UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

Analysis of bacterial diversity in Brazilian dairy samples by culture-based methods and metataxonomics

Análise da diversidade bacteriana em amostras de laticínios brasileiros através de técnica de cultivo e metataxonomia

DIEGO DE ARAÚJO FRAZILIO

Ribeirão Preto
2019

UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

DIEGO DE ARAÚJO FRAZILIO

Analysis of bacterial diversity in Brazilian dairy samples by culture-based methods and metataxonomics

Análise da diversidade bacteriana em amostras de laticínios brasileiros através de técnica de cultivo e metataxonomia

Doctoral thesis presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Doctor in Sciences.

Concentration Area: Bioagentes e Biotecnologia Aplicados à Farmácia

Supervisor: Prof. Elaine Cristina Pereira De Martinis, Ph.D.

Versão corrigida da Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biociências e Biotecnologia em 07/06/2019. A versão original encontra-se disponível na Faculdade de Ciências Farmacêuticas de Ribeirão Preto/USP

Ribeirão Preto

2019

RESUMO

FRAZILIO, D. A. **Análise da diversidade bacteriana em amostras de laticínios por meio de técnicas de cultivo e metataxonômica**. 2019. 95p. Tese. Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, 2019.

A segurança alimentar é uma questão de importância mundial e o conhecimento de microorganismos cultiváveis e não cultiváveis é de aspecto fundamental para compreender a ecologia microbiana, a fim de propor estratégias para a conservação de alimentos e prevenção de surtos de origem alimentar. O leite e os produtos lácteos são altamente perecíveis e suscetíveis à contaminação por bactérias patogênicas. Assim, é importante determinar quais microorganismos estão presentes nas diferentes etapas do processamento de laticínios e nos produtos prontos para consumo. No capítulo 3 desta tese, *Staphylococcus aureus*, *Listeria monocytogenes* e a microbiota acompanhante foram investigadas de 97 amostras de cinco fábricas de laticínios (localizadas nos estados de São Paulo e Goiás - Brasil) e os isolados foram identificados por sequenciamento do DNA utilizando o método de Sanger (gene 16S rRNA). Nenhuma amostra foi positiva para *Listeria sp*, porem a presença de *S. aureus* foi altamente prevalente. A microbiota acompanhante foi composta principalmente por bactérias produtoras de ácido lático (LAB), mas outras bactérias também estavam presentes. Esta parte da tese indicou a necessidade de um melhor controle de *S. aureus* nas fabricadas de laticínios avaliadas. No capítulo 4, amostras de fábricas de laticínios brasileiras (localizada no estado de São Paulo) previamente positivas para *S. aureus* foram analisadas pelo sequenciamento do gene 16S rRNA, dividido em quatro grupos (matéria-prima, produto final, superfícies de contato e sem-contato com o alimento). Os resultados demonstraram altos índices de diversidade alfa (produto final e superfície sem contato com o alimento), mas os índices de diversidade beta foram baixos, as amostras foram separadas em dois grupos principais onde a comunidade bacteriana era dominada por *Macrococcus*, *Alkaliphilus*, *Vagococcus*, *Lactobacillus*, *Marinilactibacillus*, *Streptococcus*, *Lisinibacillus*, *Staphylococcus*, *Clostridium*, *Halomonas*, *Lactococcus*, *Enterococcus*, *Bacillus* e *Psychrobacter*. No capítulo 5, foram analisadas 27 amostras de duas fábricas de laticínios do Estado de São Paulo para cultura da microbiota autóctone e os isolados foram identificados por meio de sequenciamento de DNA. Além disso, o DNA metagenômico foi diretamente extraído das amostras e a microbiota não cultivável foi avaliada através do sequenciamento massivo do gene 16S rRNA. Os resultados de obtidos com as culturas bacterianas, indicaram que a maioria dos isolados eram dos grupos das bactérias láticas, mas não somente essas, foram detectadas também da ordem *Enterobacterales* e das famílias *Staphylococcacceae*, *Bacillaceae*, *Pseudomonadaceae* e *Moraxellaceae*. A partir da metataxonomia, construiu-se um heatmap onde foram determinadas as 20 unidades taxonômicas operacionais (OTUs) mais abundantes, revelando uma significativa dissimilaridade da microbiota de ambos os laticínios. Foram encontradas 12 taxa bacteriana mais prevalentes na microbiota dos laticínios avaliados, com a maior abundância de OTUs de *Tolumonas auensis* e *Lactococcus fujiensis*. No geral, os resultados desta tese revelam a ecologia microbiana altamente complexa de alimentos lácteos e revelaram novas combinações de espécies mistas a partir de abordagens de bactérias cultiváveis e não culturais. A partir desses resultados, é interessante desenvolver novos estudos para avaliar

possíveis correlações positivas ou negativas entre os membros microbianos e suas possíveis implicações para a segurança alimentar.

Palavras-chave: microbiota do leite, metataxonômica, laticinios, microbiota de queijos.

ABSTRACT

FRAZILIO, D. A. **Analysis of bacterial diversity in dairy environment samples using culture dependent and metataxonomic techniques.** 2019. 95p. Thesis. Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, 2019.

Food safety is a matter of worldwide importance and the knowledge of cultivable and non-cultivable microorganisms is a key aspect to understand the microbial ecology, in order to propose strategies for food preservation and prevention of foodborne outbreaks. Milk and dairy products are highly perishable and susceptible to contamination by spoilage and pathogenic bacteria. Thus, it is important to determine the microorganisms present in different steps of dairy processing and in the readyu-to-eat products. In chapter 3 of this thesis, *Staphylococcus aureus*, *Listeria monocytogenes* and accompanying microbiota were screened from 97 samples of five dairies (located in São Paulo and Goiás states (Brazil) and the isolates were identified by Sanger DNA sequencing (16S rRNA gene). No sample was positive for *Listeria* sp. but *S. aureus* was highly prevalent. The background microbiota was composed mainly by LAB but other bacteria were also present. This part of the thesis indicated a need for a better the control of *S. aureus* in the dairies evaluated. In chapter 4, Brazilian dairy samples (from São Paulo state) that were previously known to be positive for *S. aureus* were analyzed by 16S rRNA gene sequencing, divided in four groups (raw material, final product, food-contact and non-food contact surfaces). The results showed high alpha-diversity indexes (final product and non-food contact surfaces) but, beta-diversity indexes were low. The samples were separated in two main clusters and the bacterial community was dominated by *Macrococcus*, *Alkaliphilus*, *Vagococcus*, *Lactobacillus*, *Marinilactibacillus*, *Streptococcus*, *Lysinibacillus*, Staphylococcus, Clostridium, Halomonas, Lactococcus, Enterococcus, Bacillus and Psychrobacter. In chapter 5, a total of 27 samples from two dairies of São Paulo state were sampled for culture of autochthonous microbiota and the isolates were identified by DNA sequencing. Moreover, metagenomic DNA was directly extracted from samples and the unculturable microbiota was evaluated with massive 16S rRNA gene sequencing. Culture results indicated most isolates were lactic acid bacteria, but *Enterobacterales* and families *Staphylococcacceae*, *Bacillaceae*, *Pseudomonadaceae* and *Moraxellaceae* were also detected. From metataxonomics, a heatmap was constructed and the top20 OTUs (operation taxonomics units) were determined, revealing a significant dissimilarity of the microbiota from both dairies. There were 12 most prevalent bacterial taxa in the core microbiota of the dairies evaluated, with the highest abundance of OTUs from *Tolumonas auensis* and *Lactococcus fujiensis*. Overall, the results of this thesis reveal the highly complex microbial ecology of dairy foods and revealed novel mixed species combinations from culture and non-culture based approaches. From these results, it is interesting to develop novel studies to evaluated possible positive or negative correlations among the microbial members and their possible implications for food safety.

Keywords: milk microbiota, metataxonomics, dairies, cheese microbiota.

Introduction

1.1 INTRODUCTION

Foodborne diseases are a global public health problem and their prevention has always been a challenge, which depends on the foods consumed, food processing, handling, storage and sensitivity of consumers. To reduce illnesses cases, it is important to consider besides the scientific evidences, the culture and eating habits of different populations. For example, banning unpasteurized milk may be acceptable to some countries, but not to others (ICMSF, 2006). In fact, in Brazil there is a specific legislation on artisanal Minas cheeses made from raw milk (MINAS GERAIS, 2012) and that cheesemaking process is considered a cultural Brazilian heritage (BRASIL, 2008). YOON et al. (2016) affirmed the autochthonous microbiota of raw milk cheeses contribute for products with more intense and varied flavor, preferred by many consumers. There is a large debate on the abolition of raw milk for cheese making because some advocate that even pasteurization do not necessarily ensures that the cheese will be free of pathogens, since post-pasteurization contamination of cheeses may also occur. However, the use of raw milk is of special concern for those cheeses with low acidity and high moisture, which emphasizes the need for implementation of food safety plans to protect consumer's health, and the ideal situation is always to move from responding to microbial contamination to preventing it (JOHNSON, 2017; YOON et al., 2016). According to YOON et al. (2016) the risk associated with raw milk cheeses may be minimized by appropriate aging and adoption of adequate hygienic practices, emphasizing the need for constant monitoring of hygiene in milking farm, in dairy processing facilities and at post-manufacturing stages.

1.2 CHEESE PROCESSING AND DAIRY MICROBIOTA

There are evidences of milk products being a major component of diets since prehistoric times, as shown by the studies on milk residues in ancient pottery vessels, and this suggests that production of cheese, would have been a critical development due to the preservation of milk products, providing a more digestible commodity for early prehistoric farmers (SALQUE et al., 2013).

The process of cheese production starts with the milk, which is often standardized before cheese making to optimize the protein and fat proportion, to guaranty high quality and yield. The use of heat treated milk for cheese making aims

to avoid pathogenic microorganisms, to reduce the number of spoilage microorganisms and to improve conditions for the efficiency of starter cultures (CARAFA et al., 2019; SALES et al., 2018).

There are ca. 1,000 cheese varieties worldwide and more than 80% of the production is represented by cheese of the familes Dutch, Swiss, Pasta-filata, Cheddar and Parmesan. Although no classification scheme is entirely satisfactory, many attempts have been made to classify cheese varieties, and the main characteristics considered are texture and composition, methods of milk coagulation and ripening indices. Considering the milk coagulation methods, all cheese varieties can be classified in three superfamilies: enzymatic coagulation (75% of total production); acid or lactic coagulation; and coagulation by combination of heating and addition of acid or salt (PAULA; CARVALHO; FURTADO, 2009; MCMAHON; OBERG, 2017; MCSWEENEY; OTTOGALLI; FOX, 2017). These processes allow the concentration of milk by separating solid components (mainly protein and fat) from the whey - composed of water, soluble proteins, lactose and other soluble solids (PAULA; CARVALHO; FURTADO, 2009).

Depending on the variety, cheeses can be aged from a few to several months, or even years. The maturation times of several cheeses can be seen in table 1.

Table 1 - Maturation time of some cheeses

Cheese Type	Maturation chesse – month
Parmesan (Reggiano)	14
Cheddar	3-6
Swiss cheese	2-6
Prato cheese	1
Minas Cured cheese	1
Gouda	1
Camembert	3 weeks

Source: Tronco (1996).

The dairy industry in Brazil has been highly influenced by macroeconomic changes, but milk production in Brazil has experienced a constant increase, reaching ca. 30.7 billion liters in 2010, with the largest production by Southeast region. The dairy sector has high potential to grow and there are many factors driving this scenario, such as increase in family income, population growth, and changes in consumption habits (SPERS; WRIGHT; AMEDOMAR, 2013). Dairy products are of greater

economic importance especially for some local communities, for which the production of artisanal cheeses may provide an additional family profit and to promote a better quality of life, with social inclusion (MARTINS et al., 2018).

The most popular type of cheese in Brazil is the “Minas”, which is prepared by enzymatic coagulation of milk, with no ripening. Other popular cheese varieties are mozzarella, *prato* and *requeijão*, among others. There is a very important system of sanitary inspection in place in Brazil (Ministry of Health, through the National Health Surveillance Agency - ANVISA and Ministry of Agriculture, Livestock and Food Supply - MAPA), but there are still informal sales of homemade cheeses in some regions due to cultural and economic factors (GOMES et al., 2013; USDA, 2017). Recently, a new legislation was approved (BRASIL, 2018) to allow the trade of artisanal state inspected food products of animal origin over the country (CASTRO, c2018). With regard to cheeses, there is even a specific state legislation on artisanal “Minas” cheeses, which is made by enzymatic coagulation from raw milk (MINAS GERAIS, 2012), and that cheesemaking process is considered a cultural Brazilian heritage (BRASIL, 2008).

The main steps to produce cheeses prepared with enzymatic coagulation are: 1) milk preparation (selection, filtration, clarification, standardization and pasteurization); 2) cooling (32-35ºC); 3) addition of coagulating ingredients (lactic ferment, calcium chloride and/or enzymes), addition of other ingredients (optional); 4) coagulation (32-35ºC); 5) curd cutting; 6) agitation and sineresis, with acidification and slow heating; 7) removal of whey; 8) pressing and molding; 9) brining (PAULA; CARVALHO; FURTADO, 2009).

Milk and dairy products are nutrient rich and thus provide ideal growth conditions for many microorganisms, and the large amount of cheese variety results in great part from a diverse microbiota, which can come from raw materials or can be introduced in the product from other sources, such as processing, packaging, maturation, handling and storage, depending on the type of cheese (MUNSCH-ALATOSSAVA; ALATOSSAVA, 2019; SKEIE et al., 2019). Microorganisms can contribute positively for cheese quality, for example, when used as starter cultures or probiotics. However, the cheese microbiota may include spoilage organisms that can be tolerant to heat or can survive at refrigerated storage. Besides, foodborne pathogens can also be associated with dairies which includes Gram-positive (i.e., *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*) and Gram negative bacteria (i.e.,

Salmonella spp., *Cronobacter* spp. and *Campylobacter jejuni*), among others (KENNY et al., 2013).

More recently, with the advent of “omics” technologies, there is a great potential to advance the knowledge of cheeses as complexes biological ecosystems, and this will certainly impact the understanding of cheese flavour and safity (AFSHARI et al., 2018).

1.2. MICROBIOTA OF CHEESES

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, microaerophilic or anaerobic bacteria that produce lactic acid as the major end product of sugar fermentation. LAB generally have a low GC content (<50 mol%) and are typically catalase and cytochrome negative, fastidious, aerotolerant, and acid tolerant. The most common food-related genera of LAB are *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, *Oenococcus*, *Tetragenococcus*, *Carnobacterium*, and *Weissella* (GEMECHU, 2015, PAPADIMITRIOU et al., 2016).

LAB play a prominent role in dairy food fermentation, performing a key role in bioconversions. They can metabolize hexoses by a homofermentative pathway (lactic acid is the primary product) or by a heterofermentative pathway (producing lactic acid, CO_2, acetic acid, and/or ethanol) (MAKAROVA et al., 2006).

Given that dairy food usually represent complexes biological ecosystems, the advances in culture-independent based techniques have been essential to understand

Metagenomics is a very useful molecular tool to study mixed microbial communities. According to Almeida and De Martinis (2018), metagenomics utilizes the total DNA previously extract from microbial communities of a sample of interest and analyses it without the need of culturing. Metagenomics joints concepts of statistics and of meta-analysis, and it quantitatively correlates large genomic datasets with another sequences already studied, integrating all data (SCHLOSS; HANDELSMAN, 2003, ALMEIDA; DE MARTINIS, 2018).

Meta-analysis is fundamental to understand the results obtained from high-throughput DNA-sequencing and makes possible to discuss the dynamic of microorganisms and their interactions in a microbial community (ALMEIDA; DE MARTINIS, 2019).

Metagenomics is the collection of genes and genomes from the members of a microbiota, which is obtained through shotgun DNA sequencing of a sample, followed by the assembly or mapping to a reference database and annotation. In the other hand, metataxonomics is based on the sequencing of phylogenetic markers (usually 16S *rRNA* for bacteria and ITS for fungi) providing taxonomic profiles identification of a sample (MARCHESI; RAVEL, 2015; QUINCE et al., 2017).

Metataxonomics is crucial to reveal the members of a microbial community and to monitorshifts in microbial profiles (ALMEIDA; DE MARTINIS, 2019).

Recently, metagenomics has been applied to elucidate the microbial communites of Brazilian cheeses. Kamikura et al. (2019) analyzed natural Brazilian cheese starter cultures known as “pingo”. They evaluated 11 different types of artisanal cheeses from five geographical areas of Brazil and found that LAB dominated in all cheeses although *Enterobacteriaceae* and *Staphylococcus* were also present. Sant’Anna et al. (2019) evaluated raw milk and “pingo”, from Minas artisanal cheeses. They observed *Planococcaceae* and *Streptococcaceae* dominated during ripening time, and the former family, seemed to develop strong interactions with the *Leuconostocaceae* on cheese surface. Those authors also reported environmental aspects of the region, are likely to contribute for the microbial signature of the products analyzed

With regard to the most concerning foodborne disease agents related to dairy food, *L. monocytogenes* and *S. aureus* are of great importance (KENNY et al., 2013).

L. monocytogenes was first described in 1926 but only in the 80’s it was identified as a foodborne pathogen. Since them, despite the moderately low number of infections per year reported, the lethality rate of this infection is the very high, ranging from 20 to 30% (RADOSHEVICH; COSSART, 2018).

Listeriosis is the name for a general group of disorders caused by *L. monocytogenes* and it may manifest by fever and muscle aches, which can be sometimes be preceded by diarrhea and other gastrointestinal problems. Mild to severe gastroenteritis is the symptom experienced by most healthy adults after ingestion of highly contaminated food (up to 10^9 bacteria). However, in the case of children, the elderly, immunocompromised individuals and pregnant women, severe listeriosis may manifest even after ingestion of food with low level of contamination (ca. 10^2–10^4 bacteria). The invasive disease is characterized by bacterial sepsis, Central Nervous System (CNS) infections and/or transplacental contamination, resulting in

abortion or perinatal infections (RADOSHEVICH; COSSART, 2018; CHURCHILL et al., 2019). The majority of infections caused by *L. monocytogenes* involve the serotypes 1/2a, 1/2b and 4b (ALMEIDA et al., 2017).

In the invasive disease, *L. monocytogenes* is internalized by cells into a vacuole, leading to cytoskeletal rearrangement. The bacterium disrupts the vacuolar membrane by the action of potent virulence factors (LLO and two phospholipases, PlcA and PlcB). Next, it survives and divides in the cytosol and can spread from one cell to another by co-opting actin-based motility. More recently, factors that hijack cellular processes and, in some cases, induce epigenetic changes that influence host gene expression have been reported for *L. monocytogenes* (RADOSHEVICH; COSSART, 2018).

L. monocytogenes does not form spores but it is considerably tolerant to the effects of freezing, drying and heating (ALMEIDA et al., 2017; BUCHANAN et al., 2017). Outbreaks of listeriosis have been associated mainly with ingestion of contaminated refrigerated ready-to-eat food, but diverse food items have been incriminated in outbreaks, including: meat products, raw vegetables, ice creams, milk and cheeses among others (BUCHANAN et al., 2017).

Raw or inadequately pasteurized milk, as well as soft cheeses, are food items of great concern with regard to contamination by *L. monocytogenes*. The control of this foodborne pathogen demands on-farm control measures such as hygienic husbandry and herd health management. However, there is also need to control contamination in food processing and during food handling (KENNY et al., 2013; BUCHANAN et al., 2017).

Staphylococcal food poisoning was has been know since late 19th century, and still nowadays it is one of the most common foodborne diseases. The symptoms manifest quickly after ingestion of food contaminated with enterotoxins, depending on the quantity. *S. aureus* is capable of growing in a high range of temperatures (7^{o} to 48.5^{o} C), in very dry environment and under many adverse conditions (KADARIYA; SMITH; THAPALIYA, 2014). *S. aureus* produces heat stable enterotoxins that are classified basically into serological types, mainly SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ. All staphylococcal enterotoxins (SEs) cause emesis but their mechanisms of action are not clear. It is lilkely the SEs directly affect intestinal epithelium and vagus nerve, stimulating the emetic center. Staphylococcal toxins also present superantigenic activity, interfere in the immunological system and start an

inflammatory process in intestinal cells that results in degranulation and diarrhea (KADARIYA; SMITH; THAPALIYA, 2014).

Besides the foodborne intoxication, *S. aureus* has also been implicated with hospital-acquired infections, raising special attention due to the spread of antibiotic resistant strains, particularly methicillin-resistant *S. aureus* - MRSA. In spite of the this, most of times *S. aureus* is only colonizer in humans, and ca. 20% of adults are persistent carriers (GRUMANN; NÜBEL; BRÖKER, 2014).

In the view of the growing economic and social importance of dairy food in Brazil, considering also the recent advances in the characterization of microbial communities it is crucial to investigate more in depth the composition and possible interactions of authoctonous microbiota and pathogens in Brazilian dairies.

Conclusions

- In this study, the culturable and culture-independent microbiota of samples from dairies of two Brazilian states (Goiás and São Paulo) were evaluated.

- Selected samples presumptive-positives for *Listeria* sp. and *Staphylococcus aureus* and their respective accompayning microbiota were confirmed with 16S rRNA gene sequencing by Sanger method (chapter 3). No sample was confirmed for *Listeria* sp. but *S. aureus* was highly prevalent. LAB was predominant in the background microbiota, mainly *Enterococcus casseliflavus*.

- The microbiota of dairy samples from São Paulo state were analysed by community 16S rRNA gene sequencing (chapter 4). The results revealed the samples could be grouped in two main clusters and the bacterial community was dominated by *Macrococcus, Alkaliphilus, Vagococcus, Lactobacillus, Marinilactibacillus, Streptococcus, Lysinibacillus, Staphylococcus, Clostridium, Halomonas, Lactococcus, Enterococcus, Bacillus* and *Psychrobacter*.

- The “in-house” microbiota of two Brazilian dairies were determined simultaneously by culture-dependent and culture-independent methods (chapter 5). The isolates were mainly LAB, but the order *Enterobacterales* and families *Staphylococcacceae*, *Bacillaceae*, *Pseudomonadaceae* and *Moraxellaceae* were also detected. According to metataxonomics (*16S rRNA*), there were 12 most prevalent bacterial taxa in the core microbiota of the dairies evaluated, with the highest abundance of OTUs from *Tolumonas auensis* and *Lactococcus fujiensis*.

REFERENCES

REFERENCES

ADEOLU, M.; ALNAJAR, S.; NAUSHAD, S.S.; GUPTA, R. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. **Int J Syst Evol Microbiol**, v.66, p. 5575-5599, 2016. doi: 10.1099/ijsem.0.001485.

AFSHARI, R.; PILLIDGE, C. J.; DIAS, D. A.; OSBORN, A. M.; GILL, H.; Cheesomics: the future pathway to understanding cheese flavour and quality. **Crit Rev Food Sci Nutr,** p. 1-15, 2018. doi: 10.1080/10408398.2018.1512471.

AFZAL, M. I.; JACQUETA, T.; DELAUNAYB, S.; BORGESA, F.; MILLIÈREA, JB.; REVOL-JUNELLESA, AM.; CAILLIEZ-GRIMA, C. *Carnobacterium maltaromaticum*: Identification, isolation tools, ecology and technological aspects in dairy products. **Food Microbiol**, v. 27, p. 573-579, 2010.

ALMEIDA, R.M.; BARBOSA, A.V.; LISBÔA, R.C.; SANTOS, A.F.M.; HOFER, E.; VALLIM, D.C.; HOFER, C.B. Virulence genes and genetic relationship of *L. monocytogenes* isolated from human and food sources in Brazil. **Braz J Infect Dis**, v. 21, n. 3, p. 282-289, 2017. doi.org/10.1016/j.bjid.2017.01.004.

ALMEIDA, O. G. G.; DE MARTINIS; E. C. P. Bioinformatics tools to assess metagenomic data for applied microbiology. **Appl Microbiol Biotechnol**, v. 103, n. 1, p. 69-82, 2019. doi: 10.1007/s00253-018-9464-9.

ALVES, V. F.; NINO-ARIAS, F. C.; PITONDO-SILVA, A.; FRAZILIO, D. A.; GONÇALVES, L. D.; TOUBAS, L. C.; TORRES, I. M. S.; OXARAN, V.; DITTMANN, K. K.; DE MARTINIS, E. C. P. Molecular characterization of *Staphylococcus aureus* from some artisanal Brazilian dairies. **Int Dairy J**, v. 85, p. 247-253, 2018. doi: 10.1016/j.idairyj.2018.06.008.

AMORIM, A. L. B. C.; COUTO, E. P.; SANTANA, A. P.; RIBEIRO, J. L.; FERREIRA, M. A. Avaliação da qualidade microbiológica de queijos do tipo Minas padrão de produção industrial, artesanal e informal. **Rev Inst Adolfo Lutz**, v. 73, n. 4, p. 364-367, 2014. doi: 10.18241/0073-98552014731628.

ARCURI, E. F.; SHEIKHA, A. F. E.; RYCHLIK, T.; PIRO-MÉTAYER, I.; MONTET, D. Determination of cheese origin by using 16S rDNA fingerprint of bacteria communities by PCR-DGGE: Preliminary application to traditional Minas cheese. **Food Control**, v. 30, n. 1, p. 1-6, 2013. doi:10.1016/j.foodcont.2012.07.007.

ARSÈNE-PLOETZE, F.; KOECHLER, S.; MARCHAL, M.; COPPÉE, J. Y.; CHANDLER, M.; BONNEFOY, V. Structure, function, and evolution of the *Thiomonas* spp. genome. **PLoS Genet**, v. 6, n. 2, p. 1-13, 2010.

doi: 10.1371/journal.pgen.1000859.

INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS A simplified guide to understanding and using food safety objectives and performance objectives. **International Commission on Microbiological Specifications for Foods**, p. 4-12, 2006. Available in: http://www.icmsf.org/wp-content/uploads/2018/02/GuiaSimplificadoEnglish.pdf, accessed on: march 03 of 2019.

BAGGE-RAVN, D.; NG, Y.; HJELM, M.; CHRISTIANSEN, J. N.; GRAM, L.; JOHANSEN, C. The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. **J Food Microbiol**, v. 87, n. 3, p. 239– 250, 2003.
doi: 10.1016/S0168-1605(03)00067-9.

BOKULICH, N. A.; MILLS, D. A. Facility-specific "house" microbiome drives microbial landscapes of artisan cheese making plants. **Appl Environ Microbiol**, v. 79, n. 17, p. 5214-5223, 2013. doi: 10.1128/AEM.00934.

BUCHANAN, R.L.; GORRIS, L.G.M.; HAYMAN, M.M.; JACKSON, T.C.; WHITING, R.C. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. **Food Control**, v.75, p.1-13. 2017. doi.org/10.1016/j.foodcont.2016.12.016.

BRASIL. Portaria n°358 de 04 de setembro de 1997. Fixação de identidade e qualidade do queijo prato. Diário Oficial República Federativa do Brasil. Brasília: Ministério da Agricultura Pecuária e Abastecimento. Available in: http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=1231. Accessed in: Mar. 29, 2019.

_____. **Portaria n°358 de 16 de julho de 2001**. Aprova os Regulamentos Técnicos de Identidade e Qualidade de Manteiga da Terra ou Manteiga de Garrafa; Queijo de Coalho e Queijo de Manteiga, conforme consta dos Anexos desta Instrução Normativa. Diário Oficial República Federativa do Brasil. Brasília: Ministério da Agricultura, Pecuária e Abastecimento. Available in: http://extranet.agricultura.gov.br/ sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=2194. Accessed in: Dec. 17, 2018.

BRASIL. IPHAN - Instituto do Patrimônio Histórico e Artístico Nacional. Modo Artesanal de Fazer Queijo de Minas nas Regiões do Serro, da Serra da Canastra e doSalitre,
2008.vailable:<http://portal.iphan.gov.br/bcrE/pages/folBemCulturalRegistradoE.jsf?idBemCultural=0_%5Bd36_%4018c5551n%5D8%3Am208z%40s1%5Bv8%3Ax3331

n%5D8%3Am207%2F%24ghi*%3Adef%24%3B52g0_%5B3y3p600001n%5D8%3Am209#>. Process: 01450.012192/2006-65. Date of register: Jun. 13, 2008. Accessed in: March 08 of 2019.

CALASSO, M.; ERCOLINI, D.; MANCINI, L.; STELLATO, G.; MINERVINI, F.; DI CAGNO, R.; DE ANGELIS, M.; GOBBETTI, M. Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at same dairy plant. **Food Microbiol**, v. 54, p. 115-126, 2016. doi: 10.1016/j.fm.2015.10.008.

CAMPAGNOLLO, F. B.; MARGALHO, L. P.; KAMIMURA, B. A.; FELICIANO, M. D.; FREIRE, L.; LOPES, L. S.; ALVARENGA, V. O.; CADAVEZ, V. A. P.; GONZALES-BARRON, U.; SCHAFFNER, D. W.; SANT'ANA, A. S. Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional Brazilian cheeses. **Food Microbiol**, v. 73, p. 288-297, 2018b. doi: 10.1016/j.fm.2018.02.006.

CARAFA, I.; STOCCO, G.; FRANCESCHI, P.; SUMMER, A.; TUOHY, K.M.; GIOVANNIBITTANTE, G.; FRANCIOSIA. E. Evaluation of autochthonous lactic acid bacteria as starter and non-starter cultures for the production of Traditional Mountain cheese. **Food Research International.** v. 115, p. 209-218, 2019. doi.org/10.1016/j.foodres.2018.08.069.

CASTRO, M.T. Segurança de alimentos artesanais e a nova lei nº 13.680/2018 website. Available: https://foodsafetybrazil.org/seguranca-de-alimentos-e-nova-lei-no-13-680/, accessed on: march 29, 2019.

CHAJECKA-WIERZCHOWSKA, W.; ZADERNOWSKA, A.; ŁANIEWSKA-TROKENHEIM, L. Diversity of antibiotic resistance Genes in Enterococcus strains isolated from ready-to-eat meat products. **J Food Sci**, v.81, p. 2799-2807, 2016.

CHAO, C. T.; LAI, C. F.; HUANG, J. W. *Lactococcus garvlaeae* peritoneal dialysis peritonitis. **Peritoneal Dialysis International**, v. 33, p. 100-108, 2013.

CHURCHILL, K.J.; SARGEANT, J.M.; FARBER, J.M.; O'CONNOR, A.M. Prevalence of *Listeria monocytogenes* in select ready-to-eat foods - deli meat, soft cheese, and packaged salad: a systematic review and meta-analysis. **Journal of Food Protection**, v. 82, n. 2, p. 344-357, 2019. doi.org/10.4315/0362-028X.JFP-18-158.

COPPOLA, S.; BLAIOTTA, G.; ERCOLINI, D.; MOSCHETTI, G. Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese. **Journal of Applied Microbiology**, v. 90, n. 3, p. 414-420, 2001. doi:10.1046/j.1365-2672.2001.01262. x

DALMASSO, A.; DEL RIO, M. D. S, CIVERA, T.; PATTONO, D.; CARDAZZO, B.; BOTTERO, M. T. Characterization of microbiota in Plaisentif cheese by high-throughput sequencing. **LWT - Food Sci Technol**, v. 69, p. 490-496, 2016. doi:10.1016/j.lwt.2016.02.004.

D'ANGELO, L.; CICOTELLO, J.; ZAGO, M.; GUGLIELMOTTI, D.; QUIBERONI, A.; SUAREZ, V. *Leuconostoc* strains isolated from dairy products: Response against food stress conditions. **Food Microbiol**, v. 66, p. 28-39, 2017.

DEETAE, P.; BONNARME, P.; SPINNLER, H.E.; HELINCK, S. Production of volatile aroma compounds by bacterial strains isolated from different surface-ripened French cheeses. **Appl Microbiol Biotechnol**, v.76, p.1161–117, 2007. doi.org/10.1007/s00253-007-1095-5.

DEETAE, P.; SPINNLER, H.E.; BONNARME, P.; HELINCK S. Growth and aroma contribution of *Microbacterium foliorum*, *Proteus vulgaris* and *Psychrobacter* sp during ripening in a cheese model medium. **Appl Microbiol Biotechnol**, v.82, p.169-177, 2008. doi.org/10.1007/s00253-008-1805-7.

DE FILLIPIS, F.; GENOVESE, A.; FERRANTI, P.; GILBERT, J. A.; ERCOLINI, D. Metatranscriptomics reveals temperature-driven functional changes in microbiome impacting cheese maturation rate. **Sci Rep**, v. 6, p. 1-12, 2016.
doi: 10.1038/srep21871.

_____. A Selected core nicrobiome drives the early stages of three popular Italian cheese manufactures. **PLoS One**, v. 9, n. 2, p. 1-8, 2014. doi:10.1371/journal.pone.0089680.

DE PASQUALE, I.; DI CAGNO, R.; BUCHIN S.; DE ANGELIS, M.; GOBBETTI, M. Microbial ecology dynamics reveal a succession in the core microbiota involved in the ripening of pasta filata caciocavallo pugliese cheese. **Appl Environ Microbiol**, v. 80, n. 19, p. 6243-6255, 2014. doi: 128/AEM.02097-14.

_____. Use of autochthonous mesophilic lactic acid bacteria as starter cultures for making Pecorino Crotonese cheese: Effect on compositional, microbiological and biochemical attributes. **Food Research International**, v. 116, p. 1344-1356, 2019. doi: 10.1016/j.foodres.2018.10.024.

DELCENSERIE, V.; TAMINIAU, B.; DELHALE, L.; NEZER, C.; DOYEN, P.; CREVECOEUR, S.; ROUSSEY, D.; KORSAK, N.; DAUBE, G. Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomics analysis. **J Dairy Sci**, v. 97, n. 10, p. 6046-6056, 2014. doi:10.3168/jds.2014-8225.

DELGADO, S.; RACHID, C. T.; FERNANDEZ, E.; RYCHILK, T.; ALEGRIA, A.; PEIXOTO, R. S.; MAYO, B. Diversity of thermophilic bacteria in raw, pasteurized and

selectively-cultured milk, as assessed by culturing, PCR-DGGE and pyrosequencing. **Food Microbiol**, v. 80, n. 19, p. 6243-6255, 2013. doi:10.1016/j.fm.2013.04.015.

DELGADO-BAQUERIZO, M.; MAESTRE, F. T.; REICH, P. B.; JEFFRIES, T. C.; GAITAN, J. J.; ENCINAR, D.; BERDUGO, M.; CAMPBELL, C. D.; SINGH, B. K. Microbial diversity drives multifunctionality in terrestrial ecosystems. **Nat Commun**, v. 7, p. 1-8, 2016. doi:10.1038/ncomms10541.

DITTMANN, K. K.; CHAUL, L.; LEE, S. Y.; CORASSIN, C. H, OLIVEIRA, C. A. F.; DE MARTINIS, E. C. P.; ALVES, V. F.; GRAM, L.; OXARAN, V. *Staphylococcus aureus* in some Brazilian dairy industries: changes of contamination and diversity. **Front Microbiol**, v. 8, n. 2049, p. 1-12, 2017. doi: 10.3389/fmicb.2017.02049.

DORÉ, J.; SIMRÉN, M.; BUTTLE, L.; GUARNER, F. Hot topics in gut microbiota. **United European Gastroenterol. J**, v. 1, n. 5, p. 311–318, 2013.
doi: 10.1177/2050640613502477

ERCOLINI, D. High-throughput sequencing and metagenomics: steps ahead in the culture-independent of food microbial ecology. **Appl Environ Microbiol**, v. 79, n. 10, p. 3148-3155, 2013. doi:10.1128/AEM.00256-13.

ESCOBAR-ZEPEDA, A.; SANCHEZ-FLORES, A.; BARUCH, M. Q. Metagenomics analysis of a Mexican ripened cheese reveals a unique complex microbiota. **Food Microbiol**, v. 57, p. 116-127, 2016. doi:10.1016/j.fm.2016.02.004.

FDA. Food and Drug Administration and U.S. Department of Agriculture Food Safety and Inspection Service (U.S. FDA/FSIS) U.S. Department of Health and Human Services, Center for Food Safety and Applied Nutrition.
Quantitative assessment of relative risk to public health from foodborne Listeria monocytogenes among selected categories of ready-to-eat foods
Available at: http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM197330.pdf
Accessed March 3 of 16.

FISCHER-ROMERO, C.; TINDALL, B. J.; JÜTTNER, F. *Tolumonas auensis* a toluene-producing bacterium from anoxic sediments of a freshwater lake. **Int J Syst Bacteriol**, v. 46, n. 1, p. 183-188, 1996.

FRANZ, C.M.; HOLZAPFEL, W.H.; STILES, M.E. Enterococci at the crossroads of food safety? **Int J Food Microbiol**, v.1 , p.1-24. 1999.

GEMECHU, T. Review on lactic acid bacteria function in milk fermentation and preservation. **African Journal of Food Science**, v.9, p.170-175, 2015. doi.org/10.5897/AJFS2015.1276.

GOBBETTI, M.; DI CAGNO, D.; CALASSO, M.; NEVIANI, E.; FOX, P. F.; DE ANGELIS, M. Drivers that establish and assembly the lactic acid bacteria biota in cheeses. **Trends Food Sci Technol**, v. 78, p. 244-254, 2018. doi:10.1016/j.tifs.2018.06.010.

GOMES, B. C.; FRANCO, B. D. G. M.; DE MARTINIS, E. C. P. Microbiological food safety issues in Brazil: bacterial pathogens. **Foodborne Pathog Dis**, v. 10, n. 3, p. 197-205, 2013. doi: 10.1089/fpd.2012.1222.

GOMES, B. C.; ESTEVES, C. T.; PALAZZO, I. C. V.; DARINI, A. L. C.; FELIS, G. E, SECHI, L.; FRANCO, B. D. G. M.; DE MARTINIS, E. C. P. Prevalence and characterization of *Enterococcus* spp. isolated from Brazilian foods. **Food Microbiology**, v. 25, p. 668-675, 2008. doi: 10.1016/j.fm.2008.03.008.

GOPAL, N.; HILL, C.; ROSS, P. R.; BERESFORD, T. P.; FENELON, M. A.; COTTER, P. D. The prevalence and control of Bacillus and related spore-forming bacteria in the dairy industry. **Front Microbiol**, v. 6, n. 1418, p. 1-18, 2015.
doi: 10.3389/fmicb.2015.01418.

GRUMANN, D.; NÜBEL, U.; BRÖKER, B.M. *Staphylococcus aureus* toxins—Their functions and genetics. **Infect Genet Evol**, p.583–5922014. doi: 10.1016/j.meegid.2013.03.013.

HANSEN, E. B. Commercial bacterial starter cultures for fermented foods of the future. **Int J Food Microbiol**, v. 78, n. 1-2, p. 119-131, 2002.
doi: 10.1016/S0168-1605(02)00238-6.

HAEGEMAN, B.; HAMELIN, J.; MORIARTY, J.; NEAL, P.; DUSHOFF, J.; WEITZ, J.S. Robust estimation of microbial diversity in theory and in practice. **ISME J**, (6):1092-101. 2013. doi:0.1038/ismej.2013.10.

HILL, J.; MCHIZA, Z.; PUOANE, T.; STEYN, N. Food sold by street-food vendors in Cape Town and surrounding areas: a focus on food and nutrition knowledge as well as practices related to food preparation of street-food vendors.
Journal of Hunger & Environmental Nutrition.
, doi: 10.1080/19320248.2018.1434104.

HILL, T. C. J.; WALSH, K. A.; HARRIS, A.; MOFFET, B. F. Using ecological diversity measures with bacterial communities. **FEMS Microbiol Ecol**, v. 43, n. 1, p. 1-11, 2006. doi: 10.1111/j.1574-6941. 2003.tb01040.x.

HWANHLEM, N.; IVANOVA, T.; BISCOLA, V.; CHOISET, Y.; HAERTLÉ, T. Bacteriocin producing *Enterococcus faecalis* isolated from chicken gastrointestinal tract originating from Cm Phitsanulok, Thailand: Isolation, screening, safety evaluation and probiotic properties Original Research Article. **Food Control**, v. 78, p. 187-195, 2017.

INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS. Milk and Dairy Products. *In*: INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS. **Microorganisms in Foods 8**: Use of data for assessing process control and product acceptance. New York: Springer, 2011. Chapter 23, p. 305-327.

INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS A simplified guide to understanding and using food safety objectives and performance objectives. **International Commission on Microbiological Specifications for Foods**, p. 4-12, 2006. Available in: http://www.icmsf.org/wp-content/uploads/2018/02/GuiaSimplificadoEnglish.pdf, accessed on: march 03 of 2019.

ISHIKAWA, M.; NAKAJIMA, K.; YANAGI, M.; YAMAMOTO, Y.; YAMASATO, K. *Marinilactibacillus psychrotolerans* a halophilic and alkaliphilic *marine* lactic acid bacterium isolated from marine organisms in temperate and subtropical areas of Japan. **Int J Syst Evol Microbiol**, v. 53, p. 711–720, 2003. doi: 10.1099/ijs.0.02446-0.

JOHNSON, M.E. A 100-year review: cheese production and quality. **Journal of Dairy Science**. v.100, p.9952-9965, 2017.

JONNALA, B. R. Y.; MCSWEENEY, P. L. H.; JEREMIAH, J.; SHEEHAN, J. J.; COTTER, P. D. Sequencing of the cheese microbiome and its relevance to Industry. **Front Microbiol**, v. 9, n. 1020, p. 1-12, 2018. doi:10.3389/fmicb.2018.01020.

KACI, G.; GOUDERCOURT, D.; DENNIN, V.; POT, B.; DORÉ, J.; EHRLICH, D.; RENAULT, P.; BLOTTIÈRE, H. M.; DANIEL, C.; CHRISTINE DELORME, C. Anti-Inflammatory properties of *Streptococcus salivarius*, a commensal bacterium of the oral cavity and digestive tract. **Applied and Environmental Microbiol**, v. 80, p. 928-934, 2014.

KADARIYA, J.; SMITH, T. C.; THAPALIYA, D. *Staphylococcus aureus* and Staphylococcal food-borne disease: an ongoing challenge in public health. **Biomed Res Int**, v. 2014, p. 1-9, 2014. doi: 10.1155/2014/827965.

KAMIMURA, B. A.; DE FILIPPIS, F.; SANT'ANA, A. S.; ERCOLINI, D. Large-scale mapping of microbial diversity in artisanal Brazilian cheeses. **Food Microbiology**, v. 80, p. 40-49, 2019. doi: 10.1016/j.fm.2018.12.014.

KENNY, M. - chapter 6 safety and quality. In: **Milk and dairy products in human nutrition**. Food and Agriculture Organization of the United Nations, 2013, p.243-274.

KLOOS, W.E..; BALLARD, D.N.; GEORGE, C.G.; WEBSTER, J.A.; HUBNER, R.J.; LUDWIG W.; SCHLEIFER, K.H.; FIEDLER, F.; SCHUBERT. K. Delimiting the genus Staphylococcus through description of *Macrococcus caseolyticus* gen. nov., comb. nov. and *Macrococcus equipercicus* sp. nov., and *Macrococcus bovicus* sp. nov. and *Macrococcus carouselicus* sp. nov. **Int J Syst Bacteriol**, v.48, p. 859-877, 1998.

KWAK, Y.; KHAN, A.R.; SHIN, J.H. Genome sequence of Serratia nematodiphila DSM 21420T, a symbiotic bacterium from entomopathogenic nematode. **J Biotechnology**, v.193, p.1-2, 2015.

LACERDA, I. C. A.; GOMES, F. C. O.; BORELLI, B. M.; FARIA JR, C. L. L.; FRANCO, G. R.; MOURÃO, M. M.; MORAIS, P. B.; ROSA, C. A. Identification of the bacterial community responsible for traditional fermentation during sour cassava starch, cachaça and minas cheese production using culture-independent 16s rRNA gene sequence analysis. **Braz J Microbiol**, v. 42, n. 2, p. 650-657, 2011. doi.org/10.1590/S1517-83822011000200029.

LAHTI, L.; SHET, S.; BLAKE, T.; SALOJARVI, J. **Microbiome R package, version 1.2.1**. 2018. Available in: http://microbiome.github.io/microbiome. Accessed in: 12 aug. 2018.

LANE, D. L.; PACE, B.; OLSEN, G. J.; STAHL, D. A. M. L,; SOGIN, N. R. P. “Rapid determination of 16S ribossomal RNA sequences for phylogenetic analyses. Proceedings of National Academy of Science. v. 82, 1985 .In MARANGONI P.R.D.; Caracterização de biofilmes formados em superfícies metálicas e biocorrosão. 2010.

LEMOS, L. N.; FULTHORPE, R. R.; TRIPLETT, E. W.; ROESCH, L. F. W. Rethinking microbial diversity analysis in the high throughput sequencing era. **J Microbiol Methods**, v. 86, n. 1, p. 42-51, 2011. doi:10.1016/j.mimet.2011.03.014.

LI, N.; WANG, Y.; YOU, C.; REN, J.; CHEN, W.; ZHENG, H.; LIU, Z. Variation in raw milk microbiota throughout 12 months and the impact of weather conditions. **Scientific Reports**, v. 8, n. 2371, p. 1-10, 2018. doi:10.1038/s41598-018-20862-8.

LUIZ, S. M. P.; CASTRO, R. D.; SANDES, S. H. C.; SILVA, J. G.; OLIVEIRA, L. G.; SALES, G. A.; NUNES, A. C.; SOUZA, M. R. Isolation and identification of lactic acid bacteria from Brazilian Minas artisanal cheese. **CyTA - Journal of Food**, v. 15, n. 1, p. 125-128, 2017. doi: 10.1080/19476337.2016.1219392

MACFADYEN,A.C.; ELIZABETH A. FISHER,E.A.; COSTA, B.; CULLEN, C.; PATERSON, G.K. Genome analysis of methicillin resistance in *Macrococcus caseolyticus* from dairy cattle in England and Wales. **Microb Genom,** v.4, p.8, 2018. doi:10.1099/mgen.0.000191

MACFADYEN, A. C.; FISHER, E. A.; COSTA, B.; CULLEN, C.; PATERSON, G. K. P. Genome analysis of methicillin resistence in *Macrococcus caseolyticus* from dairy cattle in England and Wales. **Microbial Genomics**, v. 4, n. 8, p. 1-8, 2018. doi: 10.1099/mgen.0.000191.

MAKAROVA, K., SLESAREV, A., WOLF, Y., SOROKIN, A., MIRKIN, B., KOONIN, E.; MILLS, D. Comparative genomics of the lactic acid bacteria. **Proceedings of the National Academy of Sciences of the United States of America**, v.103, p.15611–15616. doi:10.1073/pnas.0607117103.

MARCHAND, S.; BLOCK, J.; JONGHE, V.; COOREVITS, A.; HEYNDRICKX, M.; LIEVE, H. L. Biofilm formation in milk production and processing environments; influence on milk quality and safety. **Compr Rev Food Sc Food Saf**, v. 11, n. 2, p. 133-147, 2012. doi: 10.1111/j.1541-4337.2011.00183.x.

MARCHESI, J. R.; RAVEL, J. The vocabulary of microbiome research: a proposal. **Microbiome**, v. 3, n. 31, p. 1-3, 2015. doi: 10.1186/s40168-015-0094-5.

MARINO, M.; INNOCENTE, N.; MAIFRENI, M.; MOUNIER, J.; COBO-DÍAZ, J. F.; COTON, E.; CARRARO, L.; CARDAZZO, B. Diversity within Italian Cheese making brine-associated bacterial communities evidenced by massive parallel 16S rRNA Gene Tag Sequencing. **Front Microbiol**, v. 9, n. 1020, p. 1-12, 2017.
doi: 10.3389/fmicb.2017.02119.

MARTINS, M. C. F.; FREITAS, R.; DEUVAUX, J. C.; ELLER, M. R.; NERO, L. A.; CARVALHO, A. F. Bacterial diversity of artisanal cheese from the Amazonian region of Brazil during the dry and rainy seasons. **Food Res Int**. v. 108, p. 295-300, 2018. doi: 10.1016/j.foodres.2018.03.060.

MCMAHON, D.J.; OBERG, C.J. Chapter 40 - pasta-filata cheeses. In: **Cheese Chemistry, Physics and Microbiology**, v.4, p.1041-1068, 2017.

MCMURDIE, P. J.; HOLMES, S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. **PLoS One,** v. 8, n. 4, p. 1-11, 2013. doi: 10.1371/journal.pone.0061217.

MCSWEENEY, P.L.H.; OTTOGALLI, G.; FOX, P.F. Chapter 31 - Diversity and classification of cheese varieties: An Overview. In: **Cheese Chemistry, Physics and Microbiology**, v.4, p.781-808, 2017.

MELERO, B.; STESSL, B.; MANSO, B.; WAGNER, M.; ESTEBAN-CARBONERO, O. J.; HERNÁNDEZ, M.; ROVIRA, J.; RODRIGUEZ-LÁZARO, D. *Listeria monocytogenes* colonization in a newly established dairy processing facility. **Int J Food Microbiol**, v. 289, p. 64-71, 2019. doi: 10.1016/j.ijfoodmicro.2018.09.003.

MINAS GERAIS. **Lei nº 20549, de 18 de dezembro de 2012**. Dispõe sobre a produção e a comercialização dos queijos Artesanais de Minas Gerais. Minas Gerais: Diário do Executivo. Available in: https://www.legisweb17.com.br/legislacao/?id=248559. Accessed in: 18 dec. 2018.

MONNET, C.; LANDAUD, S.; BONNARME, P.; SWENNEN, D. Growth and adaptation of microorganisms on the cheese surface. **FEMS Microbiol Lett**, v. 362, n. 1, p. 1-9, 2014. doi: 10.1093/femsle/fnu025.

MORRIS, E. K. E.; CARUSO, T.; BUSCOT, F.; FISCHER, M.; HANCOCK, C.; MAIER, T. S.; MEINERS, T.; MÜLLER, C.; OBERMAIER, E.; PRATI, D.; SOCHER, S. A.; SONNEMANN, I.; WÄSCHKE, N.; WUBET, T.; WURST, S.; RILLIG, M. C. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. **Ecol Evol**, v. 4, n. 18, p. 3514-3524, 2014. doi: 10.1002/ece3.1155.

MUNSCH-ALATOSSAVA, P.; ALATOSSAVA, T. Quality and Safety of Bovine Raw Milk: Present Challenges and Technological Solutions. **IntechOpen,** p.1-16, 2019. doi: 10.5772/intechopen.83507.

NASSER, V. G.; VISÔTTO, L. E.; CAMPOS, F. M.; FERNANDES, R. V. B.; BOTREL, D. A. A microbiological and physical-chemical based evaluation of artisanal Minas cheese production in Rio Paranaíba city, Minas Gerais, Brazil. **Rev Acad Ciênc Anim**, v. 13, p. 47-56, 2015. doi:10.7213/academica.14.2016.

OKSANEN, J.; BLANCHET, F. G.; FRIENDLY, M.; KINDT, R.; LEGENDRE, P.; MCGLINN, D.; MINCHIN, P. R.; O'HARA, R. B.; SIMPSON, G. L.; SOLYMOS, P.; STEVENS, M. H. H.; SZOE, C. S. E.; WAGNER, H. **Community ecology package, version 2.5-2**. 2018. Available in: https://cran.ism.ac.jp/web/packages/vegan/vegan.pdf. Accessed in: 12 aug. 2018.

OXARAN, V.; DITTMANN, K. K.; LEE, S. H. I.; CHAUL, L. T.; OLIVEIRA, C. A. F.; CORASSIN, C. H.; ALVES, V. F.; DE MARTINIS, E. C. P.; GRAM, L. Behavior of

foodborne pathogens *Listeria monocytogenes* and *Staphylococcus aureus* in mixed-species biofilms exposed to biocides. **Appl Environ Microbiol**, v. 84, n. 24, 2018. doi: 10.1128/AEM.02038-18.

OXARAN, V.; LEE, S. Y.; CHAUL, L.; CORASSIN, C. H.; BARANCELLI, G. V.; ALVES, V. F.; OLIVEIRA, C. A. F.; GRAM, L. K.; DE MARTINIS, E. C. P. *Listeria monocytogenes* incidence changes and diversity in some Brazilian dairy industries and retail products. **Food Microbiol**, v. 68, p. 16-23, 2017.
doi: 10.1016/j.fm.2017.06.012.

PAPADIMITRIOU, K.; ALEGRÍA, A.; BRON, P.A.; ANGELIS, M.; GOBBETTI, M.; KLEEREBEZEM, M.; LEMOS, J.A.; LINARES, D.M.; ROSS, P.; STANTON, C.; TURRONI, F.; SINDEREN, D.V.; VARMANEN, P.; VENTURA, M.; ZÚÑIGA, M.; ETSAKALIDOU, E.; KOK, J. Stress Physiology of Lactic Acid Bacteria. **Microbiology and Molecular Biology Reviews**, v.80, p.837-890, 2016. doi:10.1128/MMBR.00076-15.

PARK, Y.G.; MUN, B.G.; KANG, S.M.; HUSSAIN, A.; SHAHZAD, R.; SEO, C.W.; KIM, A.Y.; LEE, S.U.; OH, K.Y.; LEE, D.Y.; LEE, I.J.; YUN, B.W. Bacillus aryabhattai SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. **PLoS One**, v.10; p.12, 2017. doi: 10.1371/journal.pone.0173203.

PAULA, J.C.J.; CARVALHO, A.F.C.; FURTADO, M.M. princípios básicos de fabricação de queijo: do histórico à salga. **Rev Inst Latic**, v.64, p.19-25, 2009.

PERIN, L. M.; SARDANO, M. L. S.; NERO, L. A.; NEVIANI, E.; GATTI, M. Bacterial ecology of artisanal Minas cheeses assessed by culture-dependent and independent methods. **Food Microbiology**, v. 65, p.160-169, 2017.

PURTY, S.; SARANATHAN, R.; PRASHANTH, K.; NARAYANAN, K.; ASIR, J.; DEVI, C. S.; AMARNATH, S. K. The expanding spectrum of human infections caused by Kocuria species: a case report and literature review. **Emerging Microbes Infect**, v. 71, p. 1-8, 2013.

QUIGLEY, L.; O'SULLIVAN, O.; STANTON, C.; BERESFORD, T. P.; ROSS, R. P.; GERALD, F.; FITZGERALD, G. F.; COTTER, P. D. The complex microbiota of raw milk. **FEMS Microbiology Reviews**, v. 37, n. 5, p. 664–698, 2013.
doi: 10.1111/1574-6976.12030.

QUINCE, C.; WALKER, A. W.; SIMPSON, J. T.; LOMAN, N. J.; SEGATA, N. Shotgun metagenomics, from sampling to analysis. **Nat Biotechnol**, v. 35, p. 833–844, 2017. doi: 10.1038/nbt.3935.

RADOSHEVICH, L.; COSSART, P. *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. **Nat Rev Microbiol**, v.16, p.32–46, 2018.

.

RIQUELME, C.; CÂMARA, S.; DAPKEVICIUS, M. L. N. E.; VINUESA, P.; DA SILVA, C. C. G.; MALCATA, F. X.; REGO, O. A. Characterization of bacterial biodiversity in Pico cheese (na artisanal Azorean food). **Int J Food Microbiol**, v. 192, n. 1, p. 86-94, 2015. doi: 10.1016/j.ijfoodmicro.2014.09.031.

SALOTTI, B. M.; CARVALHO, A. C. F. B.; AMARAL, L. A.; VIDAL-MARTINS, A. M. C.; CORTEZ. A. L. Qualidade microbiológica do queijo minas frescal comercializado no município de Jaboticabal, SP, Brasil. **Arq Inst Biol**, v. 73, n. 2, p. 171-175, 2016.

SALQUE, M.; BOGUCKI, P.I.; PYZEL, J.; SOBKOWIAK-TABAKA, I.; GRYGIEL, R.; SZMYT, M.; EVERSHED, R.P. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. **Nature**, v. 493, p.522–52, 2013.

SANDERSON, B. A.; ARAKI, N.; LILLEY, J. L.; GUERRERO, G.; LEWIS, K. Modification of gel architecture and TBE/TAE buffer composition to minimize heating during agarose gel electrophoresis. **Anal Biochem**, v. 454, p. 44-52, 2014. doi: 10.1016/j.ab.2014.03.003.

SANT'ANNA, F. M.; WETZELS, S.U.; SANDES, S.H.C.; FIGUEIREDO, R.C.; SALES, G.A.; FIGUEIREDO, N.C.; CANTINI, A.N.; SCHMITZ, E.S.; MANN, E.; WAGNER, M.; SOUZA, M.R. Microbial shifts in Minas artisanal cheeses from the Serra do Salitre region of Minas Gerais, Brazil throughout ripening time. **Food Microbiology**, v.82, p. 349-362, 2019. https://doi.org/10.1016/j.fm.2019.02.016.

SANTOS, D. C.; LANGE, C. C.; AVELLAR-COSTA, P.; SANTOS, K. R. N.; BRITO, M. A. V. P.; GIAMBIAGI-DEMARVALA, M. *Staphylococcus chromogenes*, a coagulase-negative *Staphylococcus* species that can clot plasma. **J Clinical Microbiol**, v. 5, p. 1372-1375, 2016.

SANTOS, V. A. Q.; HOFFMAN, F. L. Evolution of microbial contaminants in the processing line of Minas frescal and ricotta cheeses. **Rev Inst Adolfo Lutz**, v. 69, n. 1, p. 38-46, 2010.

SALES, D.C.; RANGEL, A.H.N.R.; URBANO, S.A.; TONHATI, H.; GALVÃO JÚNIOR, J.G.B.; GUILHERMINO, M.M.; AGUIAR, E.M.; BEZERRA, M.F. Buffalo milk composition, processing factors, whey constituent's recovery and yield in

manufacturing Mozzarella cheese. **Food Sci Technol**, v.38 n.2, 2018. Epub Sep 04, 2017. http://dx.doi.org/10.1590/1678-457x.04317.

SCHLOSS, P. D.; HANDELSMAN, J. Biotechnological prospects from metagenomics. **Curr Opin Biotechnol**, v. 14, n. 3, p. 303–310, 2003.

SETTANNI, L.; MOSCHETTI, G. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. **Food Microbiol**, v. 27, n. 6, p. 691-697, 2010. doi: 10.1016/j.fm.2010.05.023.

SHADE, A.; HANDELSMAN, J. Beyond the Venn diagram: the hunt for a core microbiome. **Environ Microbiol**, v. 14, n. 1, p. 4-12, 2012. doi:10.1111/j.1462-2920.2011.02585. x.

SILVA, L. F.; CASELLA, T.; GOMES, E. S.; NOGUEIRA, M. C.; LINDNER, D. D.; PENNA, A. L. Diversity of lactic acid bacteria isolated from Brazilian water buffalo mozzarella cheese. **J Food Sci**, v. 80, n. 2, p. 411-417, 2015.
doi: 10.1111/1750-3841.12771.

SUZUKI, K. 7 - Gram-positive spoilage bacteria in brewing. **Brewing Microbiology**, P.141.143, 2015.

SKEIE, S. B.; HÅLAND, M.; THORSEN, I. M.; NARVHUS, J.; PORCELLATO, D. Bulk tank raw milk microbiota differs within and between farms: A moving goalpost challenging. **Quality Control**, v. 102, n. 3, p. 1959–1971, 2019.
doi.org/10.3168/jds.2017-14083.

SPERS, R. G.; WRIGHT, J.T.C.; AMEDOMAR, A.A. Scenarios for the milk production chain in Brazil in 2020. **Rev Adm**, v. 48, n. 2, p. 254-267, 2013.

STELLATO, G.; DE FILIPPIS, F.; LA STORIA, A.; ERCOLINI, D. Coexistence of lactic Acid bacteria and potential spoilage microbiota in a dairy processing environment. **Appl Environ Microbiol**, v.1, p.7893-904, 2015. doi: 10.1128/AEM.02294-15.

STOECKEL, M.; LÜCKING, G.; EHLING-SCHULZ. M.; ATAMER, Z.; HINRICHS, J. Bacterial spores isolated from ingredients, intermediate and final products obtained from dairies: thermal resistance in milk, July 2016, Volume 96, Issue 4, pp 569–577.

SWAMINATHAN, B.; GERNER-SMIDT, P. The epidemiology of human listeriosis.
Microbes Infect. v.10, p.1236-43, 2007.

TONG S.Y.C.; DAVIS J.S.; EICHENBERGER E.; HOLLAND T.L.; FOWLER V.G. *Staphylococcus aureus* infections - epidemiology, pathophysiology, clinical manifestations, and management. **Clin Microbiol Rev**, v.28, p.603–661, 2015. doi: 10.1128/CMR.00134-14.

TULINI, F. L. **Isolation of lactic acid bacteria from milk and cheese with potential for food biopreservation and utilization for increasing whey digestibility**. Doctoral thesis presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP. Ribeirão Preto, 2014. f. 1-182.

USDA. UNITED STATES DEPARTMENT OF AGRICULTURE. **Brazil dairy and products annual dairy report. USDA Foreign Agricultural Service. Gain report number BR1719**. 2017**.** Available in: http://usdabrazil.org.br/pt-br/reports/dairy-and-products-annual-4.pdf. Accessed in: Nov. 29, 2018.

YOON, Y.; LEE, S.; CHOI, K.H. Microbial benefits and risks of raw milk cheese. **Food Control**, v.63, p. 201-215, 2016.

WULLSCHLEGER, S.; JANS, C.; SEIFERT, C.; BAUMGARTNER, S.; LACROIX, C.; BONFOH, B.; STEVENS, M. J. A.; MEILE, L. *Vagococcus teuberi* sp isolated from the Malian artisanal sour milk fènè. **Syst Appl Microbiol**, v. 41, n. 2, p. 65-72, 2018. doi: 10.1016/j.syapm.2017.11.003.

ZAKHARYUK, A.; KOZYREVA, L.; ARISKINA, E.; TROSHINA, O.; KOPITSYN, D.; SHCHERBAKOVA, V. *Alkaliphilus namsaraevii* sp. nov,; an alkaliphilic iron- and sulfur-reducing bacterium isolated from a steppe soda lake. **Int. J Syst Evol Microbiol**, v. 67, p. 1990–1995, 2018. doi 10.1099/ijsem.0.001904. 001904.

ZHENG, Q.; LIN, W.; LIU, Y.; CHEN., C.; JIAO, N. A comparison of 14 *Erythrobacter* genomes provides insights into the genomic divergence and scattered distribution of phototrophs. **Front Microbiol,** v. 7, p. 984, 2016. doi: 10.3389/fmicb.2016.00984.