Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00153

Metataxonomic analysis of bacterial and fungal communities in colonial cheese

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Abstract

Colonial cheese is one of the typical products of the southern region of Brazil, and it is known that this type of food is home to a complex microbial community. Therefore, this study aimed to evaluate the microbiota present in Colonial cheese, through the use of a high-performance system MiSeq Sequencing System, from the regions V3/V4 of the 16S rRNA using the 341F and 806R primers for bacteria and ITS1/ITS2 primers for the ITS1 region of molds and yeasts. Sequence analyses were performed using the Sentinel pipeline. The results for bacterial microbiome showed that there were 4 phyllo, 30 genera, and 57 species in the sample, among them *Lactococcus lactis* (30.63%), *Corynebacterium variable* (17.91%), *Enterococcus* sp. (17.4%), and *Bifidobacterium psychraerophilum* (7.24%), all naturally found in milk. In addition, 19 yeast species were identified, including *Diutina catelunata, Clavispora lusitaniae, Kodamaea ohmeri, Kluyveromyces marxianus*, *and Candida ethanolica* those that obtained the highest number of sequences reads. The results obtained on the microbiome are in line with other studies on cheeses across the globe, considering that the microorganisms are naturally found in both the raw material and the production environment.

Keywords: metataxonomics; metagenomic; probiotic; genetic sequencing; bacteria; 16S rRNA; ITS region; mold; yeast.

Practical application: This study presents significant findings on microbial diversity in Colonial cheese using a metataxonomic approach, suggesting the possibility of discovering potential new probiotics within this cheese variety.

1 INTRODUCTION

Worldwide, milk is among the top five most traded products, and in Brazil, the dairy sector ranks as the second most important within the food industry (Siqueira, 2019). In 2021, milk production in Santa Catarina, a southern region of Brazil, reached 3.2 billion liters, with the western region being the most productive (Epagri, 2023). In addition to being among the most exported dairy products in Brazil, cheeses have shown the highest sales growth rate in the domestic market in recent years (Carvalho & Rocha, 2019). Colonial cheese is defined as cheese obtained through milk coagulation using rennet and/or other coagulating enzymes (Santa Catarina, 2018). The production of Colonial cheese is concentrated in regions primarily settled by descendants of Italians and Germans, including western and southern Santa Catarina, southwestern and western Paraná, and the northwest region of Rio Grande do Sul (Dorigon, 2020).

Traditionally, the study of microbial communities in this type of cheese has been conducted using techniques employed in classical microbiology, such as traditional plate counting methods, isolation, and biochemical identification of microorganisms (Vanetti & Machado, 2021). However, these approaches may yield imprecise results due to the presence of injured microorganisms that cannot be detected and do not consider microorganisms that require more complex analysis methods (Rantsiou et al., 2005). As an alternative to phenotypic methods for taxonomic purposes, there has been a recognized need for rapid, reproducible, accurate, and unbiased molecular methods (Kazou et al., 2021). Molecular methods based on PCR are commonly used for the identification of bacteria and yeasts associated with dairy products, utilizing primers targeting different genomic sequences (Kazou et al., 2021). However, over the past 20 years, there has been a greater focus on the development of culture-independent methodologies, without the enrichment or isolation of strains, as a way to avoid misleading results due to the low detection of subdominant or difficult-to-cultivate organisms (Cocolin & Ercolini, 2015; Jonnala et al., 2018). These studies are primarily based on the genetic sequencing of populations present in food. Indeed, several studies have already demonstrated considerable variation between cultivated and naturally occurring species (Kazou et al., 2021).

In general, the term metataxonomic is related to the identification and characterization of microbial communities at the genomic level through culture-independent approaches (National Research Council, 2007). The highlight of this approach lies in sequencing for the identification of the entire microbiota of a sample using marker genes such as the 16S rRNA gene for bacteria and the intergenic region ITS for fungi. According to Fajarningsih (2016), the ITS region is a highly polymorphic non-coding region with the ability to differentiate fungi at the species and even subspecies level. Therefore, this

Received 10 Sept., 2023.
Accepted 7 Oct., 2023.
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Conflict of interest: nothing to declare.
Funding: Foundation for Research and Innovation Support of the State of Santa Catarina and Conselho Nacional de Desenvolvimento Científico e Tecnológico, under grant agreement no. 2021TR001446, 2022TR002005, and 402338/2023-5.

region has become the most frequently used for identifying molds and yeasts in dairy products (Banjara et al., 2015; Buehler et al., 2017). Additionally, the 16S rRNA gene contains conserved regions that allow for base pair comparisons between species (Cocolin & Rantsiou, 2007). Bacterial specificity is enhanced by the taxonomic identification of hyper-sensitive regions such as V3 and V4 (Zhang et al., 2018). The metataxonomic approach can provide taxonomic results from the phylum to the species level of the identified microorganism, using bioinformatics tools and public databases such as GreenGene and RibosomalDatabase (De Cesare, 2019; Franciosa et al., 2018). Through these methodologies, it is possible to identify the microbiota of any food, including cheeses. Thus, it is possible to understand the relationship between microorganisms, their activities, and functionalities in food products (Franciosa et al., 2018). Therefore, the objective of this study was to identify and quantify the bacterial and fungal microbiome present in Colonial cheese, using the V3–V4 regions of the 16S rRNA gene and the ITS1 and ITS2 regions of the intergenic ITS region as the basis for analysis.

2 MATERIALS AND METHODS

2.1 Sample

The Colonial cheese sample was collected from a rural property and subjected to metataxonomic evaluation after a 20-day maturation period. The producer is a member of the Cooperative of Production and Consumption of Family Producers and Agribusinesses of Seara (COOPASE). The sample was collected and frozen in its original vacuum packaging until the investigation of the bacterial and fungal community.

2.2 Metataxonomic analysis

The microbial diversity was studied based on sequenced libraries using the MiSeq Sequencing System (Illumina Inc., USA) and the V2 kit, with 300 cycles and single-end sequencing. For high-throughput sequencing of the V3/V4 regions of the 16S rRNA gene, a 25 g aliquot of the sample was weighed, which was then homogenized with 225 mL of tryptone saline solution. After this step, DNA extraction was performed using the magnetic beads technique with a proprietary protocol developed by Neoprospecta Microbiome Technologies, Brazil. Next, PCR amplification was carried out in triplicate using Platinum Taq Polymerase (Invitrogen, USA), under the following conditions: 95°C for 5 min, 25 cycles of 95°C for 45 s, 55°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 2 min. Amplification was performed with primers 341F (CCTACGGGRSGCAGCAG) (Wang & Quian, 2009) and 806R (GGACTACHVGGGTWTCTAAT) universal for the V3/V4 region of the 16S rRNA gene for bacteria (Caporaso et al., 2012). For fungi, amplification was generated with primers for the ITS1 region, primer ITS1 (GAACCWGCGGARGGATCA) (Schmidt et al., 2013), and primer ITS2 (GCTGCGTTCTTCATCGATGC) (White et al., 1990). Sequences were analyzed through a pipeline, and libraries were prepared, both following a proprietary protocol (Neoprospecta Microbiome Technologies, Brazil).

2.2.1 Bioinformatics

The Phred quality scores (QP) of the fastq files in the Sentinel pipeline were assessed using the FastQC program v.0.11.8 (Andrews, 2010). Subsequently, these files underwent primer screening and the removal of low-quality sequences (Phred < 20) through proprietary software built in Python v.3.6, inspired by the capabilities of the BioPython project (Cock et al., 2009). In general, clusters with abundances less than 2 are associated with chimera sequences (Smyth et al., 2010). Therefore, they were excluded from the analyses. blastn v.2.6.0+ (Altschul et al., 1990) was used to obtain identifications, with a proprietary database as the reference. The species designation was established through a Python statement that assessed whether one of the following criteria was met by the hits: (1) higher bit score; (2) lower e-value; and (3) taxonomies with greater representation. The representative species were selected from the hits that met one of the criteria. The obtained DNA sequences were compared with proprietary or public databases (Quast et al., 2013) and Greengenes (DeSantis et al., 2006) containing various previously characterized DNA sequences.

3. RESULTS AND DISCUSSION

3.1 Bacterial diversity in colonial cheese

A total of 76,695 reads were identified through bacterial metataxonomic analysis. Taxonomically, four phyla were found: Firmicutes (65.44%), Actinobacteria (26.38%), Proteobacteria (8.09%), and Bacteroidetes (0.1%). Gill et al. (2006) identified microorganisms from the external environment in the teat canal, with the predominant phyla being Firmicutes, Actinobacteria, and Proteobacteria. Delbès et al. (2007) examined microbial diversity in Saint-Nectaire cheese, while Zhong et al. (2016) investigated microbial variations in fermented milk from different regions of China, Mongolia, and Russia. Both research groups observed that the phyla Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes are abundantly present in both raw materials and fermented products. Additionally, Gill et al. (2006) suggested that initial contamination of raw materials occurs in the udder due to microorganisms present in the environment, with the Firmicutes phylum being the most prevalent.

In relation to the abundance of bacterial genera in the cheese sample (Figure 1A), it was possible to observe that the most abundant genera were *Lactococcus*, *Enterococcus*, *Bifidobacterium*, and *Corynebacterium*. These genera can contribute positively to milk processing or aid in the deterioration of raw materials (Quigley et al., 2013). *Lactococcus* and *Enterococcus* are naturally found in raw milk and are part of the microbial composition of cheeses. Both groups are responsible for lactate production and contribute to the sensory characteristics of cheeses, as they are involved in proteolysis and lipolysis processes (Cavanagh et al., 2015; Quigley et al., 2013).

Furthermore, *Enterococcus*, *Lactobacillus*, and *Leuconostoc* are heterofermentative species, meaning that they produce gases and, in conjunction with the action of some yeasts, create holes in cheeses (Beresford et al., 2001), which is a desirable characteristic in Colonial cheese (Santa Catarina, 2018). Similar to our

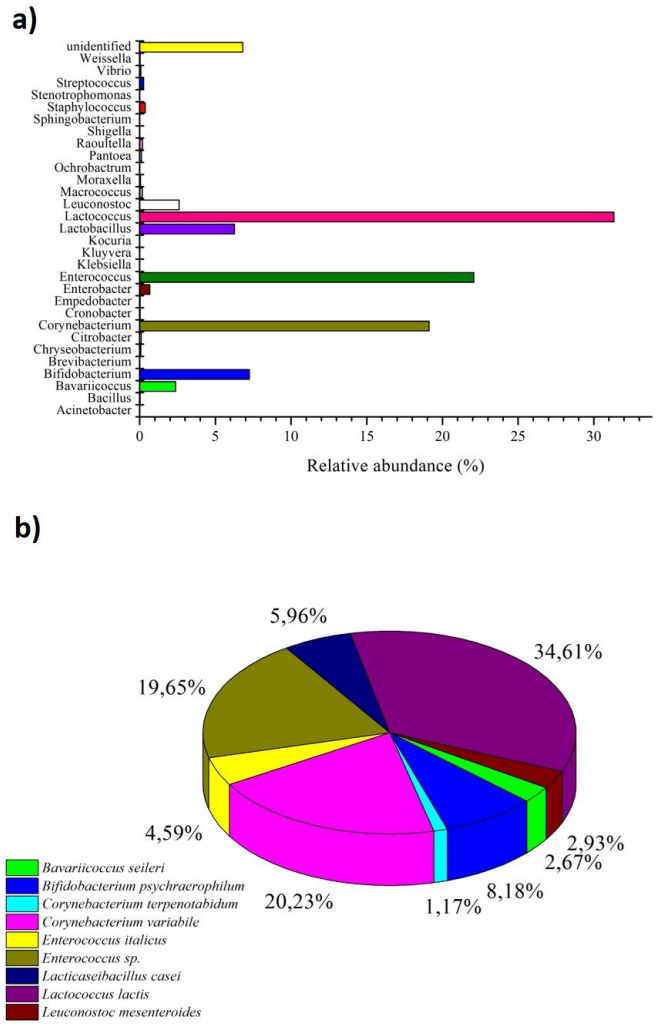

Figure 1. Relative abundance (%) of (A) bacterial genera and (B) lactic acid bacteria and bifidobacteria species present in Colonial cheese.

study, other research has also found *Corynebacteria* in milk and its by-products. Non-pathogenic species of *Corynebacterium*, such as *Corynebacterium casei* and *Corynebacterium variabile*, which have beneficial functions in food processing, are frequently isolated from dairy products (Fricker et al., 2001; Hahne et al., 2018).

Additionally, the presence of the genus *Bifidobacterium* sp. may represent a potential probiotic action by specific strains. Studies and evidence regarding bifidobacteria in biotechnological and biomedical applications have a long history. In cheeses, these microorganisms not only influence sensory characteristics due to their ability to hydrolyze or metabolize carbohydrates but also act as natural antimicrobials (Yasmin et al., 2020).

A total of 57 species of bacteria were identified in the Colonial cheese analyzed. Among the species of lactic acid bacteria (LAB) detected in the analyzed Colonial cheese sample, the most abundant was *Lactococcus lactis* (Figure 1B), as also observed in the studies by Alegría et al. (2009) and Lima et al. (2009). This abundance may be attributed to the widespread distribution of this species in various environments, as it has previously been isolated from rainwater, plants, the human gastrointestinal tract, and cow udders (Cavanagh et al., 2015). Its proven safety status as Generally Recognized as Safe (GRAS) (Cavanagh et al., 2015) makes it a widely used culture in cheese production (Picon et al., 2010), as it plays a significant role in the sensory characteristics of this product. These strains degrade proteins through the action of the enzyme lactocepin, which hydrolyzes casein; they ferment lactose also through the enzymatic action of phospho-β-galactosidase, producing lactose-6-phosphate and glucose; and they produce organic acids, primarily lactic acid (Cavanagh et al., 2015). It is through the enzymatic degradation of amino acids that aromatic compounds related to cheese flavors are formed (Centeno et al., 2002), and in artisanal cheeses, this characteristic is of utmost importance as it imparts uniqueness to the product (Smit et al., 2005). *L. lactis* is also applied in food as a next-generation probiotic with effects on inflammatory bowel diseases, autoimmune disease (type 1 diabetes), and allergen sensitivity (Barros et al., 2020). They can also be used to inhibit the growth of pathogenic strains in food, as they have the capacity to produce antimicrobial compounds called bacteriocins, with nisin being the primary representative used in food, acting against *Staphylococcus aureus*, *Listeria monocytogenes*, and *Clostridium* spp. (Khelissa et al., 2020).

The detection of significant quantities of *C. variabile* reads in the sample indicates possible contamination during milking or cheese production, as it is a species frequently found on the skin and in the gastrointestinal tract of animals and humans (Braem et al., 2012). However, it is not considered a pathogenic species (Schröder et al., 2011). In a study conducted by Chombo-Morales et al. (2016), samples of artisanal Cotija cheese were also contaminated with *C. variabile*, and the authors associated the presence of this species with its relevance in the cheese maturation process. This significance is attributed to the ability of the species to metabolize lactate, as well as its capacity to produce lipolytic enzymes such as lipases (lipA1–lipA3) and hydrolases (SGNH-hydrolase), which lead to the release of volatile compounds such as sulfur, esters, aldehydes, and ketones resulting from the degradation of lactose and citrate, thereby influencing cheese flavor (Yvon & Rijnen, 2001). Proteolytic enzymes (serine protease, aminopeptidase, and proline iminopeptidase) are related to the degradation of proteins contributing to the texture and flavor of cheeses, as aromatic compounds are derived from amino acids (Deetae et al., 2007; Schröder et al., 2011). Another member of the *Corynebacterium* genus detected in this study was *Corynebacterium terpenotabidum*. When comparing the genomic sequencing of this species with the previously mentioned one, the similarity of the 16S rRNA gene is over 97%, and it also lacks pathogenicity (Rückert et al., 2014).

The third most frequently appearing LAB species in terms of read numbers (Table 1) in the metataxonomic analysis belongs to the *Enterococcus* spp. genus. Although some *Enterococcus* strains are not declared GRAS due to their potential to transfer antibiotic resistance genes (Cambronel et al., 2020), they are often isolated from cheeses because they play a crucial role in the characteristics that cheese acquires during the maturation stage (Barros et al., 2020; Castro et al., 2016; Renye et al., 2011). This is because this genus is associated with lipolysis, proteolysis, and diacetyl production reactions (Moraes et al., 2012). Additionally, they can inhibit the growth of pathogens such as

Table 1. Quantity of identified sequences of the most abundant bacterial species in Colonial cheese.

Species	Reads
Lactococcus lactis	22,576
Corynebacterium variabile	13,199
Enterococcus sp.	12,821
Bifidobacterium psychraerophilum	5,333
Enterobacteriaceae bacterium	5,015
Lacticaseibacillus casei	3,886
Enterococcus italicus	2,996
Leuconostoc mesenteroides	1,914
Bavariicoccus seileri	1,743
Corynebacterium terpenotabidum	766
Lactococcus garvieae	507
Enterobacter cloacae	427
Lactiplantibacillus plantarum	393
Enterococcus saccharolyticus	365
Staphylococcus saprophyticus	256
Streptococcus thermophilus	188
Lactobacillus helveticus	169
Raoultella ornithinolytica	132
Macrococcus caseolyticus	124
Corynebacterium flavescens	112
Levilactobacillus brevis	94
Pantoea agglomerans	88
Citrobacter freundii	70
Enterobacter hormaechei	65
Vibrio furnissii	48
Enterococcus durans	44
Moraxella osloensis	44
Chryseobacterium bovis	33
Lentilactobacillus parabuchneri	27
Cronobacter sakazakii	23
Companilactobacillus farciminis	22
Enterococcus pseudoavium	18
Bacillus cereus sp. *group*	16
Weissella paramesenteroides	16
Empedobacter brevis	15
Enterococcus thailandicus	13
Staphylococcus xylosus	12
Acinetobacter ursingii	10
Brevibacterium iodinum	9
Bifidobacterium mongoliense	8
Chryseobacterium anthropi	8
Limosilactobacillus fermentum	8
Kluyvera cryocrescens	7
Kocuria kristinae	7
Lactobacillus rossiae	7
Shigella flexneri	7
Lactobacillus crispatus	6
Raoultella planticola	6
Chryseobacterium flavum	5
Corynebacterium casei	5
Empedobacter falsenii	5
Enterococcus casseliflavus	5
Ochrobactrum anthropi	5
Sphingobacterium multivorum	5
Klebsiella oxytoca	4
Shigella dysenteriae	4
Stenotrophomonas maltophilia	3
Acinetobacter lwoffii	1

L. monocytogenes and *S. aureus* (Silvetti et al., 2014). Strains *Enterococcus faecium* SF-68 and *E. faecium* M74, which are used in dietary supplementation, exhibit probiotic effects (Barros et al., 2020; Franz et al., 2011; Waheed et al., 2021).

In view of their natural presence in broad ecological niches, whether connected or not to the gastrointestinal tract, bifidobacteria have co-evolved with their human and animal hosts (Duranti et al., 2020). Bifidobacterial species have also been isolated from the oral cavity and fermented foods (Lugli et al., 2019; Okamoto et al., 2008). Studies based on the 16S rRNA gene sequencing of bifidobacteria isolated from pig ceca have proposed new species, with *Bifidobacterium psychraerophilum* being one of them (Simpson et al., 2003). This strain was found during the genetic sequencing of bacterial populations in the colonial cheese sample of this study. Because of being one of the first organisms to colonize the human intestine, there are numerous studies investigating their presence and the production of health-promoting compounds, leading to recent exploration as probiotic strains (Duranti et al., 2020). Among the health-promoting benefits attributed to gastrointestinal bifidobacteria are the development of the immune system, protection against pathogens, and the production of B-complex vitamins (Wong et al., 2020).

Another lactic species exhibiting a high relative abundance in metataxonomic analysis is *Lacticaseibacillus casei*, possibly due to their natural presence in raw milk and their use as non-starter LAB (NSLAB) (Bottari et al., 2018). This species, along with *Bifidobacterium animalis*, is referred to as allochthonous or transient bacteria because when ingested in fermented foods, especially fermented milk and yogurts, they can incorporate and survive in the intestinal flora, thereby providing health benefits to humans (Derrien & Vlieg, 2015; Kok & Hutkins, 2018). Despite the long-standing studies on the probiotic potential of *Lactobacillus* and *Bifidobacterium* genera, characterizing new strains with unique characteristics is of utmost importance (Sharma et al., 2014).

Lactiplantibacillus plantarum, Lactobacillus helveticus, Levilactobacillus brevis, Companilactobacillus farciminis, Limosilactobacillus fermentum, and *Leuconostoc mesenteroides*, along with other species of LAB identified in this study in lower relative abundances, include *Lactobacillus crispatus* and *Lactobacillus rossiae*. A recent *in vitro* study conducted by Wu and Shah (2017) with the strain *L. fermentum* JL-3, isolated from a fermented dairy product called Jiangshui, demonstrated its efficient ability to degrade uric acid (UA) in mice. One solution to the increasing cases of individuals with high UA levels in the body is the introduction of probiotics into the diet that have the capacity to degrade acid through the intestinal microbiota (Nishita et al., 2017). Furthermore, *L. rossiae*, a heterofermentative lactic acid bacterium (Ilse et al., 2009) with ecological adaptability and genotypic and phenotypic diversity (Di Cagno et al., 2007), is found in various environments such as fermented meat, pineapple, and the human and animal gastrointestinal tracts (De Angelis et al., 2014). Considering this great diversity observed, the isolation and characterization of these strains demonstrate great potential for use as potential probiotics.

Moreover, numerous studies have linked diet, intestinal microbiota diversity, and immune responses (Barrea et al., 2020). However, the immune system must exhibit good specificity and function optimally to act against pathogens without eliminating the benefits caused by some microorganisms (Chaplin, 2010). Venter et al. (2020) reported that individuals with good nutritional status consequently have a well-functioning immune system. Due to these factors, the relative abundance of fewer than 100 reads that some pathogenic bacteria presented in our work, such as *Shigella dysenteriae, Shigella flexneri, Bacillus cereus, Klebsiella oxytoca, Cronobacter sakazakii,* and *Moraxella osloensis*, may not be a problem for individuals with a healthy immune system. Additionally, the presence of potentially probiotic LAB, used as ingredients or naturally found in some fermented foods, has the capacity to re-establish both innate and adaptive immunities (Kok & Hutkins, 2018).

3.2 Fungal diversity in colonial cheese

For most of cheeses, bacteria constitute the primary microbiota, while halophilic bacteria, molds, and yeasts comprise the secondary microbiota (Pereira, 2018). Metataxonomic analysis of Colonial cheese revealed a total of 321,083 fungal reads, identifying 19 yeast species (Table 2). The two fungal phyla identified were Basidiomycota and Ascomycota. In this study of fungal identification in Colonial cheese, the five most abundant microorganisms were yeasts: *Diutina catenulata, Clavispora lusitaniae, Kodamaeae ohmeri, Kluyveromyces marxianus*, and *Candida ethanolica*, as presented in Table 2. *D. catenulata* is an ascomycetous yeast, known to contaminate dairy products and isolate them from humans, animals, and environmental sources (O'Brien et al., 2018). In artisanal cheese, it has been observed as one of the most prevalent yeasts (Pereira, 2018), a finding consistent with this study, where it exhibited the highest number of reads. In a study of the natural microbiota of artisanal Serrano cheese, Pereira (2018) identified *D. catenulata* in 14 samples throughout the maturation process. Similarly, in Minas Serro cheese, this yeast was detected during three maturation periods, as reported by Cardoso et al. (2015). Welthagen and Viljoen (1999) reported intense proteolytic and lipolytic activity of *D. catenulata* in Gouda cheese. In Stilton blue cheese, *D. catenulata* was identified on the outer rind through culture-independent methods, although its detection was not possible using culture-dependent methods (Gkatzionis et al., 2014).

Table 2. Quantity of identified sequences of yeast species in Colonial artisanal cheese.

Species	Reads
Diutina catenulata	245,460
Clavispora lusitaniae	42,560
Kodamaea ohmeri	20,882
Kluyveromyces marxianus	5,350
Candida ethanolica	3,875
Tricholoma matsutake	851
Galactomyces sp. *BPY-54*	707
Candida pararugosa	402
Geotrichum bryndzae	359
Starmerella etchellsii	313
Yarrowia lipolytica	123
Trichosporon coremiiforme	101
Trichosporon ovoides	23
Phanerochaete sordida	23
Torulaspora delbrueckii	20
Kluyveromyces lactis	8
Candida intermedia	5
Sterigmatomyces halophilus	5
Debaromyces hansenii	1

C. lusitaniae emerged as the second most abundant yeast in this study. In a study of traditional artisanal dairy products from Bangladesh, *C. lusitaniae* was also found to be the second most predominant yeast in the microflora, trailing only behind *K. ohmeri* (Nahidul-Islam et al., 2018). In turn, *K. ohmeri* occupied the third place as the most abundant yeast. The identification of this yeast associated with cheese production was initially conducted by Borelli et al. (2006) in a study of the yeast population related to artisanal cheese production in the Serra da Canastra region. *K. ohmeri* was also prevalent in a study of artisanal cheese from the Serro de Minas during all studied maturation periods and exhibited low lipolytic activity and β-galactosidase production (Cardoso et al., 2015). This yeast has also been identified in endogenous yeast cultures (pingo and rala) used in the production of Minas Artisanal Cheese (QMA) in the Serro-MG region (Miranda, 2020). In the same study, the author suggests that *K. ohmeri* may be a part of the natural microbiota in initiation in many properties, a phenomenon that may have repeated itself in the cheese analyzed in this study.

Among the identified genera, the genus *Kluyveromyces* stands out in dairy products, with the species *K. lactis* and *K. marxianus*, which are the only lactose fermenting species and are important for cheese maturation and aroma formation (Belloch et al., 2011). Similar to this study, in a study of the microbial community of Minas cheese, Cardoso et al. (2015) also found *Debaromyces hansenii*, *K. ohmeri*, and *K. marxianus* as the predominant yeasts. Meanwhile, in raw milk Tête de Moine cheese, *C. lusitaniae, D. hansenii, Pichia pseudocactophila, K. marxianus, Rhodotorula mucilaginosa, Debaryomyces capitatus*, and *Pichia jadinii* were identified (Buchl & Seiler, 2011). Due to its low pH, low moisture content, high salt concentration, and storage conditions, the occurrence of yeast in cheese is expected (Fleet, 1990). Most of the time, the presence of yeast in food is beneficial for flavor development. The proteolytic and lipolytic activity carried out by yeast contributes to the release of amino acids, fatty acids, and esters, which act as aroma precursors (Lima et al., 2009). In a yeast characterization study of curd cheese, *Yarrowia lipolytica* was found to be the best producer of lipolytic and proteolytic enzymes (Almeida, 2011). Considering the lipolytic and proteolytic activities of this yeast, *Y. lipolytica* may contribute to the cheese maturation process by imparting organoleptic characteristics of aroma, body, taste, and texture (Groenewald et al., 2013), a phenomenon that may have also occurred in the cheese analyzed in this study. Overall, lipolytic and proteolytic properties, aroma component formation, lactose fermentation, lactate assimilation, positive interactions with starter cultures, and osmotolerance are desirable technological characteristics in yeast used as spontaneous or controlled starter cultures for cheeses (Jakobsen & Narvhus, 1996).

In addition, in cheese production, there may exist a synergistic relationship between yeasts and LAB, wherein the survival of lactobacilli is enhanced by the presence of yeasts (Buchl & Seiler, 2011). Yeasts elevate the pH of the environment (resulting from LAB metabolism) by utilizing lactate and generating alkaline substances through proteolysis, thereby facilitating the growth of acid-sensitive bacteria. Moreover, yeasts provide vitamins and amino acids to bacteria (Buchl & Seiler, 2011). Finally, it is noteworthy that the application of genotypic methods has enabled the identification of a substantial number of microorganisms. Such a specific classification might not have been achievable using conventional microbiological methods, which are reliant on cultivation due to their intrinsic limitations (Sharma et al., 2020).

4 CONCLUSION

In this study, the microorganisms identified through metataxonomic analysis are those naturally occurring in milk and various cheese varieties. Furthermore, many of the species identified are currently undergoing extensive investigation due to their potential probiotic properties when employed as ingredients and natural antibacterials in fermented dairy products, such as *L. casei, L. helveticus, Bifidobacterium psychraerophilum, Enterococcus durans, L. plantarum*, and *L. lactis*. Additionally, next-generation potential probiotics, including those from the *Enterococcus* spp. genus, were discerned within this sample. Furthermore, the analysis revealed the presence of 19 yeast species, with *D. catenulata, C. lusitaniae, K. ohmeri, K. marxianus*, and *C. ethanolica* being the most abundant. During the cheese maturation process, certain yeasts contribute to desirable consumer attributes such as aroma, flavor, and texture. Hence, the presence of these microorganisms can significantly enhance the quality of the final product. Ultimately, the metataxonomic approach proved to be pivotal in conducting a preliminary screening of the sample's microbiota, guiding the isolation of species of interest.

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