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# Microbiological profile of raw refrigerated and processed bovine milk at dairy industries from Vale do Taquari, Rio Grande do Sul, Brazil

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**ABSTRACT**
Milk is an essential food, widely consumed by the population. Brazil is one of the world's largest producers of milk. Milk quality is influenced by several factors in all its stages of production. The aim of this study was to determine the microbiological profile of refrigerated and processed raw bovine milk from industries in Vale do Taquari, state of Rio Grande do Sul, Brazil, using metagenomic analysis. A total of six samples were collected, one of refrigerated raw milk from the tanker truck, one of pasteurized milk and one of milk sterilized by the ultra-high temperature (UHT) process, in each of the industries. The identification of the milk microbiota was performed by sequencing the 16S rRNA gene. The results show that refrigerated raw milk has a greater number of microorganisms, followed by pasteurized milk and sterilized milk, successively. Processed milk showed the presence of beneficial microorganisms such as *Streptococcus thermophilus* and *Streptococcus macedonicus*. Nevertheless, even UHT milk showed the presence of microorganisms considered harmful, such as the *Bacillus cereus* group, *Aeromonas dhakensis*, *Enterobacter bacterium* and *Acinetobacter haemolyticus*. Metagenomics is a valuable tool for the thorough evaluation of the milk microbiota in order to implement the processing stages in industries.

**Keywords:** microorganisms; microbiome; genetic sequencing.

## INTRODUCTION

Brazil is considered the fifth largest world milk producer and obtained a production of 6,555,592 thousand liters in the first quarter of 2021, with Rio Grande do Sul accounting for 840,063 thousand liters (IBGE, 2021). The Vale do Taquari region is responsible for much of the state production and this activity is the basis of the economy in the small municipalities that compose it.

Milk is a rich food that has nutritional characteristics essential to humans. The refrigerated raw milk must remain at a maximum temperature of 5 °C until it reaches the industry (BRAZIL, 2018a) and to be consumed, it needs to go through processing stages. This processing can occur in two ways: pasteurization or ultra-high temperature (UHT) sterilization. The Ministry of Agriculture, Livestock and Supply's Normative Instruction No. 76 provides information on the identity and quality characteristics that the refrigerated raw milk, pasteurized milk and pasteurized milk type A should present (BRAZIL, 2018b), and Ordinance No. 370 regulates the characteristics that UHT milk must present (BRAZIL, 1997).

Milk microbiota can be composed of microorganisms beneficial to human health or even deteriorating or pathogenic microorganisms. Bovine milk microbiota has been intensively used with the objective of evaluating and improving animal

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health and ensuring milk quality, additionally to its consumption safety. High-throughput sequencing has been extensively used in determining the microbial community in milk and dairy products, with the objective of identifying microorganisms present that may be difficult to cultivate or are in low concentration (RUSSO et al., 2020).

High-throughput sequencing is used to perform metagenomics, a research field which main objective is to verify the total content of microorganisms present in the analyzed sample (SUDARIKOV et al., 2017). The use of metagenomics can be of great value in the food industry, as it allows the identification of microbiological amplitude, with possible applications in the improvement of products or identification of failures in the processing stages (YAP et al., 2020).

The aim of this study was to determine the microbiological profile of refrigerated and processed raw bovine milk from industries in Vale do Taquari, Rio Grande do Sul, using metagenomic analysis. Refrigerated raw milk of tanker trucks, pasteurized milk and milk sterilized by the UHT process of two industries in the region were tested. The characterization of the milk microbiota was performed by sequencing the 16S rRNA gene, which allows the identification of multiple microorganisms.

## MATERIAL AND METHODS

The study was conducted in the Vale do Taquari region, Rio Grande do Sul, Brazil. The sampling and analyses occurred in March and April of the current year. A total of six samples were collected in two dairy industries in two municipalities, one of refrigerated raw milk from the tanker truck, one of pasteurized milk and one of milk sterilized by the UHT process, in each of the industries. The companies received the name C1 (company 1) and C2 (company 2) and the milk types received their initial, being "R" for refrigerated raw milk, "P" for pasteurized milk, and "S" for sterilized milk. At the time of sampling, the sample temperature was measured using an Incoterm thermometer (model 5135). The samples were collected with 100-mL sterilized plastic bottles and packed in a polystyrene box with ice, which kept the temperature of the samples below 12 °C.

Bacteria identification was performed using high-throughput sequencing of the 16S rRNA gene V3/V4 regions. Amplification with primers for region V3-V4 of the rRNA 16S, 341F (CCTACGGGRSGCAGCAG), and 806R (GGACTACHVGGGTWTCTAAT) gene was performed. polymerase chain reaction reactions were performed in triplicates, with the following conditions: 95 °C for 5 min, 25 cycles of 95 °C for 45 s, 55 °C for 30 s and 72 °C to 45 s and a final extension of 72 °C for 2 min.

Genomic libraries were sequenced using the MiSeq Sequencing System. For single-end sequencing, the V2 kit with 300 cycles was used. The sequences were analyzed through the Sentinel pipeline. In the Sentinel pipeline fastq files were evaluated for Phred quality using the FastQC v.0.11.8 software. Therefore, fastq files were subjected to the trimming of primers and sequences with low quality (Phred < 20). The software used for this purpose was built in Python v.3.6, this being inspired by the features of the BioPython project. For paired-end data, before the trimming step, two pairs of files (R1 and R2) were joined in a single file using pandaseq v.2.11. Clusters with abundance smaller than two were removed from the analyses, as such structures are usually related to chimera sequences. Taxonomic identifications were performed with BLASTn v.2.6.0, using a proprietary or public database as reference. As for the definition of a species, among the 20 hits returned for each cluster, a Python instruction evaluated whether one of the three items would be met by the hits: (i) higher bit-score; (ii) lower value; and (iii) greater representation taxonomies. The data were tabulated using excel and biostatistical analyses were performed using the Past software.

## RESULTS AND DISCUSSION

The analysis of milk samples collected in the industries of Vale do Taquari (RS) confirmed the presence of 51,401 sequences from a single kingdom, Bacteria. Three phyla (Bacteroidetes, Firmicutes and Proteobacteria), nine classes (Alphaproteobacteria, Bacilli, Bacteroidia, Betaproteobacteria, Clostridia, Epsilonproteobacteria, Flavobacteriia, Gammaproteobacteria and Negativicutes), 15 orders, 21 families, and 41 genera were found (Table 1). RYU et al. (2021), in their study with refrigerated raw milk, reported having found microbiota with the prevalence of the phyla Proteobacteria, Bacteroidetes and Firmicutes, Actinobacteria—three in common with this study.

**Table 1.** Orders, families and genera found in samples of refrigerated, pasteurized and sterilized raw milk from the companies of Vale do Taquari, Rio Grande do Sul, Brazil.

| Orders | Families | Genera | |
|---|---|---|---|
| Aeromonadales | Acetobacteraceae | *Acetobacter* | *Lysinibacillus* |
| Alteromonadales | Aeromonadaceae | *Acinetobacter* | *Macrococcus* |
| Bacillales | Bacillaceae | *Aeromonas* | *Morganella* |
| Bacteroidales | Burkholderiaceae | *Bacillus* | *Pantoea* |
| Burkholderiales | Campylobacteraceae | *Campylobacter* | *Prevotella* |
| Campylobacterales | Carnobacteriaceae | *Chryseobacterium* | *Proteus* |
| Clostridiales | Clostridiaceae | *Citrobacter* | *Providencia* |
| Enterobacteriales | Comamonadaceae | *Clostridium* | *Pseudomonas* |
| Flavobacteriales | Enterobacteriaceae | *Comamonas* | *Rahnella* |
| Lactobacillales | Enterococcaceae | *Cronobacter* | *Ralstonia* |
| Pseudomonadales | Erwiniaceae | *Elizabethkingia* | *Raoultella* |
| Rhodospirillales | Flavobacteriaceae | *Empedobacter* | *Serratia* |
| Selenomonadales | Moraxellaceae | *Enterobacter* | *Shewanella* |
| Sphingomonadales | Planococcaceae | *Enterococcus* | *Sphingomonas* |
| Xanthomonadales | Prevotellaceae | *Escherichia* | *Staphylococcus* |
| | Pseudomonadaceae | *Granulicatella* | *Stenotrophomonas* |
| | Shewanellaceae | *Hafnia* | *Streptococcus* |
| | Sphingomonadaceae | *Klebsiella* | *Vagococcus* |
| | Staphylococcaceae | *Kluyvera* | *Veillonella* |
| | Streptococcaceae | *Kurthia* | *Viridibacillus* |
| | Veillonellaceae | *Lactococcus* | |
| | Xanthomonadaceae | | |

The analyses also demonstrated the presence of 87 species (Table 2). Of the total species, 59% (52 species) had abundance smaller than 50 sequences, that is, less than 1% of the total individuals found (51,401). For TAPONEN et al. (2019), microorganisms appearing in small quantities should not be considered as they may indicate sporadic species. In addition, the microbiota of bovine milk is an extremely complex issue, as the results obtained for bovine milk using the same sampling on the same day of collection differed significantly in their study. For PARENTE et al. (2020), although diverse, the milk microbiota shows some similarity in some studies, especially at the phylum and genus level.

The total amount of microorganisms in raw, pasteurized and sterilized milk shows that refrigerated raw milk has the highest number of microorganisms (29,334), followed by pasteurized milk (14,674) and sterilized milk (7,393). This represents a reduction of 49.97% of the total microorganisms from refrigerated raw milk to pasteurized milk and 74.79% from refrigerated raw milk to sterilized milk. This decrease indicates that milk processing steps are being effective in the general decrease of the present microorganisms. The decrease is necessary for milk to have microbiological levels safe to human health (ROSENBERG, 2020). However, the beneficial food microbiota also ends up being eliminated or decreased considerably, such as important probiotic microorganisms.

Regarding the frequency of microorganisms in the samples, it can be observed that a single microorganism, *Sphingomonas echinoides*, was present in all six samples. Despite being the most frequent microorganism, *S. echinoides* is not a microorganism abundant in the samples, and the sum of all sequences obtained is nine, that is, four of the six samples analyzed presented only one sequence of this microorganism. VRIES et al. (2018), this microorganism is a biofilm-former and can metabolize a wide range of substrates. These characteristics may be related to the fact that this microorganism appears in all samples, even with negligible abundance, because the temperature variations of the samples at the time of collection were from 1.2 to 30 °C. Nine other microorganisms were present in five of the six samples analyzed: *Bacillus cereus* group*, Enterobacter bacterium, Enterobacter hormaechei, Kluyvera intermedia, Streptococcus macedonicus, Acinetobacter johnsonii, Kurthia gibsonii, Staphylococcus epidermidis, Acinetobacter baumannii*.

The most abundant microorganisms found in the three types of milk analyzed in this study are shown in Figure 1a. The microorganism with the highest number of sequences found was *K. gibsonii* (9,569), followed by *Priestia megaterium*, (8,696) and *S. macedonicus* (8,459). Additionally, *K. gibsonii, A. baumannii, B. cereus* group and *E. bacterium* were present in five of the six samples analyzed—they are therefore frequent microorganisms. *Kurthia gibsonii* is in five of the six samples analyzed, besides being abundant in the three types of milk, raw refrigerated, pasteurized and sterilized (Fig. 1).

**Table 2.** Total of each species sequences found in the samples of refrigerated, pasteurized and sterilized raw milk from the industries of Vale do Taquari (RS).

| Species | Total | Species | Total | Species | Total |
|---|---|---|---|---|---|
| *Kurthia gibsonii* | 9569 | *Comamonas aquatica* | 110 | *Pseudomonas fragi* | 24 |
| *Priestia megaterium* | 8696 | *Streptococcus oralis* | 102 | *Streptococcus agalactiae* | 21 |
| *Streptococcus macedonicus* | 8459 | *Morganella morganii* | 88 | *Prevotella melaninogenica* | 19 |
| *Enterobacter cloacae* | 5510 | *Pantoea agglomerans* | 84 | *Providencia alcalifaciens* | 19 |
| *Bacillus cereus group* | 5450 | *Pseudomonas aeruginosa* | 83 | *Serratia marcescens* | 19 |
| *Lysinibacillus sphaericus* | 3475 | *Pseudomonas putida group* | 81 | *Ralstonia insidiosa* | 18 |
| *Enterobacter bacterium* | 2201 | *Proteus vulgaris* | 79 | *Acinetobacter johnsonii* | 17 |
| *Lactococcus garvieae* | 1325 | *Acinetobacter bereziniae* | 66 | *Citrobacter braakii* | 17 |
| *Acinetobacter baumannii* | 1097 | *Enterobacter hormaechei* | 66 | *Comamonas kerstersii* | 17 |
| *Aeromonas hydrophila* | 858 | *Lysinibacillus fusiformis* | 55 | *Streptococcus porcorum* | 17 |
| *Aeromonas dhakensis* | 427 | *Acinetobacter junii* | 52 | *Staphylococcus epidermidis* | 16 |
| *Aeromonas caviae* | 418 | *Proteus mirabilis* | 45 | *Lactococcus lactis* | 15 |
| *Acinetobacter haemolyticus* | 354 | *Raoultella ornithinolytica* | 42 | *Enterobacter ludwigii* | 14 |
| *Streptococcus equinus* | 262 | *Aeromonas sanarellii* | 38 | *Shewanella xiamenensis* | 13 |
| *Enterococcus sp.* | 238 | *Vagococcus fluvialis* | 34 | *Acinetobacter sp.* | 12 |
| *Viridibacillus arenosi* | 206 | *Klebsiella oxytoca* | 33 | *Chryseobacterium oncorhynchi* | 12 |
| *Streptococcus uberis* | 173 | *Aeromonas veronii* | 30 | *Granulicatella adiacens* | 12 |
| *Streptococcus thermophilus* | 163 | *Klebsiella aerogenes* | 30 | *Kluyvera intermedia* | 12 |
| *Providencia stuartii* | 157 | *Macrococcus caseolyticus* | 28 | *Pseudomonas azotoformans* | 12 |
| *Hafnia alvei* | 147 | *Acetobacter pasteurianus* | 27 | *Empedobacter brevis* | 11 |
| *Acinetobacter calcoaceticus* | 137 | *Enterococcus italicus* | 25 | *Lactococcus piscium* | 11 |
| *Escherichia hermannii* | 131 | *Acinetobacter nosocomialis* | 24 | *Streptococcus cristatus* | 11 |
| *Enterobacter mori* | 127 | *Kluyvera cryocrescens* | 24 | *Kluyvera ascorbata* | 10 |
| *Citrobacter freundii* | 122 | | | | |

Species found in the samples of industries from Vale do Taquari - RS that obtained a total number of sequences greater than 0.2% (10 sequences) of the total microorganisms and the total sequences found for each species.

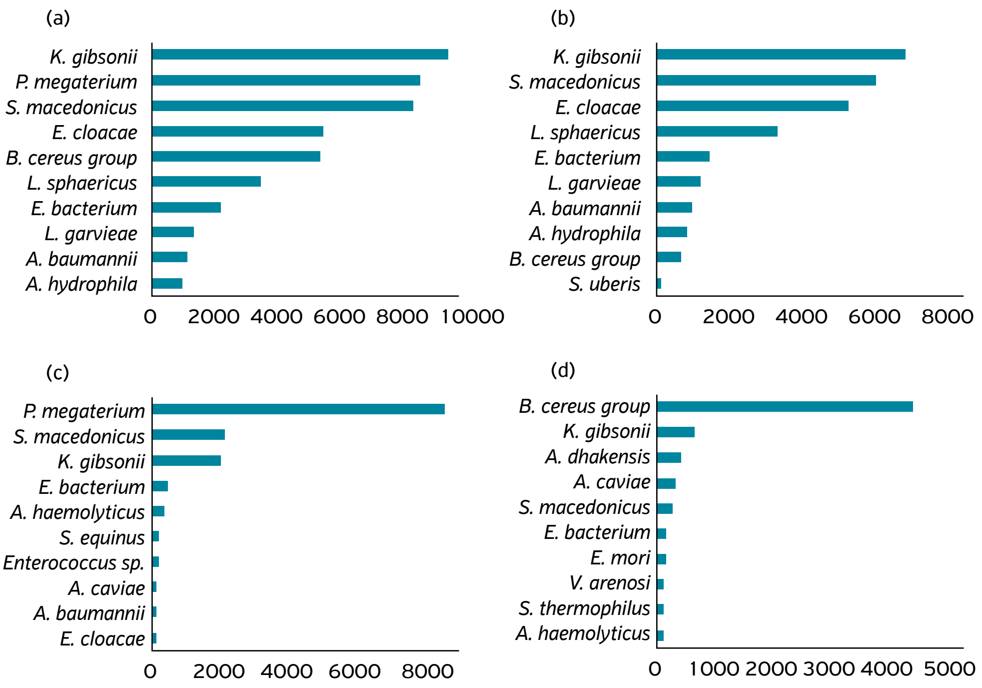

**Figure 1.** Species and quantities of microorganisms more abundant in (a) the three types of milk; (b) ten most abundant microorganisms in raw refrigerated milk; (c) ten most abundant microorganisms in pasteurized milk; and (d) ten most abundant microorganisms in sterilized milk (d) in the two industries from Vale do Taquari, Rio Grande do Sul, Brazil.

According to SEKOAI et al. (2022), *K. gibsonii* is a gram-positive bacterium of the phylum Firmicutes found in decomposing organic matter, meat products and milk. In their study on the microbial population of wood cutting board used for meat, they found prevalence of *Lactococcus garvieae*, *Weissella hellenica* and *K. gibsonii*. Because this is a microorganism commonly found in foods, such as meat products and milk, its presence in the analyzed samples is within the expected.

The second most abundant microorganism, *P. megaterium*, formerly known as *Bacillus megaterium*, is a Gram-positive endospore-forming microorganism found in seawater, soil and dry foods. It is an industrially relevant species to produce enzymes and vitamins and can also be used for decontamination of toxic waste. *Priestia megaterium* represents a great tool for cell biology studies, mainly as a bacterium promoting growth in plants (BIEDENDIECK et al., 2021).

The third most abundant microorganism in the three types of milk analyzed is *S. macedonicus*, an acid-lactic bacterium commonly used as an initial culture in industrial dairy fermentations due to its ability to rapidly acidify milk and prevent deterioration (EL HATMI et al., 2018).

*Acinetobacter baumannii*, *B. cereus* group and *E. bacterium*, as mentioned, are frequent, besides being abundant in the samples. To PAKHARUKOVA et al. (2018), *A. baumannii* is one of the main causes of nosocomial infections, having the ability to persist in the environment, as it easily forms biofilms, in addition to possessing resistance to antibiotics. This microorganism has been isolated from various animal, human and environmental sources, where it plays a role in the decomposition of organic matter. The existence of *A. baumannii* in milk samples can be explained by this being a microorganism present in several sources such as animals, besides being a psychrotrophic species, of easily proliferation in refrigerated milk, such as raw and pasteurized milk. The presence of this microorganism, especially in processed milk, deserves attention.

The *B. cereus* group consists of at least 12 Gram-positive, optionally mobile, saprophyte and facultative anaerobic bacteria. This group is common in nature in both endospore forms as in vegetative cells. Endospores are resistant to extreme environmental conditions and are commonly associated with food poisoning. Some species of the *B. cereus* group are psychrotrophic and can grow at temperatures lower than 7 °C, causing concerns for the food industry due to its capacity to cause a deterioration in refrigerated foods, such as raw milk and pasteurized milk (TAKAHASHI et al., 2021). The fact that this microorganism strains are resistant to extreme environmental conditions may explain their abundance and frequency in milk samples from industries in Vale do Taquari, whether in milk refrigerated or exposed to the thermal process, as is the case of pasteurized and UHT.

According to MUENSRITHARAM et al. (2016), *Enterobacter* species are associated with a variety of environmental habitats, usually found in soil and water, in addition to containing the main antibiotic-resistant bacterial pathogens. These microorganisms are not recognized as important food-borne pathogens, but may be found in a wide variety of foods. The species found in milk are usually eliminated by pasteurization, but members of this genus were found in pasteurized milk and milk cream. In the present study, the pasteurized milk showed the presence of *E. bacterium*. The fact that this microorganism is found in several environments, including water, may explain its presence in the milk samples from this study, as many animals use surface water for their drinking or may still ingest this microorganism in their diet.

For being microorganisms capable of causing damage to human health, the presence and abundance of *K. gibsonii*, *A. baumannii, B. cereus* group and *E. bacterium* in samples deserve attention; moreover, *E. bacterium* and *A. baumannii* show resistance to antibiotics and biofilm formation capacities, making it more difficult for the treatment and disposal on the environment. According to MORADI; TAJIK (2017), biofilm formation promotes the growth and survival of pathogenic microorganisms within food processing units and are major risks to public health.

In addition to the microorganisms mentioned, *Lysinibacillus sphaericus*, *L. garvieae* and *A. hydrophila* were also abundant in the total number of analyzed samples, with respectively 3,475, 1,325 and 858 sequences. *Lysinibacillus sphaericus* is a spore-forming bacterium, considered plant growth promoter (RODRÍGUEZ et al., 2019). According to ERACLIO et al. (2017), *L. garvieae* is one of the most important pathogens in the aquaculture sector, for causing infections in fish. This species is found in different types of food, besides colonizing different types of environments. *Aeromonas hydrophila* is a Gram-negative bacterium also found in various aquatic environments, which can cause septicemia in humans. The contamination of milk samples by *L. garvieae* and *A. hydrophila*, microorganisms causing diseases in fish, may be tied to the environment where the animals are raised, as often there are other activities in milk-producing properties, such as fish ponds, and animals can roam near the site or use the water for their drinking (LI et al., 2021).

When analyzing the ten most abundant microorganisms in each type of milk (raw refrigerated milk up to milk sterilized by the UHT process), it can be seen that *K. gibsonii, E. bacterium* and *S. macedonicus* appear as abundant in the three types. By adding the number of sequences of the ten most abundant in each type of milk, it is possible to observe a gradual decrease from the raw refrigerated milk to sterilized milk, as observed in the sum of the total amount of sequences. The ten most abundant total were 27,294 sequences in raw refrigerated milk, 13,941 in pasteurized milk, and 6,939 in sterilized milk. As already observed in studies such as that of TAPONEN et al. (2019), there is a great diversity in microorganisms observed in the samples of the same type of milk in each of the collected companies. The microorganisms described in this study as the most abundant for each type of milk were almost totally restricted to only one of the industries, except for *S. macedonicus*, in raw refrigerated milk. Therefore, there was no sum of sequences of the most abundant species for milk types.

The most abundant microorganism in raw refrigerated milk was *K. gibsonii* with 6,907 sequences, followed by *S. macedonicus* (6,107, being 1,067 at C1 and 5,040 at C2), *Enterobacter cloacae* (5,339) and *L. sphaericus* (3,402) (Fig. 1b). Seven (70%) of the ten most abundant microorganisms were in the sample from Company 1. *Enterobacter cloacae* is included as a common nosocomial pathogen capable of producing a variety of infections and having resistance to broad-spectrum antibiotics, but its presence in milk is not a concern as the heat treatment of milk by UHT safely inactive members of the Enterobacteriaceae family (ANNAVAJHALA et al., 2019).

When analyzing the ten most abundant microorganisms in pasteurized milk (Fig. 1c), the microorganism with the highest number of sequences was *P. megaterium*, with 8,696, followed by *S. macedonicus* (2,091) and *K. gibsonii* (1,992), being the other seven less expressive sequence numbers, lower than 400. Seven of the ten most abundant microorganisms belonged to C2, but the most abundant microorganism (*P. megaterium*) was present only in the pasteurized milk sample of C1-P. When comparing the abundance in the analyzed samples, it can be seen that *P. megaterium* (with 8,696 sequences) is restricted to pasteurized milk.

In this study, *E. cloacae* and *A. baumannii* appear to be abundant in raw and pasteurized milk. These microorganisms, being found in pasteurized milk cause concern, because although little consumed, this milk is used to produce yogurts and other products in the dairy industries. Additionally, pasteurized milk also presented *Streptococcus equinus*, which is a microorganism belonging to the *Streptococcus bovis/S. equinus* complex, a diverse group of bacteria that includes inhabitants of the gastrointestinal tract of humans and animals, being an opportunistic pathogen found in food (KAINDI et al., 2018).

When analyzing the ten most abundant microorganisms in sterilized milk (Fig. 1d) it was found that the B. cereus group was the most abundant, with 4,743 sequences identified. All other microorganisms obtained a much lower number of sequences, being the second *K. gibsonii*, with 661, and the last placed *Acinetobacter haemolyticus*, with only 83 sequences. The most abundant microorganism, as well as 80% of the total abundant from sterilized milk were present in the sample from C1.

*Bacillus cereus* group and *A. haemolyticus* were abundant in the samples of raw refrigerated milk and in the sterilized milk samples. The latter was defined as a Gram-negative, strictly aerobic and non-fermentative coccobacillus widely distributed in nature and commonly found in soil, water, and hospitals and constitutes a challenge for public health (BAI et al., 2020). Because it is a microorganism found in soil and water it may have been ingested by the animals and therefore found in the milk samples of the present study.

Milk sterilized by the UHT process also presented sequences of microorganisms such as *Aeromonas dhakensis* (400), *Aeromonas caviae* (321), *Enterobacter mori* (120), *Viridibacillus arenosi* (113) and *Streptococcus thermophilus* (105). *Streptococcus thermophilus*, as well as *S. macedonicus* commented earlier, is a widely known microorganism and used in the food industry. It is a probiotic of lactic acid widely used in dairy products as an initial culture for the manufacturing of cheeses and yogurts (PHILIPPE et al., 2020). *Aeromomas dhakensis* is a pathogen, infection-causing, widely distributed in the environment and causing a variety of infections in humans (CHEN et al., 2017). *Aeromomas caviae* is also a microorganism found in the environment, usually in places with high salinity (CARDOZO et al., 2019). *Viridibacillus arenosi* is an aerobic bacterium that forms spores, psychrotolerant and deteriorating. This microorganism is predominant in foods such as milk (THAKUR et al., 2017). *Enterobacter mori* is a plant pathogenic microorganism, being described as the causing agent by bacterial wilt in *Morus alba* (white mulberry), a serious disease in orchards. This microorganism is not commonly associated with human diseases, but in its study is reported a case of acute extreme otitis, in Austria, whose isolate demonstrated *in vitro* resistance to carbapenems (HARTL et al., 2019).

Heat treatment in milk has the function of eliminating all pathogenic bacteria that can cause infections to consumers, in addition to inactivating enzymes and reducing the total amount of microorganisms, so that it is possible to extend the shelf-life of the product. The UHT processing allows to achieve commercial sterility, with minimal impact on the milk nutritional value (ROSENBERG, 2020). The Ordinance No. 370 (BRAZIL, 1997) brings as microbiological criteria that UHT milk must present up to 100 CFU/mL of mesophilic aerobics. The levels observed in this study are above the maximum limit allowed by the ordinance, but metagenomics is a more sensitive method, which allows the identification of a more accurate number of microorganisms in the samples than the methods used for conventional microbiological analysis.

The presence of *B. cereus* group, *A. haemolyticus*, *A. dhakensis* and *A. caviae* deserves attention because they are microorganisms causing infections in humans and their presence in the samples indicates that the thermal process was not completely efficient. STRÖHER et al. (2021) concluded in their study that UHT milk with lower quality came from raw material with low quality, i.e., raw refrigerated milk with high microorganism count, or high total bacterial count. According to ROSENBERG (2020), inappropriate milk storage has a significant impact on the composition of the microbial community. This impact is greater for species of the *Streptococcus*, *Staphylococcus*, *Macrococcus*, and *Corynebacterium* genera and deteriorating bacteria, such as *Acinetobacter* and *Pseudomonas*, psychrotrophic bacteria that form spores (PARENTE et al., 2020). In this study, several species of the *Streptococcus* and *Acinetobacter* genera were observed in the samples collected in the dairy industries.

Among the ten most abundant in the three types of milk and raw refrigerated milk there are nine common microorganisms. *Priestia megaterium* appears only in pasteurized milk, as already mentioned and *S. uberis* in raw refrigerated milk; the latter being one of the main causes of clinical mastitis worldwide, being considered a barrier in its control due to its epidemiology not being fully understood (TOMAZI et al., 2019). Because it is a mastitogenic microorganism, its presence is common in raw refrigerated milk.

By observing the different types of microorganisms present between raw refrigerated milk and processed milk (pasteurized and sterilized), it can be noticed that the processing is efficient in the elimination of microorganisms such as *L. sphaericus*, *L. garvieae* and *A. hydrophilic* and, in addition to these, sterilization (UHT) is still efficient for *E. cloacae*, *A. baumannii*. Spore-forming microorganisms such as *B. cereus* group, *V. arenosi* and *P. megaterium* may be more difficult to eliminate by the thermal process.

The samples Shannon Diversity Index ranged from 0.2112 in the pasteurized milk sample from company 1 (C1-P) to 2.18 in the raw refrigerated milk sample also from company 1 (C1-R). The indexes of each sample were: raw refrigerated milk: 2.18 (C1-R) and 1.153 (C2 -R); pasteurized milk: 0.2112 (C1-P), 1.919 (C2-P) and sterilized milk: 1,399 (C1-S) and 1,817 (C2-S). The multivariate principal component analysis (Fig. 2) shows that the component 1 (x-axis) explains 33.29% of the data variability and component 2 (y-axis) explains 17.67% of the variability. Together these two components explain 50.96% of the results. Through the analysis of the perceptual map, it can be verified that the samples from company of raw refrigerated milk 1 (C1 – R), sterile milk (C1 – S) and the pasteurized milk sample of company 2 (C2 – P) are correlated. The company 2 sample of raw refrigerated milk (C2 – R) and sterilized milk (C2 – S) also have correlation, indicating similarity in the microorganisms found in both samples and their quantity.

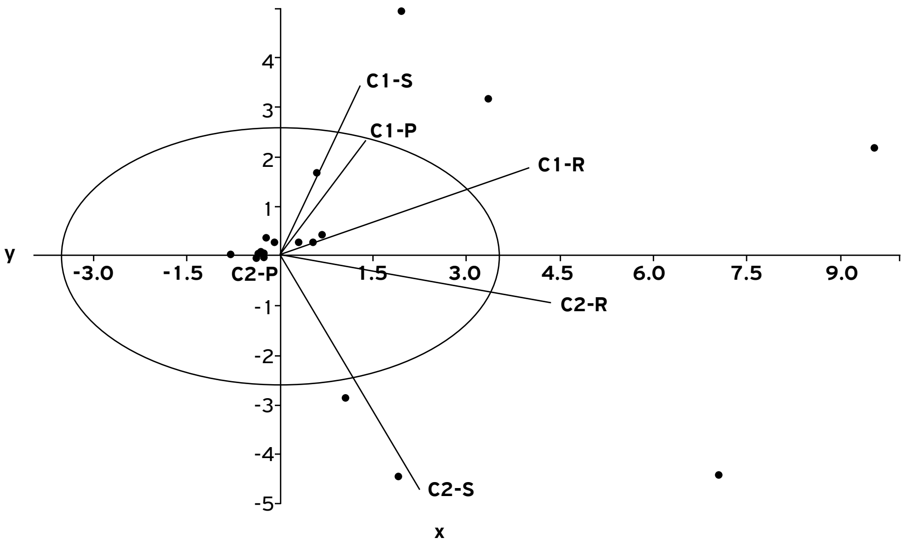

**Figure 2.** Perceptual map of the multivariate analysis of milk samples collected in industries from Vale do Taquari, Rio Grande do Sul, Brazil. Sort chart using multivariate analysis in Past. C1-R: Raw milk from Company 1; C2-R: Raw milk from Company 2; C1-P: Pasteurized milk from Company 1; C2-P: Pasteurized milk from Company 2; C1-S: Sterilized milk from Company 1; C2-S: Sterilized milk from Company 2.

When analyzing the samples for species diversity, it can be observed that the sample of raw refrigerated milk from company 1 (C1-R) has the largest number of different species with 62 species of microorganisms, followed by the pasteurized milk sample from company 2 (C2-P). The sample with the smallest number of different species is raw refrigerated milk from company 2 (C2-R), with only 17 different species (Fig. 3a).

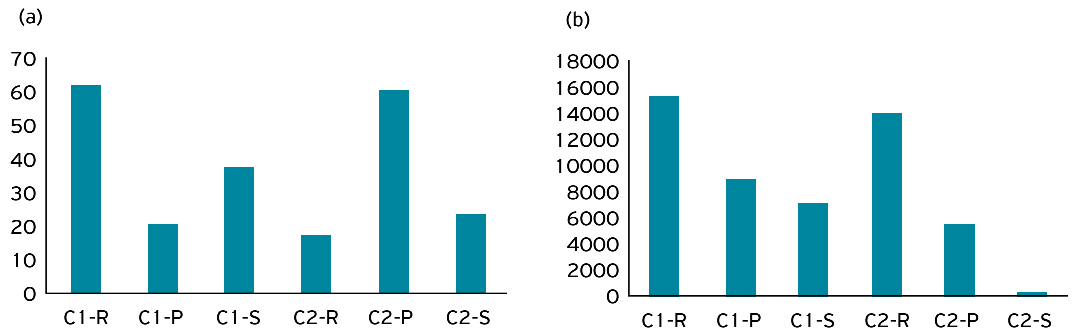

**Figure 3.** Abundance and diversity of different species found in each milk sample collected in the industries of Vale do Taquari, Rio Grande do Sul, Brazil. (a) Species diversity found in refrigerated, pasteurized and sterilized raw milk samples; and (b) Abundance of species found in refrigerated, pasteurized and sterilized raw milk samples. C1-R: Raw milk from Company 1; C2-R: Raw milk from Company 2; C1-P: Pasteurized milk from Company 1; C2-P: Pasteurized milk from Company 2; C1-S: Sterilized milk from Company 1; C2-S: Sterilized milk from Company 2.

When analyzing the samples separately regarding microbiological abundance, it is possible to notice that the two samples with the highest abundance are of raw refrigerated milk from company 1 and company 2, with a total of 15,384 (C1-R) and 13,950 (C2-R) sequences, respectively. The sterile milk sample from company 1 (C1-S) has greater abundance than the pasteurized milk sample from company 2 (C2-P). However, when the number of sequences in the samples from the same company was analyzed, there was a gradual decrease in the two samples of raw refrigerated milk for the two types of processed milk, pasteurized and sterilized, respectively (Fig. 3b).

## CONCLUSION

The analysis of milk collected from the industries in Vale do Taquari showed a significant decrease in the number of microorganisms from raw refrigerated milk to milk processed by pasteurization and sterilization processes (UHT). This indicates that the processes are effective in reducing the total amount of microorganisms, in addition to eliminating microorganisms that cause milk deterioration or problems to the consumer health, such as *L. sphaericus, L garvieae, A. hydrophilic, E. cloacae* and *A. baumannii*. The processed milk demonstrated the presence of microorganisms beneficial to human health, such as *S. thermophilus* and *S. macedonicus*, but also microorganisms considered harmful as the *B. cereus* group, *A. dhakensis, E. bacterium* and *A. haemolyticus*, showing that thermal processes have not been completely efficient. Metagenomics allows the identification of the microbiota present in milk and, consequently, a more accurate evaluation of its quality. These studies can be used to improve the processing steps used by industries and to trace possible contaminant sources. Improving the quality of the milk produced is essential to ensure the health of the consumer.

### AUTHORS' CONTRIBUTIONS

**Conceptualization:** Müller, T.; Rempel, C.; Maciel, M.J.; Lunardi, L. **Data curation:** Müller, T.; Rempel, C. **Formal analysis:** Müller, T.; Rempel, C. **Funding acquisition:** Rempel, C.; **Investigation:** Müller, T. **Methodology:** Müller, T.; Maciel, M.J. **Project administration:** Rempel, C. **Resources:** Müller, T.; Rempel, C.; Maciel, M.J. **Supervision:** Rempel, C.; Maciel, M.J. **Validation:** Rempel, C.; Maciel, M.J. **Visualization:** Rempel, C.; Maciel, M.J. **Writing – original draft:** Müller, T.; Rempel, C.; Maciel, M.J.; Lunardi, L. **Writing – review & editing:** Rempel, C.; Maciel, M.J.

### AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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### CONFLICTS OF INTEREST

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

### ETHICAL APPROVAL

Not applicable.

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