.copbio.2010.01.005.

GÄNZLE, M. G., VERMEULEN, N., AND VOGEL, R. F. (2007). Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. **Food Microbiol**. 24, 128–138. doi:10.1016/j.fm.2006.07.006.

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** 116:137–44. doi:10.1016/j.foodres.2018.11.056.

GAO, Z., DALIRI, E. B. M., WANG, J. U. N., LIU, D., CHEN, S., YE, X., & DING, T. (2019). Inhibitory effect of lactic acid bacteria on foodborne pathogens: A review. **Journal of Food Protection**, 82(3), 441–453. https://doi.org/10.4315/0362-028X.JFP-18-303

GARMIENE, G., SALOMSKIENE, J., JASUTIENE, I., MACIONIENE, I., AND MILIAUSKIENE, I. (2010). Production of benzoic acid by lactic acid bacteria from *Lactobacillus, Lactococcus* and *Streptococcus* genera in milk. **Milchwissenschaft** 65, 295–298.

GAROFALO, C., A. OSIMANI, V. MILANOVIĆ, L. AQUILANTI, F. DE FILIPPIS, G. STELLATO, S. DI MAURO, ET AL. 2015. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. **Food Microbiology** 49:123–33. doi:10.1016/j.fm.2015.01.017.

GATTI, M., J. D. LINDNER, A. DE LORENTIIS, B. BOTTARI, M. SANTARELLI, V. BERNINI, AND E. NEVIANI. 2008. Dynamics of whole and lysed bacterial cells during parmigiano-reggiano cheese production and ripening. **Applied and Environmental Microbiology** 74:6161-7.

GAVRILOVA, E., ANISIMOVA, E., GABDELKHADIEVA, A., NIKITINA, E., VAFINA, A., YARULLINA, D., BOGACHEV, M., & KAYUMOV, A. (2019). Newly isolated lactic acid bacteria from silage targeting biofilms of foodborne pathogens during milk fermentation. **BMC Microbiology**, 19(1), 1–12. https://doi.org/10.1186/s12866-019-1618-0

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.

GENNARI, M., AND DRAGOTTO, F. (1992). A study of the incidence of different fluorescent *Pseudomonas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. **J. Appl. Bacteriol**. 72, 281–288. doi:10.1111/j.1365-2672.1992.tb01836.x.

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** 67:19–34. doi:10.1016/S0168-1605(01)00445-7.

GLAXO GROUP LTD. 1998. Method of nucleic acid amplification. WO 98/44151, filled April 1, 1998.

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):431–36. 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., BRANCO, G. F., CRUZ, A. G., FARIA, J. DE A. F., AND SHAH, N. P. (2010). Probiotic dairy products as functional foods. Compr. Rev. **Food Sci. Food** Saf. 9, 455–470. doi:10.1111/j.1541-4337.2010.00120.x.

GRANDOS CONDE, C., ACEVEDO CORREA, D., AND TORRES, R. (2013). Calidad de la leche y del suero costeño de los municipios Turbaco, Arjona y Carmen de Bolívar - Colombia. **Rev. Lasallista Investig.** 9, 132–137.

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.

HAGHSHENAS, B., HAGHSHENAS, M., NAMI, Y., KHOSROUSHAHI, A. Y., ABDULLAH, N., BARZEGARI, A., ROSLI, R., & HEJAZI, M. S. (2016). Probiotic assessment of *Lactobacillus plantarum* 15HN and *Enterococcus mundtii* 50H isolated from traditional dairies microbiota. **Advanced Pharmaceutical Bulletin**, 6(1), 37–47. https://doi.org/10.15171/apb.2016.07

HARRINGTON, C. T., E. I. LIN, M. T. OLSON, AND J. R. ESHLEMAN. 2013. Fundamentals of pyrosequencing. **Archives of Pathology and Laboratory Medicine** 137(9):1296–03. 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** 23:125–33. doi:10.1007/s11274-006-9201-x.

HASHEMI, S.M.B., MOUSAVI KHANEGHAH, A., KONTOMINAS, M.G., EŞ, I., SANT'ANA, A.S., MARTINEZ, R.R., DRIDER, D., 2017. Fermentation of sarshir (kaymak) by lactic acid bacteria: antibacterial activity, antioxidant properties, lipid and protein oxidation and fatty acid profile. **J. Sci. Food Agric**. 97, 4595–4603. https://doi.org/10.1002/jsfa.8329

HEAD, S. R., H. K. KOMORI, S. A. LAMERE, T. WHISENANT, F. VAN 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: **Genes, Genomes, Genetics** 8:79–89. 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** 4:50–8. doi:10.1089/wound.2014.0542.

HONG, J. Y., N. K. LEE, S. H. YI, S. P. HONG, AND H. D. PAIK. 2019. Physicochemical features and microbial community of milk kefir using a potential probiotic Saccharomyces cerevisiae KU200284." **Journal of Dairy Science** 102(12):10845–49. doi:10.3168/jds.2019-16384.

HORNÍČKOVÁ, Š., DRAGOUNOVÁ, H., HEJTMÁNKOVÁ, K., AND MICHLOVÁ, T. (2014). Production of benzoic acid in fermented goat's and sheep's milk. **Sci. Agric. Bohem**. 45, 247–253. doi:10.1515/sab-2015-0005.

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): 4765–4774. doi:10.1128/JB.180.24.6793-6793.1998.

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

HUMBLOT, C., AND GUYOT, J. P. (2009). Pyrosequencing of tagged 16S rRNA gene amplicons for rapid deciphering of the microbiomes of fermented foods such as pearl millet slurries. **Appl. Environ. Microbiol**. 75, 4354–4361. 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.

ISHIDA, T., YOKOTA, A., UMEZAWA, Y., TODA, T., AND YAMADA, K. (2005). Identification and characterization of lactococcal and Acetobacter strains isolated from traditional Caucasusian fermented milk. **J. Nutr. Sci. Vitaminol**. (Tokyo). 51, 187–193. doi:10.3177/jnsv.51.187.

ISSA, A. T., AND R. TAHERGORABI. 2019. Milk bacteria and gastrointestinal tract: Microbial composition of milk. In Dietary Interventions in Gastrointestinal Diseases, eds. R. R. Watson and V. R. Preedy, 265–275. **Boca Raton: Academic Press.** doi:10.1016/B978-0-12-814468-8.00022-3.

ITO, A., SATO, Y., KUDO, S., SATO, S., NAKAJIMA, H., AND TOBA, T. (2003). The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychrotrophic food-borne pathogens. **Curr. Microbiol**. 47, 231–236. doi:10.1007/s00284-002-3993-1.

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):239. 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):560–67. 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, 159–182. 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.

JUNQUEIRA, A. C. O., PEREIRA, G. V. M., MEDINA, J. D. C., ALVEAR, M. C. R., ROSERO, R., CARVALHO NETO, D. P., ET AL. (2019). First description of bacterial and fungal communities in Colombian coffee beans fermentation analysed using Illumina-based amplicon sequencing. **Sci. Rep**. 9, 1–10. doi:10.1038/s41598-019-45002-8.

KANDLER, O. (1983). Carbohydrate metabolism in lactic acid bacteria. **Antonie Van Leeuwenhoek** 49, 209–224. doi:10.1016/j.proeng.2016.11.092.

KANG, S. M., KHAN, A. L., HAMAYUN, M., SHINWARI, Z. K., KIM, Y. H., JOO, G. J., ET AL. (2012). *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemicals. **Pakistan J. Bot**. 44, 365–372.

KEBA, A., ROLON, M. L., TAMENE, A., DESSIE, K., VIPHAM, J., KOVAC, J., & ZEWDU, A. (2020). Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. **International Dairy Journal**, 104762. https://doi.org/10.1016/j.idairyj.2020.104762

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. **Journal of Food Science** 76(5):M276-83. doi:10.1111/j.1750-3841.2011.02191.x.

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:e12728. doi:10.1111/jfs.12728.

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.

KIRYU, T., KISO, T., NAKANO, H., OOE, K., KIMURA, T., AND MURAKAMI, H. (2009). Involvement of *Acetobacter orientalis* in the production of lactobionic acid in Caucasian yogurt ("Caspian Sea yogurt") in Japan. **J. Dairy Sci**. 92, 25–34. doi:10.3168/jds.2008-1081.

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 and Technology** 94(4):397–408. doi:10.1007/s13594-014-0167-4.

KORKEALA, H. J., & JOHANNA BJÖRKROTH, K. (1997). Microbiological spoilage and contamination of vacuum-packaged cooked sausages. **Journal of Food Protection**, 60(6), 724–731. https://doi.org/10.4315/0362-028X-60.6.724

KORSAK, N., B. TAMINIAU, M. LECLERCQ, C. NEZER, S. CREVECOEUR, C. FERAUCHE, E. DETRY, V. DELCENSERIE, AND G. DAUBE. 2015. 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.

KUMARESAN, G., AND VILLI, R. A. (2008). Incidence of Pseudomonas species in pasteurized milk. Tamilnadu **J. Vet. Anim. Sci**. 4, 56–59.

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** 71:187–221. 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.

LANE, D. J., PACE, B., OLSEN, G. J., STAHL, D. A., SOGIN, M. L., & PACE, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. **Proceedings of the National Academy of Sciences of the United States of America**, 82(20), 6955–6959. https://doi.org/10.1073/pnas.82.20.6955

LEITE, A. M. O., M. A. L. MIGUEL, R. S. PEIXOTO, A. S. ROSADO, J. T. SILVA, V. MARGARET, AND F. PASCHOALIN. 2013. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. **Brazilian Journal of Microbiology** 349(2):341-9.

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.

LEITNER, G., SILANIKOVE, N., JACOBI, S., WEISBLIT, L., BERNSTEIN, S., AND MERIN, U. (2008). The influence of storage on the farm and in dairy silos on milk quality for cheese production. **Int. Dairy J.** 18, 109–113. doi:10.1016/j.idairyj.2007.09.001.

LEONARD, S. R., MAMMEL, M. K., LACHER, D. W., & ELKINS, C. A. (2015). Application of metagenomic sequencing to food safety: Detection of shiga toxin-producing *Escherichia coli* on fresh bagged spinach. **Applied and Environmental Microbiology**, 81(23), 8183– 8191. https://doi.org/10.1128/AEM.02601-1

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** 100:1822–6. 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.

LINARES, D. M., DEL RÍO, B., LADERO, V., MARTÍNEZ, N., FERNÁNDEZ, M., MARTÍN, M. C., ET AL. (2012). Factors influencing biogenic amines accumulation in dairy products. **Front. Microbiol**. 3, 1–10. doi:10.3389/fmicb.2012.00180.

LIU, A., LI, X., PU, B., AO, X., ZHOU, K., HE, L., CHEN, S., & LIU, S. (2017). Use of psychrotolerant lactic acid bacteria (*Lactobacillus spp*. and *Leuconostoc spp*.) isolated from Chinese traditional Paocai for the quality improvement of Paocai products. **Journal of Agricultural and Food Chemistry**, 65(12), 2580–2587. https://doi.org/10.1021/acs.jafc.7b00050

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., Z. H. SUN, 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., M. ZHANG, J. XIE, H. WANG, X. ZHAO, BI. 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, 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, 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.

LÓPEZ-DÍAZ, T. M., ALONSO, C., ROMÁN, C., GARCÍA-LÓPEZ, M. L., AND MORENO, B. (2000). Lactic acid bacteria isolated from a hand-made blue cheese. **Food Microbiol**. 17, 23–32. doi:10.1006/fmic.1999.0289.

ŁOPUSIEWICZ, Ł., DROZŁOWSKA, E., SIEDLECKA, P., MĘŻYŃSKA, M., BARTKOWIAK, A., SIENKIEWICZ, M., ET AL. (2019). Development, characterization, and bioactivity of non-dairy kefir-like fermented beverage based on flaxseed oil cake. **Foods** 8, 544.

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** 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** 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.

MARTIN, N. H., BOOR, K. J., AND WIEDMANN, M. (2018). Symposium review: Effect of post-pasteurization contamination on fluid milk quality. **J. Dairy Sci**. 101, 861–870. doi:10.3168/jds.2017-13339.

MARTÍNEZ, I., MULLER, C. E., AND WALTER, J. (2013). Long-term temporal analysis of the human fecal microbiota revealed a stable core of dominant bacterial species. **PLoS One** 8, e69621. doi:10.1371/journal.pone.0069621.

MATAMOROS, S., PILET, M. F., GIGOUT, F., PRÉVOST, H., AND LEROI, F. (2009). Selection and evaluation of seafood-borne psychrotrophic lactic acid bacteria as inhibitors of pathogenic and spoilage bacteria. **Food Microbiol**. 26, 638–644. doi:10.1016/j.fm.2009.04.011.

MAYO, B., ALEKSANDRZAK-PIEKARCZYK, T., FERNÁNDEZ, M., KOWALCZYK, M., ÁLVAREZ-MARTÍN, P., AND BARDOWSKI, J. (2010). "Updates in the metabolism of lactic acid bacteria," in Biotechnology of Lactic Acid Bacteria: Novel Applications, eds. F. Mozzi, R. R. Raya, and G. M. Vignolo (Iowa: Wiley-Blackwell), 3–33. doi:10.1017/CBO9781107415324.004.

MAYO, B., RACHID, C., ALEGRIA, A., LEITE, A., PEIXOTO, R., AND DELGADO, S. (2014). Impact of next generation sequencing techniques in food microbiology. **Curr. Genomics** 15, 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.

MELEH, H. U., CHOO, S., MOHD DESA, M. N., CHEW, S. Y., RANGASAMY, P., HASSAN, H., & THAN, L. T. L. (2020). Isolation and safety characterization of lactobacilli strains with antimicrobial properties as potential probiotics for human use. Lwt, 131, 109796. https://doi.org/10.1016/j.lwt.2020.109796

MESSENS, W., & DE VUYST, L. (2002). Inhibitory substances produced by Lactobacilli isolated from sourdoughs - A review. **International Journal of Food Microbiology**, 72(1–2), 31–43. https://doi.org/10.1016/S0168-1605(01)00611-0

MIGUEL, M. G. C. P., P. G. CARDOSO, L. A. 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.

MORALES, P., FERNÁNDEZ-GARCÍA, E., AND NUÑEZ, M. (2003). Caseinolysis in cheese by Enterobacteriaceae strains of dairy origin. **Lett. Appl. Microbiol**. 37, 410–414. doi:10.1046/j.1472-765X.2003.01422.x.

MORANDI, S., CREMONESI, P., SILVETTI, T., & BRASCA, M. (2013). Technological characterisation, antibiotic susceptibility and antimicrobial activity of wild-type *Leuconostoc* strains isolated from north Italian traditional cheeses. **Journal of Dairy Research**, 80(4), 457–466. https://doi.org/10.1017/S0022029913000447

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.

MOTATO, K. E., MILANI, C., VENTURA, M., VALENCIA, F. E., RUAS-MADIEDO, P., AND DELGADO, S. (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 Microbiol**. 68, 129–136. 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** 111:431–7. doi:10.1016/j.foodres.2018.05.048.

NAKAI, S. A., AND SIEBERT, K. J. (2004). Organic acid inhibition models for *Listeria innocua, Listeria ivanovii, Pseudomonas aeruginosa* and *Oenococcus oeni*. **Food Microbiol**. 21, 67–72. doi:10.1016/S0740-0020(03)00043-1.

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.

NEUMANN, B., POSPIECH, A., & SCHAIRER, H. U. (1992). Rapid isolation of genomic DNA from Gram-negative bacteria. **Trends in Genetics**, 8(10), 332–333. https://doi.org/10.1016/0168-9525(92)90269-A

NIETO-ARRIBAS, P., SESEÑA, S., POVEDA, J. M., PALOP, L., & CABEZAS, L. (2010). Genotypic and technological characterization of Leuconostoc isolates to be used as adjunct starters in Manchego cheese manufacture. **Food Microbiology**, 27(1), 85–93. https://doi.org/10.1016/j.fm.2009.08.006

OBERMAN, H., AND Z. LIBUDZISZ. 1998. Fermented Milks. In Microbiology of Fermented Foods, ed. B. J. B. Wood, 308–350. 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.

OLANIYI, O. I., ADENIRAN, H. A., & ABIOSE, S. H. (2019). Antimicrobial characteristics of lactic acid bacteria in African yam bean-based drink. **International Food Research Journal**, 26(2), 1733–1740.

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. **Kidlington: Academic Press**. doi:10.1016/B978-0-12-805134-4.00015-8.

ÖZOGUL, F., & HAMED, I. (2018). The importance of lactic acid bacteria for the prevention of bacterial growth and their biogenic amines formation: A review. **Critical Reviews in Food Science and Nutrition**, 58(10), 1660–1670. https://doi.org/10.1080/10408398.2016.1277972

PANESAR, P. S. (2011). Fermented dairy products: Starter cultures and potential nutritional benefits. **Food Nutr. Sci**. 02, 47–51. doi:10.4236/fns.2011.21006.

PARKER, M., S. ZOBRIST, C. DONAHUE, C. EDICK, K. MANSEN, M. H. Z. NADJARI, M. HEERIKHUISEN, 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.

PARRY-HANSON KUNADU, A., ABOAGYE, E. F., COLECRAFT, E. K., OTOO, G. E., ADJEI, M. Y. B., ACQUAAH, E., AFRIFA-ANANE, E., & AMISSAH, J. G. N.

(2019). Low consumption of indigenous fresh dairy products in Ghana attributed to poor hygienic quality**. Journal of Food Protection**, 82(2), 276–286. https://doi.org/10.4315/0362-028X.JFP-18-146

PEREIRA, G. V. M., CARVALHO NETO, D. P., JUNQUEIRA, A. C. D. O., KARP, S. G., LETTI, L. A. J., MAGALHÃES JÚNIOR, A. I., ET AL. (2019). A review of selection criteria for starter culture development in the food fermentation industry. **Food Rev**. Int. doi:10.1080/87559129.2019.1630636.

PICOLI, T., C. M. PETER, J. L. ZANI, ST. 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.

POORNACHANDRA RAO, K., CHENNAPPA, G., SURAJ, U., NAGARAJA, H., CHARITH RAJ, A. P., & SREENIVASA, M. Y. (2015). Probiotic Potential of *Lactobacillus* Strains Isolated from Sorghum-Based Traditional Fermented Food. **Probiotics and Antimicrobial Proteins**, 7(2), 146–156. https://doi.org/10.1007/s12602-015-9186-6

QUAST, C., PRUESSE, E., YILMAZ, P., GERKEN, J., SCHWEER, T., YARZA, P., ET AL. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. **Nucleic Acids Res**. 41, 590–596. doi:10.1093/nar/gks1219.

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.

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'SULLIVAN, O., STANTON, C., BERESFORD, T. P., ROSS, R. P., FITZGERALD, G. F., ET AL. (2013). The complex microbiota of raw milk. **FEMS Microbiol**. Rev. 37, 664–698. doi:10.1111/1574-6976.12030.

RAAK, N., ROHM, H., JAROS, D., 2017. Cross-linking with microbial transglutaminase: Isopeptide bonds and polymer size as drivers for acid casein gel stiffness. **Int. Dairy J**. 66, 49–55. https://doi.org/10.1016/j.idairyj.2016.10.015.

RAVESCHOT, C., CUDENNEC, B., DERACINOIS, B., FRÉMONT, M., VAEREMANS, M., DUGERSUREN, J., DEMBEREL, S., DRIDER, D., DHULSTER, P., COUTTE, F., FLAHAUT, C., 2020. Proteolytic activity of Lactobacillus strains isolated from Mongolian traditional dairy products: A multiparametric analysis. **Food Chem**. 304, 125415. https://doi.org/10.1016/j.foodchem.2019.125415

RAY, B., AND A. BHUNIA. 2007. **Fundamental Food Microbiology**. 4th ed. Boca Raton: CRC Press.

REICHLER, S. J., TRMČIĆ, A., MARTIN, N. H., BOOR, K. J., AND WIEDMANN, M. (2018). *Pseudomonas fluorescens* group bacterial strains are responsible for repeat and sporadic postpasteurization contamination and reduced fluid milk shelf life. J. **Dairy Sci.** 101, 7780–7800. doi:10.3168/jds.2018-14438.

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.

RHEE, S. J., LEE, J. E., & LEE, C. H. (2011). Importance of lactic acid bacteria in Asian fermented foods. **Microbial Cell Factories**, 10(SUPPL. 1), 1–13. https://doi.org/10.1186/1475-2859-10-S1-S5

ROTHBERG, J. M., W. HINZ, T. M. REARICK, J. SCHULTZ, W. MILESKI, M. DAVEY, J. H. LEAMON, ET AL. 2011. An integrated semiconductor device enabling non-optical genome sequencing. **Nature** 475:348–52. doi:10.1038/nature10242.

RUSSO, P., FIOCCO, D., ALBENZIO, M., SPANO, G., & CAPOZZI, V. (2020). Microbial populations of fresh and cold stored donkey milk by high-throughput sequencing provide indication for a correct management of this high-value product. **Applied Sciences** (Switzerland), 10(7). https://doi.org/10.3390/app10072314.

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.

SÁEZ, G. D., FLOMENBAUM, L., & ZÁRATE, G. (2018). Lactic acid bacteria from argentinean fermented foods: Isolation and characterization for their potential use as starters for fermentation of vegetables. **Food Technology and Biotechnology**, 56(3), 398–410. https://doi.org/10.17113/ftb.56.03.18.5631

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.

SALQUE, M., BOGUCKI, P. I., PYZEL, J., SOBKOWIAK-TABAKA, I., GRYGIEL, R., SZMYT, M., ET AL. (2013). Earliest evidence for cheese making in the sixth millennium BC in northern Europe. **Nature** 493, 522–525. doi:10.1038/nature11698.

SAMELIS, J., & GEORGIADOU, K. G. (2000). The microbial association of Greek taverna sausage stored at 4 and 10 °C in air, vacuum or 100% carbon dioxide, and its spoilage potential. **Journal of Applied Microbiology**, 88(1), 58–68. https://doi.org/10.1046/j.1365-2672.2000.00936.x

SAMET-BALI, O., FELFOUL, I., LAJNAF, R., ATTIA, H., AND AYADI, M. A. (2013). Study of proteolytic and lipolytic activities of *Pseudomonas spp*. isolated from pasteurized milk in Tunisia. **J. Agric. Sci.** 5, 46–50. doi:10.5539/jas.v5n7p46.

SASAKI, M., B. W. BOSMAN, AND P. S. T. TAN. 1995. Comparison of proteolytic activities in various lactobacilli. **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.

SAVAIANO, D. A. (2014). Lactose digestion from yogurt: Mechanism and relevance. Am. **J. Clin. Nutr.** 99, 1251S-1255S. doi:10.3945/ajcn.113.073023.1.

SAVELEV, S. U., PERRY, J. D., BOURKE, S. J., JARY, H., TAYLOR, R., FISHER, A. J., ET AL. (2011). Volatile biomarkers of *Pseudomonas aeruginosa* in cystic fibrosis and noncystic fibrosis bronchiectasis. **Lett. Appl. Microbiol**. 52, 610–613. doi:10.1111/j.1472-765X.2011.03049.x.

SAXER, S., SCHWENNINGER, S. M., AND LACROIX, C. (2013). Characterization of the microflora of industrial Mexican cheeses produced without added chemical preservatives. LWT - **Food Sci. Technol**. 53, 314–320. doi:10.1016/j.lwt.2013.01.016.

SCATAMBURLO, T. M., YAMAZI, A. K., CAVICCHIOLI, V. Q., PIERI, F. A., AND NERO, L. A. (2015). Spoilage potential of Pseudomonas species isolated from goat milk. **J. Dairy Sci.** 98, 759–764. doi:10.3168/jds.2014-8747.

SERAFINI, F., TURRONI, F., RUAS-MADIEDO, P., LUGLI, G. A., MILANI, C., DURANTI, S., ET AL. (2014). Kefir fermented milk and kefiran promote growth of *Bifidobacterium bifidum* PRL2010 and modulate its gene expression. **Int. J. Food Microbiol**. 178, 50–59. doi:10.1016/j.ijfoodmicro.2014.02.024.

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** 8:1532. 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.

SHARMA, P. K. (2019). Antimicrobial Profiling of Lactobacillus spp. Isolated from Indian Fermented Food. **International Journal of Pure & Applied Bioscience**, 7(2), 182–189. https://doi.org/10.18782/2320-7051.7389

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. 2016. Molecular methods developed for the identification and characterization of Candida species. **International Journal of Open Access Ophtalmology** 1(3):6. doi:10.15226/2377-4274/4/1/00114.

SINGH, P. K., AND SHAH, N. P. (2017). "Other fermented dairy products: Kefir and koumiss," in **Yogurt in Health and Disease Prevention**, ed. N. P. Shah (Boca Raton: Academic Press), 87–106. doi:10.1016/B978-0-12-805134-4.00005-5.

SLATTERY, L., J. O'CALLAGHAN, G. F. FITZGERALD, T. BERESFORD, AND R. P. ROSS. 2010. 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., L. L. A. IN, S. H. E. LIM, AND R. A. RAHIM. 2017. A review on Lactococcus lactis: From food to factory. **Microbial Cell Factories** 16:55. 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** 7:108. 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, AND Z. SUN. 2020. Profiling of koumiss microbiota and organic acids and their effects on koumiss taste. **BMC Microbiology** 20:85. doi: 10.1186/s12866-020-01773-z

TAŞ, TUĞBA KÖK, F. YEŞIM EKINCI, AND ZEYNEP B. GUZEL-SEYDIM. 2012. "Identification of Microbial Flora in Kefir Grains Produced in Turkey Using PCR." **International Journal of Dairy Technology** 65 (1): 126–131. 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. http://www.arpnjournals.com/jabs/research_papers/rp_2011/jabs_0411_259.pdf.

THARMARAJ, N., AND N. P. SHAH. 2003. Selective Enumeration of *Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus,* and *propionibacteria*." **Journal of Dairy Science** 86 (7). Elsevier: 2288–2296. doi:10.3168/jds.S0022-0302(03)73821-1.

THITARAM, S. N., C. H. CHUNG, D. F. DAY, A. HINTON, J. S. BAILEY, AND G. R. SIRAGUSA. 2005. Isomaltooligosaccharide increases cecal *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., AKASHI, K., MOTOSHIMA, H., URASHIMA, T., ARAI, I., AND SAITO, T. (2009). Microbiota analysis of Caspian Sea yogurt, a ropy fermented milk circulated in Japan. **Anim. Sci.** J. 80, 187–192. doi:10.1111/j.1740-0929.2008.00607.x.

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. **Fermented Beverages** 5:473-527. doi:10.1016/b978-0-12-815271-3.00012-9.

VARDJAN, T., P. MOHAR LORBEG, I. ROGELJ, AND A. ČANŽEK 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.

VIDAL, A. M. C., NETTO, A. S., VAZ, A. C. N., CAPODIFÓGLIO, E., GONÇALVES, A. C. S., ROSSI, G. A. M., ET AL. (2017). *Pseudomonas spp.*: Contamination sources in bulk tanks of dairy farms. **Pesqui. Vet. Bras.** 37, 941–948. doi:10.1590/s0100-736x2017000900008.

VIEIRA, C. P., C. C. CABRAL, B. R. C. C. 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. doi:10.1016/j.jff.2017.01.047.

VIESSER, J. A., DE MELO PEREIRA, G. V., DE CARVALHO NETO, D. P., VANDENBERGHE, L. P. DE S., AZEVEDO, V., BRENIG, B., ROGEZ, H., GÓES-NETO, A., & SOCCOL, C. R. (2020). Exploring the contribution of fructophilic lactic acid bacteria to cocoa beans fermentation: isolation, selection and evaluation. **Food Research International**, 109478. https://doi.org/10.1016/j.foodres.2020.109478

WALSH, A. M., CRISPIE, F., KILCAWLEY, K., O'SULLIVAN, O., O' SULLIVAN, M. G., CLAESSON, M. J., ET AL. (2016). Microbial succession and flavor production

in the fermented dairy beverage kefir. **Appl. Environ. Sci**. 1, e00052-16. doi:10.1128/mSystems.00052-16.Editor.

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.

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

WANIKAWA, A., SHOJI, H., HOSOI, K., AND NAKAGAWA, K. I. (2002). Stereospecificity of 10-hydroxystearic acid and formation of 10-ketostearic acid by lactic acid bacteria. J**. Am. Soc. Brew. Chem**. 60, 14–20. doi:10.1094/asbcj-60-0014.

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, 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.

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, D. SUN, S. LI, T. GU, L. SUN, Y. LUO, M. XU, AND B. ZHANG. 2019. Direct metatranscriptome RNA-Seq and multiplex RT-PCR amplicon sequencing on Nanopore MinION – Promising strategies for multiplex identification of viable pathogens in food. **Preprint.** doi:10.1101/700674.

YANG, J.-M., & MOON, G.-S. (2018). Isolation of a Lactococcus lactis Strain Producing Anti-staphylococcal Bacteriocin. **Korean Journal for Food Science of Animal Resources,** *38*(6), 1315–1321. https://doi.org/10.5851/kosfa.2018.e67

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., HAMEED, S., JAVED, R., AHMED, S., AND IMRAN, M. (2017). Inactivation of foodborne pathogens on food packaging and in cow milk by exposure to a Nd:YAG laser. Can. **J. Phys**. 95, 662–669. 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, 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, 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.

ZECHMAN, M. (1985). Volatiles of Pseudomonas aeruginosa and related species by automated headspace concentration - gas chromatography. **Can. J. Microbiol**. 31, 232–237.

ZHANG, J., & ZHAO, J. (2018). Immuno-biosensor. In Nano-inspired Biosensors for Protein Assay with Clinical Applications. **Elsevier** Inc. https://doi.org/10.1016/B978-0-12-815053-5.00005-2

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.

ZHOU, X. G., L. F. REN, Y. T. LI, M. ZHANG, Y. DE YU, AND J. YU. 2010. The next-generation sequencing technology: A technology review and future perspective. **Science China Life Sciences** 53(1):44–57. doi:10.1007/s11427-010-0023-6.

ZIADI, M., WATHELET, J. P., MARLIER, M., HAMDI, M., AND THONART, P. (2008). Analysis of volatile compounds produced by 2 strains of *Lactococcus lactis* isolated from leben (Tunisian fermented milk) using solid-phase microextraction-gas chromatography. **J. Food Sci**. 73, S247–S252. doi:10.1111/j.1750-3841.2008.00846.x.

ANNEXES

CHAPTER ONE

TABLE A1.1 - MICROBIAL GROUPS IDENTIFIED IN TRADITIONAL FERMENTED MILK PRODUCTS BY CULTURE-INDEPENDENT METHODS.

Culture-independent technique	Fermented milk	Country	Identified bacteria	Reference
PCR-DGGE	*Nyarmie*	Ghana	*Enterococcus durans* *Lactobacillus acidophilus* *Lactobacillus delbrueckii* subsp. *delbrueckii* *Lactobacillus fermentum* *Streptococcus salivarius* *Streptococcus thermophilus*	(Obodai & Dodd, 2006)
PCR-DGGE	*Zabady*	Egypt	*Escherichia coli* *Klebsiella terrigena* *Lactobacillus reuteri* *Lactobacillus casei* *Streptococcus thermophilus*	(El-Baradei et al., 2008)
PCR-DGGE	*Airag*	Mongolia	*Enterococcus* sp. *Lactococcus* sp. *Lactococcus raffinolactis* *Lactococcus lactis* subsp. *lactis* *Lactobacillus helveticus* *Lactobacillus kefiri/buchneri* *Leuconostoc citreum* *Leuconostoc mesenteroides* *Streptococcus parauberis* *Streptococcus thermophilus*	(Miyamoto et al., 2010)

PCR-DGGE	*Undaa*	Mongolia	*Lactococcus raffinolactis/piscium* *Lactococcus lactis* subsp. *lactis* *Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri/buchneri* *Streptococcus parauberis* *Leuconostoc citreum* *Leuconostoc mesenteroides* *Leconostoc lactis*	(Miyamoto et al., 2010)
PCR-DGGE	*Hoormog*	Mongolia	*Enterococcus* sp. *Lactococcus lactis* subsp. *lactis* *Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri/buchneri* *Leuconostoc mesenteroides* *Leconostoc lactis* *Streptococcus parauberis*	(Miyamoto et al., 2010)
PCR-DGGE	Kefir grains	Brazil	*Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactococcus lactis*	(Leite et al., 2012)
PCR-DGGE	*Matsoni*	Georgia	*Streptococcus thermophilus* *Lactococcus raffinolactis* *Lactococcus lactis* subsp. *lactis* *Lactobacillus delbrueckii* subsp. *bulgaricus* *Lactobacillus. paracasei* *Staphylococcus pasteuri*	(Quero et al., 2014)
PCR-DGGE	*Koumiss*	Mongolia	*Acinetobacter johnsonii* *Clostridium acidurci* *Dickeya* sp. *Enterobacter* sp. *Lactococcus lactis* subsp. *lactis* *Pseudomonas* sp. *Raoultella* sp.	(Ringø et al., 2014)

			Ruminococcus sp.	
PCR-DGGE	Kefir grains	Italy	*Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus buchneri* *Lactobacillus sunkii* *Lactobacillus otakiensis*	(Garofalo et al., 2015)
PCR-DGGE	*Suero costeño*	Colombia	*Lactobacillus* sp. *Lactococcus lactis* *Streptococcus salivarius* *Streptococcus* sp.	(Motato et al., 2017)
PCR-DGGE	Yak's milk	China	*Bacterium* sp. *Escherichia coli* *Enterobacter cloacae* *Enterobacter ludwigii* *Lactobacillus bulgaricus* *Lactobacillus helveticus* *Lactococcus lactis* subsp. *cremoris* *Streptococcus thermophilus*	(Ren et al. 2017)

PCR-TTGE	*Zabady*	Egypt	*Lactococcus garvieae* *Lactococcus raffinolactis* *Lactococcus lactis* *Lactobacillus johnsonii* *Lactobacillus gasseri* *Lactobacillus delbrueckii subsp. bulgaricus* *Leuconostoc citreum* *Staphylococcus hyicus* *Streptococcus thermophilus*	(El-Baradei et al., 2008)
qPCR	Kefir grains and kefir milk	Korea	*Lactobacillus* sp. *Lactococcus* sp. *Streptococcus* sp. *Enterococcus* sp.	(Kim et al., 2015)
qPCR	*Lait caillé*	Senegal	*Lactobacillus* sp. *Streptococcus* sp. *Lactococcus* sp. *Weisella* sp. *Enterococcus* sp. *Leuconostoc* sp. *Vagococcus* sp. *Pediococcus* sp.	(Parker et al., 2018)
qPCR	*Doogh*	Iran	*Lactobacillus acidophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus* *Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus amylovorus*	(Sayevand et al., 2018)
qPCR	Mare milk	China	*Bifidobacterium* sp. *Lactobacillus helveticus* *Lactobacillus acidophilus* *Lactobacillus paracasei* *Lactobacillus sakei* *Lactobacillus fermentum*	(Mo et al., 2019)

			Lactobacillus	*plantarum*	
			Lactobacillus delbrueckii subsp.	*bulgaricus*	
T-RFLP	*Kurut*	China	*Enterococcus faecalis* *Enterococcus faecium* *Lactobacillus* *Lactobacillus casei* *Lactobacillus* *Lactobacillus* *Lactococcus lactis* *Leuconostoc mesenteroides* *Leuconostoc* *Pediococcus acidilactici* *Pediococcus pentosaceus*	*fermentum* *plantarum* *helveticus* *lactis*	(Yu et al., 2009)
T-RFLP	Mare's milk	Indonesia	*Lactobacillus* *Lactobacillus plantarum* *Ochrobactrum* *Ochrobactrum* *Staphylococcus* *Staphylococcus* *Staphylococcus*	*rhamnosus* sp. *oryzae* *epidermidis* *aureus* *hominis*	(Mulyawati et al., 2019)

RAPD	*Dahi*	Nepal	*Lactobacillus delbrueckii* subsp. *bulgaricus* *Lactobacillus paracasei* *Lactobacillus rhamnosus* *Lactobacillus fermentum* *Lactobacillus parabuchneri* *Lactobacillus helveticus* *Lactobacillus brevis* *Lactobacillus coryniformis* *Lactobacillus harbinensis* *Lactobacillus plantarum*	(Koirala et al., 2014)

TABLE A1.2 - LIST OF BACTERIAL GROUPS IDENTIFIED IN TRADITIONAL FERMENTED MILK PRODUCTS BY HIGH-THROUGHPUT SEQUENCING.

NGS platform	**Fermented milk**	**Country**	**Identified lactic acid bacteria**	**Other bacteria**	**Reference**
Illumina	Kefir grains and milk	China	*Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactococcus* sp. *Lactococcus lactis* *Leuconostoc* sp. *Leuconostoc mesenteroides*	*Acetobacter* sp. *Acetobacter okinawensis* *Acetobacter orientalis* *Lactobacillus* sp. *Enterobacter* sp. *Porphyromonas* sp.	(Gao & Zhang, 2019)
454 Roche (Pyrosequencing)	Kefir grains and milk	Ireland, United Kingdom, EUA, Spain, France, Italy, Canada, and Germany	*Enterococcus* sp. *Lactobacillus* sp.	*Acetobacter* sp. *Bifidobacterium* sp. *Alistipes* sp. *Allobaculum* sp. *Bacteroides* sp. *Clostridium* sp. *Faecalibacterium* sp.	(Marsh et al., 2013)

454 Roche (Pyrosequencing)	Kefir grains and milk	Italy	*Enterococcus* sp. *Lactobacillus crispatus* *Lactobacillus intestinalis* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactococcus lactis* *Sterptococcus* sp. *Streptococcus thermophilus*	*Acetobacter fabarum* *Acetobacter lovaniensis* *Acetobacter orientalis* *Acetobacter* sp. *Bacillus* sp. *Fusobacterium* sp. *Porphyromonas* sp.	(Garofalo et al., 2015)
Illumina	Kefir milk	South Korea	*Lactobacillus buchneri* *Lactobacillus crispatus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus psittaci*	*Acetobacter fabarum* *Gluconobacter cerevisiae*	(Hong et al., 2019)
Illumina	Kefir grains	China	*Lactobacillus kefiri* *Lactobacillus sunkii* *Lactobacillus* sp. *Lactococcus* sp. *Lactococcus lactis* *Leuconostoc* sp. *Leuconostoc mesenteroides*	*Acetobacter* sp. *Acetobacter orientalis* *Acinetobacter* sp. *Pseudomonas* sp. *Tanticharoenia aidae*	(Han et al., 2018)
Illumina	Kefir grains	South Korea	*Lactobacillus crispatus* *Lactobacillus farraginis* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus psittaci* *Lactococcus lactis* *Leuconostoc mesenteroides*	*Acetobacter fabarum*	(Kim et al., 2020)

454 Roche (Pyrosequencing)	Kefir grains and milk	Belgium	*Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus parakefiri* *Lactococcus kefiri* *Lactococcus lactis* ssp. *cremosis* *Leuconostoc mesenteroides*	*Acetobacter lovaniensis* *Acetobacter orientalis* *Gluconobacter cerinus* *Gluconobacter frateurii* *Enterobacter amnigeus* *Enterobacter* sp. LH-CAB10	(Korsak et al., 2015)
454 Roche (Pyrosequencing)	Kefir grains	Brazil	*Lactobacillus* sp. *Lactobacillus amilovorus* *Lactobacillus buchneri* *Lactobacillus crispatus* *Lactobacillus kefiranofaciens* subsp. *kefiranofaciens* *Lactobacillus kefiranofaciens* subsp. *kefirgranum* *Lactobacillus kefiri* *Lactobacillus parabuchner* *Lactobacillus parakefiri* *Lactococcus* sp. *Lactococcus lactis* subsp. *cremosis* *Leuconostoc* sp. *Streptococcus* sp.	*Acetobacter* sp. *Bifidobacterium* sp. *Pseudomonas* sp. *Solirubacter* sp.	(Leite et al., 2012)
Illumina	Kefir grains	China	*Lactobacillus* sp. *Lactobacillus delbrueckii* subsp. *bulgaricus* *Lactobacillus kefiranofaciens* subsp. *kefirgranum* *Lactobacillus kefiri*	*Acetobacter* sp. *Acetobacter syzygii*	(Wenwen Liu et al., 2019)

454 Roche (Pyrosequencing)	Kefir grains and milk	Ireland, United Kingdom, USA, Spain, France, Italy, Canada, and Germany	*Enterococcus* sp. *Lactobacillus* sp. *Lactococcus* sp. *Leuconostoc* sp.	*Acetobacter* sp. *Bifidobacterium* sp. *Alistipes* sp. *Allobaculum* sp. *Bacteroides* sp. *Clostridium* sp. *Faecalibacterium* sp.	(Marsh et al., 2013)
Illumina	Kefir grains	United Kingdom, France, and Ireland	*Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus sanfranciscensis* *Lactobacillus* sp. *Lactococcus* sp. *Leuconostoc carnosum* *Leuconostoc citreum* *Leuconostoc gelidum* *Leuconostoc kimchii* *Leuconostoc mesenteroides* *Leuconostoc* sp. *Streptococcus* sp.	*Acetobacter pasteurianus* *Acetpbacter* sp. *Gluconobacter diazotrophicus* *Gluconobacter oxydans* *Bifidobacterium* sp. *Acinetobacter* sp. *Anoxybacillus* sp. *Brochothrix* sp. *Clostridium* sp. *Enterobacter* sp. *Escherichia* sp. *Geobacillus* sp. *Propinobacterium* sp. *Pseudomonas* sp. *Serratia* sp. *Thermus* sp. *Turicibacter* sp.	(Walsh et al., 2016)

454 Roche (Pyrosequencing)	Kefir grains	Turkey	*Lactobacillus* sp. *Lactobacillus acidophilus* *Lactobacillus amylovorus* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus casei* *Lactobacillus crispatus* *Lactobacillus delbrueckii* *Lactobacillus diolivorans* *Lactobacillus gallinarum* *Lactobacillus gasseri* *Lactobacillus helveticus* *Lactobacillus johnsonii* *Lactobacillus kalixensis* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus otakiensis* *Lactobacillus parabuchneri* *Lactobacillus paracasei* *Lactobacillus parafarraginis* *Lactobacillus parakefiri* *Lactobacillus pentosus* *Lactobacillus plantarum* *Lactobacillus rapi* *Lactobacillus reuteri* *Lactobacillus rhamnosus* *Lactobacillus rossiae* *Lactobacillus sakei* *Lactobacillus salivarius* *Lactobacillus sunkii* *Lactococcus garvieae* *Lactococcus lactis* *Leuconostoc mesenteroides*	Not identified	(Nalbantoglu et al., 2014)

			Oenococcus oeni *Pediococcus* sp. *Pediococcus claussenii* *Pediococcus damnosus* *Pediococcus halophilus* *Pediococcus lolii* *Pediococcus pentosaceus* *Tetragenococcus halophilus*		
Illumina	Kefir milk	USA	*Lactobacillus acidophilus* *Lactobacillus crispatus* *Lactobacillus delbrueckii* *Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri*	Not identified	(Dallas et al., 2016)
Illumina	Kefir grains	Turkey	*Enterococcus* sp. *Enterococcus lactis* *Lactobacillus apis* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus* sp. *Lactobacillus ultunensis* *Lactococcus* sp. *Pseudomonas aeruginosa* *Pseudomonas azotoformans* *Pseudomonas otitidis* *Sphingomonas* sp.	*Acinetobacter* sp. *Acinetobacter calcoaceticus* *Acinetobacter rhizosphaerae* *Enterobacter* sp. *Enterobacter amnigenus* *Enterobacter hormaechei* *Enterobacter soli* *Enterobacteriaceae* *Halomonas* sp. *Propionibacterium acnes* *Pseudomonas* sp.	(Dertli & Çon, 2017)

454 Roche (Pyrosequencing)	Kefir grains	Ireland	*Lactobacillus acidophilus* *Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiranofaciens ssp. kefirgranum* *Lactobacillus kefiri* *Lactobacillus parabuchneri* *Lactobacillus* sp. *Lactobacillus parakefiri* *Lactococcus garvieae* *Lactococcus lactis* *Leucoconstoc* sp.	*Bifidobacterium* sp. *Bifidobacterium breve* *Bifidobacterium choerinum* *Bifidobacterium longum* *Bifidobacterium pseudolongum*	(Dobson et al., 2011)

Illumina	Kefir grains	Malaysia	*Lactobacillus* sp. *Lactobacillus acidophilus* *Lactobacillus animalis* *Lactobacillus apis* *Lactobacillus buchneri* *Lactobacillus casei* *Lactobacillus crispatus* *Lactobacillus dulbrueckii* *Lactobacillus faeni* *Lactobacillus gigeriorum* *Lactobacillus helveticus* *Lactobacillus hilgardi* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus lactis* *Lactobacillus nagelii* *Lactobacillus parakefiri* *Lactobacillus rhamnosus* *Lactobacillus senmaizukei* *Lactobacillus taiwanensis* *Lactobacillus thailandensis* *Lactobacillus tucceti* *Lactobacillus ultunensis* *Lactococcus* sp. *Pediococcus* sp. *Pediococcus argentinicus* *Pediococcus cellicola* *Streptococcus* *Streptococcus thermophilus*	*Acinetobacter* sp. *Acinetobacter* sp. *Acinetobacter johnsonii* *Acinetobacter tjernbergiae* *Bacteroides* sp. *Cohnella soli* *Mesoplasma entomophilum* *Phyllobacterium* *Phylobacterium myrsinacearum* *Rhodococcus erythropolis* *Rhodococcus qingshengii* *Rothia amarae* *Staphylococcus cohnii*	(Zamberi et al., 2016)
Illumina	*Dahi*	India	*Lactococcus lactis* *Lactobacillus helveticus* *Leuconostoc mesenteroides*	*Acetobacter pasteurianus* *Gluconobacter oxydans*	(Shangpliang et al., 2018)

			Staphylococcus cohnii *Enterococcus* sp.	*Pseudomonas* sp. *Bacillus* sp. *Clostridium* sp. *Streptococcus thernophilus* *Acetobacter lovaniensis* *Acetobacter syzygii*	
SMRT PacBio	*Koumiss*	Mongolia	*Lactobacillus helveticus* *Lactococcus lactis* *Streptococcus parauberis* *Lactobacillus kefiranofaciens* *Lactobacillus raffinolactis* *Streptococcus thermophilus* *Lactobacillus gallinarum* *Enterococcus durans*	*Rothia nasimurium* *Macrococcus casolyticus* *Acetobacter pasteurianus*	(Gesudu et al., 2016)
454 Roche (Pyrosequencing)	Buttermilk	India	*Enterococcus faecalis* *Lactobacillus delbrueckii* *Streptococcus thermophilus* *Streptococcus mutans*	*Nesterenkonia* sp. *Flavobacterium* sp. *Macrococcus caseolyticus* *Methylobacterium populi* *Ralstonia solanacearum* *Aeromonas hydrophila* *Aeromonas salmonicida* *Dictyglomus turgidum* *Methylobacterium raiotolerans*	(Jayashree et al., 2013)

				Psychrobacter arcticus *Bartonella quintana*	
454 Roche (Pyrosequencing)	Natural fermented cow's milk	Russia	*Lactococcus* sp. *Enterococcus* sp. *Lactobacillus* sp. *Streptococcus* sp. *Leuconostoc* sp.	*Macrococcus* sp. *Bacillus* sp. *Kluyvera* sp. *Acinetobacter* sp. *Citrobacter* sp. *Enterobacter* sp. *Acetobacter* sp.	(Wenjun Liu et al., 2015)
454 Roche (Pyrosequencing)	*Airag*	Mongolia	*Enterococcus durans* *Enterococcus italicus* *Kocuria rhizophila* *Komagataeibacter europaeus* *Lactobacillus crustorum* *Lactobacillus delbrueckii* *Lactobacillus diolivorans* *Lactobacillus helveticus* *Lactobacillus hilgardii* *Lactobacillus kefiranfaciens* *Lactobacillus kefiri* *Lactobacillus paracasei* *Lactobacillus parakefiri* *Lactobacillus* sp. *Lactococcus lactis* *Lactococcus raffinolactis* *Lactococcus* sp. *Leuconostoc mesenteroides* *Streptococcus parauberis* *Streptococcus thermophilus*	*Acetobacter ghanensis* *Acetobacter malorum* *Acetobacter pasteurians* *Arthrobacter russicus* *Brevundimonas nasdae* *Bifidobacterium mongoliense* *Citrococcus* sp. *Enhydrobacter aerosaccus* *Shigella flexneri*	(Oki et al., 2014)

454 Roche (Pyrosequencing)	*Khoormog*	Mongolia	*Lactobacillus helveticus* *Lactobacillus kefiranofaciens* *Lactobacillus kefiri* *Lactobacillus paracasei* *Lactobacillus pentosus* *Lactococcus lactis* *Leuconostoc mesenteroides* *Streptococcus gallolyticus*	*Acetobacter ghanensis* *Acetobacter malorum* *Acetobacter pasteurianus* *Aeromonas media* *Arthrobacter russicus* *Bacillus cereus* *Brevundimonas nasdae* *Macrococcus caseolyticus* *Shigella flexneri* *Yokenella regensburgei*	(Oki et al., 2014)
454 Roche (Pyrosequencing)	*Tarag* (Cow's milk)	Mongolia	*Lactobacillus delbrueckii* *Lactobacillus fermentum* *Lactobacillus helveticus* *Lactococcus lactis* *Streptococcus thermophilus*	*Acetobacter malorum* *Arthobacter russicus* *Brevundimonas nasdae*	(Oki et al., 2014)
Illumina	*Lait caillé*	Senegal	*Streptococcus* sp. *Lactobacillus* sp. *Lactococcus* sp. *Enterococcus* sp. *Weissella* sp. *Leuconostoc* sp. *Pediococcus* sp.	*Acetobacter* sp. *Acinetobacter* sp. *Kocuria* sp. *Kurthia* sp. *Macrococcus* sp. *Shigella* sp. *Bacillus* sp. *Staphylococcus* sp. *Pseudomonas* sp. *Vagococcus* sp. *Gluconobacter* sp. *Stenotrophomonas* sp. *Salmonella* sp. *Bifidobacterium* sp. *Chryseobacterium* sp. *Clostridium* sp.	(Parker et al., 2018)

				Enhydrobacter sp. *Corynebacterium* sp. *Pantoea* sp. *Exiguobacterium* sp. *Enterobacter* sp. *Aeromonas* sp. *Undibacterium* sp. *Viridibacillus* sp. *Actinomyces* sp. *Aquabacterium* sp. *Rothia* sp. *Geobacillus* sp. *Xanthomonas* sp. *Streptophyta* sp. *Citrobacter* sp.	
454 Roche (Pyrosequencing)	*Tarag* (Cow's milk)	Mongolia	*Lactobacillus* sp. *Streptococcus* sp. *Lactococcus* sp. *Leuconostoc* sp.	*Acinetobacter* sp. *Enhydrobacter* sp. *Cloacibacterium* sp. *Acetobacter* sp. *Macrococcus* sp. *Chryseobacterium* sp. *Delftia* sp. *Escherichia* sp. *Rhizobium* sp. *Roseomonas* sp. *Kurthia* sp. *Enterobacter* sp. *Klebsiella* sp. *Bacillus* sp. *Citrobacter* sp. *Bifidobacterium* sp.	(Sun et al., 2014)

454 Roche (Pyrosequencing)	*Tarag* (Cow's milk)	China	*Lactobacillus* sp. *Streptococcus* sp. *Lactococcus* sp. *Leuconostoc* sp.	*Acinetobacter* sp. *Enhydrobacter* sp. *Cloacibacterium* sp. *Acetobacter* sp. *Macrococcus* sp. *Chryseobacterium* sp. *Delftia* sp. *Escherichia* sp. *Rhizobium* sp. *Roseomonas* sp. *Kurthia* sp. *Enterobacter* sp. *Klebsiella* sp. *Bacillus* sp. *Citrobacter* sp. *Bifidobacterium* sp.	(Sun et al., 2014)
Illumina	*Nunu*	Ghana	*Pediococcus* sp. *Weissella confusa* *Lactobacillus fermentum* *Lactobacillus plantarum* *Leuconostoc pseudomesenteroides* *Enterococcus faecium* *Lactococcus lactis* *Streptococcus infantarius*	*Pseudomonas* sp. *Acetobacter* sp. *Exiguobacterium* sp. *Staphylococcus* sp. *Citrobacter* sp. *Enhydrobacter* sp. *Kurthia* sp. *Gluconobacter* sp. *Rhodococcus* sp. *Kocurcia* sp. *Macrococcus caseolyticus* *Bacillus* sp. *Acinetobacter baumannii* *Enterobacter* sp. *Klebsiella pneumoniae*	(Walsh et al., 2017)

Illumina	*Koumiss*	Mongolia	*Lactobacillus helveticus* *Streptococcus parauberis* *Lactococcus lactis* *Lactobacillus kefiri* *Leuconostoc mesenteroides* *Lactobacillus plantarum* *Lactobacillus acetotolerans* *Streptococcus pluranimalium* *Streptococcus dysgalactia* *Lactobacillus brevis* *Lactococcus raffinolactis* *Lactobacillus casei* str. *Zhang*	*Rothia nasimurium* *Acetobacter pasteurians* *Acinetobacter johnsonii* *Enterobacter cloaceae* *Staphylococcus aureus* *Raoutella ornithinolytica* *Escherichia coli* *Serratia grimesii* *Prevotella* sp. *Microbacterium esteraromaticum* *Rhizobium radiobacter* LBA4213 *Parabacteroides merdae* *Bacteroides caccae* *Neisseria sicca* *Bacteroides uniformis* *Bacteroides ovatus* *Bacteroides plebeius* *Bacteroides coprocola* *Brevundimonas diminuta*	(Wurihan et al., 2019)
Illumina	Fermented mare's milk	Indonesia	*Lactobacillus* sp. *Lactococcus* sp.	*Brachybacterium* sp. *Chroococcidiopsis* sp. *Acetobacter* sp. *Reyranella* sp. *Novosphingobium* sp. *Ralstonia* sp. *Enterobacter* sp. *Shigella* sp. *Acinetobacter* sp.	(Jatmiko et al., 2019)

Illumina	Fermented yak's milk	China	*Lactobacillus helveticus* *Lactobacillus delbrueckii* *Lactobacillus acidophilus* *Lactobacillus casei* *Lactobacillus fermentum* *Lactobacillus plantarum* *Lactobacillus amylovorus* *Streptococcus* sp. *Lactococcus* sp.	*Acetobacter* sp. *Bifidobacterium* sp. *Aggregatibacter* sp.	(Jiang et al., 2020)
Illumina	*Suero costeño*	Colombia	*Lactobacillus* sp. *Streptococcus* sp. *Leuconostoc* sp. *Lactococcus* sp.	*Bifidobacterium* sp. *Staphylococcus* sp. *Acetobacter* sp. *Aromonas* sp. *Enterobacter* sp. *Shigella* sp. *Klebsiella* sp. *Pantoea* sp.	(Motato et al., 2017)

CHAPTER TWO

FIGURE A2.1 - ALPHA RAREFACTION CURVES OF OBSERVED BACTERIAL OTUS (OPERATIONAL TAXONOMIC UNITS) FROM THE TEMPORAL SAMPLES OF CASPIAN SEA-STYLE FERMENTED MILK **(A)**. RAW MILK AND VIABILITY TESTS **(B)**.

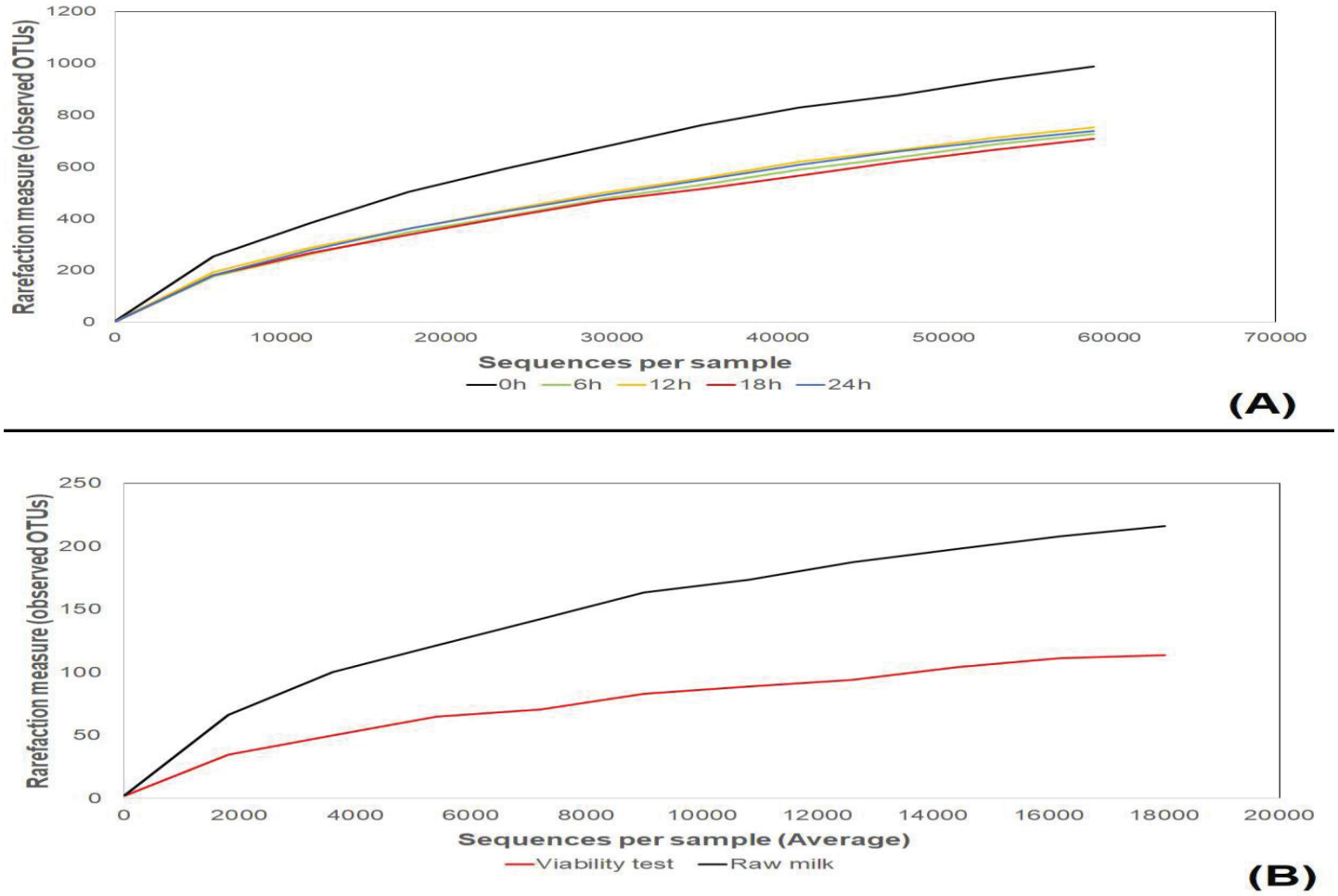


TABLE A2.1 - RELATIVE ABUNDANCE (%) OF LOW ABUNDANCE BACTERIA AT FAMILY AND GENUS LEVEL DURING CASPIAN SEA-STYLE FERMENTATION PROCESS. ND = NOT DETERMINED; VT = VIABILITY TEST.

Family	**Genera**	**Fermentation time (h)**						
		Raw milk	0h	6h	12h	18h	24h	VT
Bacteria								
Nitrososphaeraceae	*Candidatus Nitrososphaera*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Erysipelotrichaceae	*[Eubacterium]*	-	0.01	<0.01	<0.01	<0.01	<0.01	-
[Paraprevotellaceae]	*[Prevotella]*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Lachnospiraceae	*[Ruminococcus]*	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bacteroidaceae		-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Acetobacteraceae	*Acetobacter*	<0.01	0.01	<0.01	<0.01	0.01	0.01	0.01
Actinomycetaceae	*Actinomyces*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Nocardioidaceae	*Actinopolymorpha*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Aerococcaceae	*Aerococcus*	<0.01	-	-	-	-	-	<0.01
Bradyrhizobiaceae	*Afipia*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Erysipelotrichaceae	*Allobaculum*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Paenibacillaceae	*Ammoniphilus*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Anaplasmataceae	*Anaplasma*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Xanthomonadaceae	*Aquimonas*	-	<0.01	0.01	<0.01	<0.01	<0.01	-
Rhodospirillaceae	*Azospirillum*	-	<0.01	<0.01	<0.01	<0.01	<0.01	-
Bacteroidaceae	*Bacteroides*	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bifidobacteriaceae	*Bifidobacterium*	-	0.02	0.02	0.01	0.02	0.02	-

Lachnospiraceae	*Blautia*	-		0.01	<0.01	<0.01	0.01	0.01	-
Dermabacteraceae	*Brachybacterium*		0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Paenibacillaceae	*Brevibacillus*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Brevibacteriaceae	*Brevibacterium*		0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
Burkholderiaceae	*Burkholderia*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
[Odoribacteraceae]	*Butyricimonas*		0.01	-	-	-	-	-	<0.01
Halomonadaceae	*Candidatus Portiera*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Carnobacteriaceae	*Carnobacterium*	-		0.04	0.02	0.02	<0.01	0.01	-
Erysipelotrichaceae	*Catenibacterium*	-		<0.01	<0.01	<0.01	<0.01	0.01	-
[Paraprevotellaceae]	ND	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
[Weeksellaceae]	*Cloacibacterium*	-		0.01	<0.01	<0.01	<0.01	<0.01	-
Clostridiaceae	*Clostridium*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Coriobacteriaceae	*Collinsella*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Conexibacteraceae	*Conexibacter*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Erysipelotrichaceae	*Coprobacillus*		0.01	-	-	-	-	-	<0.01
Lachnospiraceae	*Coprococcus*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Corynebacteriaceae	*Corynebacterium*		0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Oxalobacteraceae	*Cupriavidus*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Lachnospiraceae	*Dorea*		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Neisseriaceae	*Eikenella*	-		<0.01	<0.01	<0.01	<0.01	<0.01	-
Moraxellaceae	*Enhydrobacter*	-		0.01	<0.01	<0.01	<0.01	<0.01	-

Enterobacteriaceae	*Enterobacter*		0.01	-		-		-		-		-			<0.01
Enterococcaceae	*Enterococcus*		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01
Enterobacteriaceae	*Erwinia*		0.01		0.03		0.01		0.01		0.01		0.01		0.03
Erythrobacteraceae	*Erythrobacter*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Euzebyaceae	*Euzebya*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Ruminococcaceae	*Faecalibacterium*		0.04		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01
Fibrobacteraceae	*Fibrobacter*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Flavobacteriaceae	*Flavobacterium*		0.03	-		-		-		-		-			<0.01
Fusobacteriaceae	*Fusobacterium*	-			0.03		<0.01		<0.01		0.01%		<0.01		-
Geobacteraceae	*Geobacter*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Bogoriellaceae	*Georgenia*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Halomonadaceae	*Halomonas*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Hyphomonadaceae	*Hyphomonas*	-			<0.01		0.05		<0.01		<0.01		<0.01		-
Oxalobacteraceae	*Janthinobacterium*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Staphylococcaceae	*Jeotgalicoccus*		0.01	-		-		-		-		-			<0.01
Enterobacteriaceae	*Klebsiella*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Lactobacillaceae	*Lactobacillus*		0.07		0.04		0.03		0.03		0.03		0.04		0.03
Burkholderiaceae	*Lautropia*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-
Bacillaceae	*Lentibacillus*		0.01	-		-		-		-		-			<0.01
Microbacteriaceae	*Leucobacter*		0.03	-		-		-		-		-			<0.01
Xanthomonadaceae	*Luteimonas*	-			<0.01		<0.01		<0.01		<0.01		<0.01		-

Veillonellaceae	*Megamonas*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Veillonellaceae	*Megasphaera*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Methylobacteriaceae	*Methylobacterium*		0.15		0.01		<0.01		<0.01		<0.01		<0.01		0.01
Moraxellaceae	*Moraxella*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Micrococcaceae	*Nesterenkonia*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Ruminococcaceae	*Oscillospira*		0.01	-		-		-		-		-			<0.01
Paenibacillaceae	*Paenibacillus*		0.07	-		-		-		-		-			<0.01
Enterobacteriaceae	*Pantoea*	-			0.01		<0.01		<0.01		<0.01		<0.01		-
Hyphomicrobiaceae	*Parvibaculum*	-		<0.01		0.01		<0.01		<0.01		<0.01		-	
Pasteurellaceae	*Pasteurella*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Sphingobacteriaceae	*Pedobacter*	-			0.01		<0.01		<0.01		<0.01		<0.01		-
Peptococcaceae	*Peptococcus*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Veillonellaceae	*Phascolarctobacterium*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Comamonadaceae	*Polaromonas*		0.01	-		-		-		-		-			<0.01
Porphyromonadaceae	*Porphyromonas*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Nocardiopsaceae	*Prauseria*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Enterobacteriaceae	*Proteus*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Enterobacteriaceae	*Providencia*		0.04	-		-		-		-		-			<0.01
Pseudonocardiaceae	*Pseudonocardia*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	
Eubacteriaceae	*Pseudoramibacter*	-		<0.01		<0.01		<0.01		<0.01		<0.01		-	

Oxalobacteraceae	*Ralstonia*		0.01	-		-		-		-	
Nocardiaceae	*Rhodococcus*	-			<0.01		<0.01		<0.01		<0.01
Planococcaceae	*Rummeliibacillus*	-			<0.01		<0.01		<0.01		<0.01
Staphylococcaceae	*Salinicoccus*		<0.01		<0.01		<0.01		<0.01		<0.01
Sanguibacteraceae	*Sanguibacter*		<0.01		<0.01		<0.01		<0.01		<0.01
Planococcaceae	*Solibacillus*		0.03	-		-		-		-	
Sphingobacteriaceae	*Sphingobacterium*	-			<0.01		<0.01		<0.01		<0.01
Xanthomonadaceae	*Stenotrophomonas*		<0.01		0.01		<0.01		<0.01		<0.01
Succinivibrionaceae	*Succinivibrio*		0.01	-		-		-		-	
Alcaligenaceae	*Sutterella*		0.03	-		-		-		-	
Symbiobacteriaceae	*Symbiobacterium*	-			<0.01		<0.01		<0.01		<0.01
Synechococcaceae	*Synechococcus*	-			<0.01		<0.01		<0.01		<0.01
Spirochaetaceae	*Treponema*		0.05		<0.01		<0.01		<0.01		<0.01
Turicibacteraceae	*Turicibacter*	-			0.01		0.02		0.01		0.01
Enterococcaceae	*Vagococcus*	-			0.01		<0.01		<0.01		<0.01
Vibrionaceae	*Vibrio*	-			<0.01		0.01		0.01		<0.01
Bacillaceae	*Virgibacillus*		<0.01	-		-		-		-	
[Weeksellaceae]	*Wautersiella*	-			0.01		<0.01		<0.01		0.01
Yaniellaceae	*Yaniella*		<0.01		<0.01		<0.01		<0.01		<0.01
[Marinicellaceae]	ND	-			<0.01		<0.01		<0.01		<0.01
[Weeksellaceae]	ND	-			<0.01		<0.01		<0.01		<0.01

Acetobacteraceae	ND		<0.01		0.03		0.02		0.02		0.05		0.03	0.01
Aerococcaceae	ND		0.01	-		-		-		-		-		<0.01
Aeromonadaceae	ND	-			0.01		<0.01		<0.01		<0.01		<0.01	-
Bacillaceae	ND		0.08		<0.01		<0.01		<0.01		<0.01		<0.01	0.01
Bradyrhizobiaceae	ND	-			0.01		0.02		<0.01		0.01		<0.01	-
Cardiobacteriaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Caulobacteraceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	
Clostridiaceae	ND		0.02		0.01		0.01		0.01		0.01		0.01	<0.01
Coxiellaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Enterococcaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Erysipelotrichaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Flavobacteriaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Gaiellaceae	ND		<0.01	-		-		-		-		-		<0.01
Hyphomicrobiaceae	ND	-			<0.01		0.01		<0.01		<0.01		<0.01	-
Hyphomonadaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Iamiaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Intrasporangiaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Koribacteraceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-
Lachnospiraceae	ND		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01	<0.01
Lactobacillaceae	ND		0.00	-		-		-		-		-		0.01
Microbacteriaceae	ND	-			<0.01		<0.01		<0.01		<0.01		<0.01	-

Micrococcaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Micromonosporaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Moraxellaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Neisseriaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Nitriliruptoraceae	ND	-	<0.01	0.01	<0.01	<0.01	<0.01	-	
Nitrosomonadaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Nitrospiraceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Nocardiopsaceae	ND	0.03	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01
Pasteurellaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Patulibacteraceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Peptostreptococcaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Phyllobacteriaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Pirellulaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Pseudoalteromonadaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Pseudonocardiaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Rhodobacteraceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Rhodospirillaceae	ND	0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01
Rikenellaceae	ND	<0.01	-	-	-	-	-		<0.01
Thermomonosporaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	
Xanthomonadaceae	ND	0.01%	<0.01	0.01%	<0.01	<0.01	<0.01		<0.01
Xenococcaceae	ND	-	<0.01	<0.01	<0.01	<0.01	<0.01	-	