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Large-scale mapping of microbial diversity in artisanal Brazilian cheeses

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ABSTRACT

Brazilian artisanal cheeses are characterized by the use of raw milk and in some cases, natural starter cultures, known as "pingo", as well as following simple and traditional manufacturing technology. In this study, a large-scale screening of the microbial ecology of 11 different types of artisanal cheeses produced in five geographical areas of Brazil was performed. Besides, the specific origin-related microbial signatures were identified. Clear geography- and technology-based differences in the microbiota were observed. Lactic acid bacteria dominated in all cheeses although *Enterobacteriaceae* and *Staphylococcus* also occurred in North, Northeast and Central cheeses. Differences in the lactic acid bacteria patterns were also highlighted: *Streptococcus*, *Leuconostoc*, *Lactococcus* and *Lactobacillus* were differently combined in terms of relative abundance according to product type and region of production. This study provides a comprehensive, unprecedented microbiological mapping of Brazilian cheeses, highlighting the impact of geographical origin and mode of production on microbial diversity. The results obtained will help to plan an evaluation of microbial contamination sources that will need to be studied for the improvement of cheese quality and safety.

1. Introduction

Cheeses are highly appreciated dairy products throughout the world. Most of cheese making has evolved in the last 100 years from "traditional- and art-based processes" to recent "scientific- and technological-based processes" (Johnson, 2017). A variety of cheeses exists in almost every country of the world, characterized by their own chemical and sensorial properties.

Brazilian artisanal cheeses are made employing traditional methods developed by cheesemakers, and both social and cultural aspects influence procedures. These cheeses normally have typical features, such as texture and flavor, as a result of the origin and composition of the starter cultures, raw materials employed and ripening conditions (Almena-Aliste and Mietton, 2014; Fialho et al., 2018; González-Córdova et al., 2016; Montel et al., 2014; Soares et al., 2017; Zheng et al., 2018).

In Brazil, several artisanal cheeses are produced, with inter-regional differences in the types of products and in the manufacturing practices. Marajó cheese, obtained from raw buffalo's milk, is produced on the island of Marajó, in the North of the country (Ferreira et al., 2017). Curd and Butter cheeses are traditional dairy products from the Northeast (Medeiros et al., 2016; Nassu et al., 2009), while Caipira cheese is produced in Central Brazil. In the state of Minas Gerais higher intra-regional diversity exists, with at least six different traditional cheeses produced (Araxá, Campo das Vertentes, Canastra, Cerrado, Serro and Triângulo Mineiro) (Pinto et al., 2009; Santos et al., 2017). In the South of Brazil, Serrano (Cruz and Menasche, 2014) and Colonial cheeses can be highlighted (Funck et al., 2015). Most of the Brazilian traditional cheeses are often produced using raw milk, except Butter and Curd cheeses, which are made with pasteurized milk (Andrade et al., 2011; Nassu et al., 2009).

While some of them achieved widespread recognition, such as the Minas cheeses, recognized as Brazilian immaterial cultural heritage (IPHAN, 2014), there are still some sanitary concerns regarding their commercialization throughout the country. Until recently, the Brazilian regulation stated that cheeses made from raw milk and ripened for < 60 days could be sold throughout Brazil if scientific studies were performed to support that safety risks were low. Then, the commercialization could take place following the approval of the Ministry of Agriculture, including the state and/or municipal body of industrial and sanitary inspection recognized by Brazilian System of Animal Product Inspection (SISBI/POA) (MAPA, 2013). However, recently the Brazilian regulation was updated and the commercialization of these products is now allowed if they are subjected *"to the supervision of public health*

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Table 1
Technological characteristics of Brazilian artisanal cheeses production.

Region	Type of cheese	Production Technology	Reference
Non-ripened cheeses			
North	Marajó	Fusion of the curd mass desorbed of buffalo milk and/or buffalo milk mixed with bovine milk in the maximum proportion of 40%, washed with water or buffalo or bovine milk, obtained by spontaneous coagulation and added cream milk or butter, salting, cooking and molding.	Figueiredo (2006) Simões et al. (2014)
Northeast	Butter	Usually skimmed raw bovine milk, spontaneously coagulated, whose mass is subjected to desorption, washing with water and/or milk, salting, melting with addition of "Manteiga da Terra" and molding.	Cavalcante and Costa (2005) MAPA (2001) Nassu et al. (2009)
	Curd	Cow raw milk with addition of industrial rennet, clotting (35 °C, 35–45 min), curd cutting, stirring, curd cooking and whey drainage, salting, molding, pressing, cold storage (2–12 °C) and package.	Fontenele et al. (2017)
Ripened cheeses			
Central	Caipira	Filtration of fresh raw bovine milk; addition of rennet and natural starter; coagulation, curd cutting; stirring; syneresis; molding; pressing; dry salting and ripening.	Menezes (2009) Silva (2007)
Southeast	Araxá	The endogenous culture is obtained from cheese whey and is added to the raw milk in concentrations between 0.1% and 2%. The industrial rennet also is added; after, the curd is cutting, stirring, molding in plastic forms, pressing, salting, turning and ripening in shelves during 22 days.	IMA (2013) Luiz et al. (2016) Sobral (2012)
	Campo das Vertentes	Raw milk, with added endogenous starter cultures and clotting agents. The endogenous starter culture comes from the whey drained from previously made cheeses, which is collected and used for cheesemaking on the next day. During the production, the cheeses are turned and ripened (22 days).	Castro et al. (2016) IMA (2013)
	Canastra	Cow raw milk inoculated with the commercial rennet and "pingo" (Natural Starter), salting and manual pressing, dry salting and ripening for 22 days.	IMA (2013) Nóbrega (2012)
	Cerrado	Filtration of raw bovine milk, addition of rennet and "pingo", curd cutting, stirring, molding, superficial salting, turning cheese and ripening (22 days).	IMA (2013) Lima et al. (2009)
	Serro	Cow raw milk with addition of rennet and "pingo", coagulated in natural temperature, obtaining a firm mass, which is compressed into forms, eliminating the whey. The salting is superficial on both sides and then the cheese is ripened during 17 days.	IMA (2013) Lima and Rocha (2016) Martins et al. (2015)
South	Colonial	Coagulation of raw bovine milk with addition of calcium chloride, lactic ferment and industrial rennet, pressing, syneresis, salting molding and ripening (30 days).	Ide and Benedet (2001) Mello and Armachuk (2013)
	Serrano	Raw milk obtained from beef cattle breeds, mixed breeds, and fermented by natural microbial populations. Filtration of milk, salting, coagulation with addition of rennet, curd cutting, syneresis, molding, pressing and ripening for 15 days.	Córdova and Schlickmann (2015) Pereira et al. (2014)

authorities of the States and the Federal District" (Anonymous, 2018).

In fermented foods, beneficial microbial communities can be responsible for rheological and organoleptic traits. Moreover, undesirable microorganisms may also be present and may affect the quality of food (De Filippis et al., 2018a).

The microbial community is one the relevant factors in the manufacturing of (artisanal) cheeses produced from raw milk and with the use of natural cultures (Almena-Aliste and Mietton, 2014). During milk fermentation and cheese ripening, complex interactions occur in the microbial community (Montel et al., 2014). In the past years, the microbial ecology of different types of cheeses has been studied worldwide (Calasso et al., 2016; De Filippis et al., 2014, 2016; De Pasquale et al., 2014b; Guidone et al., 2016; Masoud et al., 2011; Riquelme et al., 2015). In each study, both common and unique microbiological traits have been highlighted, suggesting the importance of microbial mapping in order to plan improvements of cheese quality and safety (De Filippis et al., 2018b, 2017).

Even though the artisanal cheeses produced in Brazil are widely appreciated and consumed, the microbiota involved in their production and ripening have never been described in depth. In addition, the diversity existing in the manufacturing practices in the different geographical areas makes a microbiological mapping of these cheeses surely relevant. The contribution of different microbial groups to the microbiota of Brazilian raw milk cheeses needs to be elucidated, with the final aim to establish quality standards for these products.

Few previous reports investigated the lactic acid bacteria populations of Brazilian Minas cheeses produced in different regions (Arcuri et al., 2013; Perin et al., 2017); however, a systematic and wide-range study of microbial ecology of Brazilian artisanal cheeses produced in different regions is not available.

This study aimed to characterize the complex microbiota of Brazilian artisanal cheeses produced in different regions, with emphasis on the differences existing according to the manufacturing practice and the geographical origin. The results generated will be extremely important to assist in improving cheese quality and safety, and possibly in the achievement of certifications where a specific legislation or regulation is still lacking.

2. Materials and methods

2.1. Sampling

In total, 578 samples of Brazilian artisanal cheeses were collected in local supermarkets, fairs, cooperatives and producers, stored frozen and lyophilized at – 60 °C with a pressure of 40 UH for 72 h. Subsequently, 10 g of each cheese were pooled in order to obtain 200 homogeneous sample groups, according to the type of cheese, the technology of production and the geographical origin. The pooled samples were used for DNA extraction and sequencing.

The cheeses were produced in different areas of Brazil: North (Marajó cheese); Northeast [Curd ("*queijo de coalho*") and Butter ("*queijo manteiga*") cheeses); Central (Caipira cheese); Southeast (Araxá, Campo das Vertentes, Cerrado, Canastra and Serro cheeses) and South (Colonial and Serrano cheeses).

Most of the cheeses were produced with raw milk and only few samples of Butter, Curd, Campo das Vertentes, Serrano and Colonial cheeses were obtained with pasteurized milk (analyzed separately). A list of the cheese analyzed is reported in Table 1.

2.2. Physicochemical analysis

For the determination of pH, 20 g of crushed cheese sample were weighted into a 50 mL beaker, and diluted 1:1 with deionized water. After homogenization, the pH was measured using KASVI digital potentiometer; model K39−2014B (KASVI, Shanghai, China). The water activity (a_w) of cheeses was determined at 25 °C ± 4 °C, using the AquaLab equipment, model CX2T (Decagon Devices, Pullman, USA), following the manufacturer's instructions.

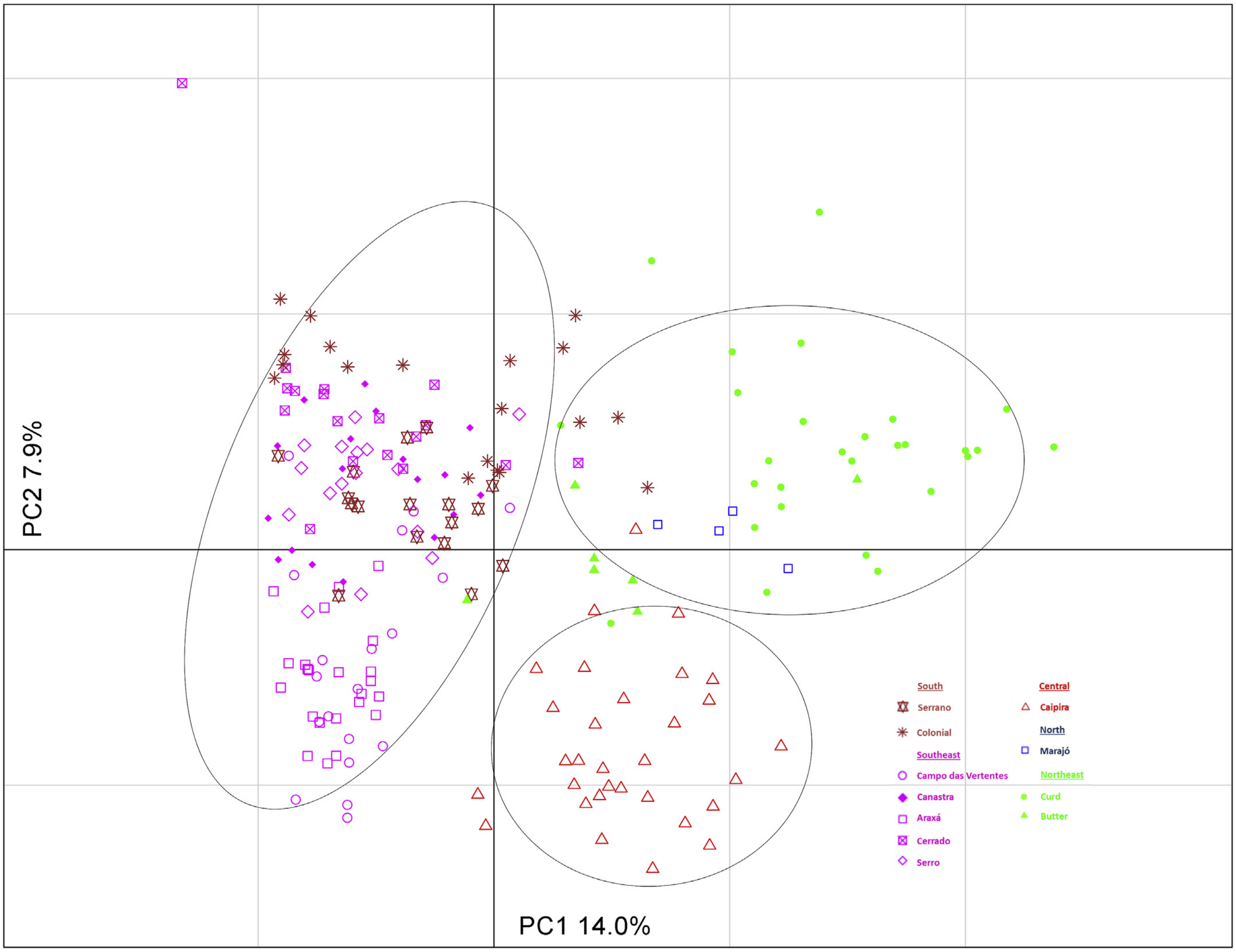

Fig. 1. Principal Component Analysis (PCA) based on the bacterial community composition. Samples are coloured according to the geographical origin and shaped according to the type of cheeses (Serrano n = 17, Colonial n = 19, Campo das Vertentes n = 19, Canastra n = 18, Araxá n = 23, Cerrado n = 18, Serro n = 16, Caipira n = 31, Marajó n = 4, Curd n = 28 and Butter n = 7). Circles group the samples according to production regions (North/North-East, Central, South/South-East).

2.3. DNA extraction

Total DNA extraction from the cheese samples was carried out by using the Biostic bacteremia DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). The cheese samples were 3-fold diluted in PBS solution (Peptone Buffered Saline, pH 7.4) and homogenized in a stomacher (Seward Stomacher 400 LabSistem, PBI International, Milan, Italy). The extraction protocol was applied to the pellet (12,000 × *g*) of 2 mL of homogenate.

2.4. 16S rRNA gene amplicon library preparation and sequencing

Microbial diversity was studied by sequencing of the amplified V3—V4 region of the 16S rRNA gene by using primers S-D-Bact-0341-b-S-17: 5′-CCTACGGGNGGCWGCAG-3′ and S-D-Bact-0785-a-A-21: 5′-GACTACHVGGGTATCTAATCC-3′ amplifying a fragment of 464 bp (Klindworth et al., 2013). Library preparation and sequencing was carried out as recently described (Berni Canani et al., 2017). The amplicons were combined in an equimolar pool and sequenced on an Illumina MiSeq platform, leading to 2 × 250 bp reads.

2.5. Bioinformatics data analysis

After demultiplexing, paired-end reads were joined by FLASh (Magoč and Salzberg, 2011) and a quality filtering was carried out by PRINSEQ (Schmieder and Edwards, 2011). Reads were trimmed at the first base with a Phred score lower than 20 and those shorter than 300 bp were discarded. Data were analyzed by using QIIME 1.9.1 software (Caporaso et al., 2010), with a pipeline recently reported (Berni Canani et al., 2017).

Reads assigned to the *Lactobacillus* genus were extracted and entropy analysis and oligotyping were carried out as described by the developers (Eren et al., 2013). After the initial round of oligotyping, high entropy positions were chosen (—C option): 2, 67, 108, 109, 120, 123, 124, 241, 242, 247, 269, 280, 281, 295, 296, 297, 298, 362, 379, 396. To minimize the impact of sequencing errors, we required an oligotype to be represented by at least 100 reads (-M option). Moreover, rare oligotypes present in less than 10 samples were discarded (-s option). These parameters led to 162,832 (46.49%) sequences remaining in the dataset. BLASTn was used to query the representative sequences against the NCBI nr database, and the top hit was considered for taxonomic assignment.

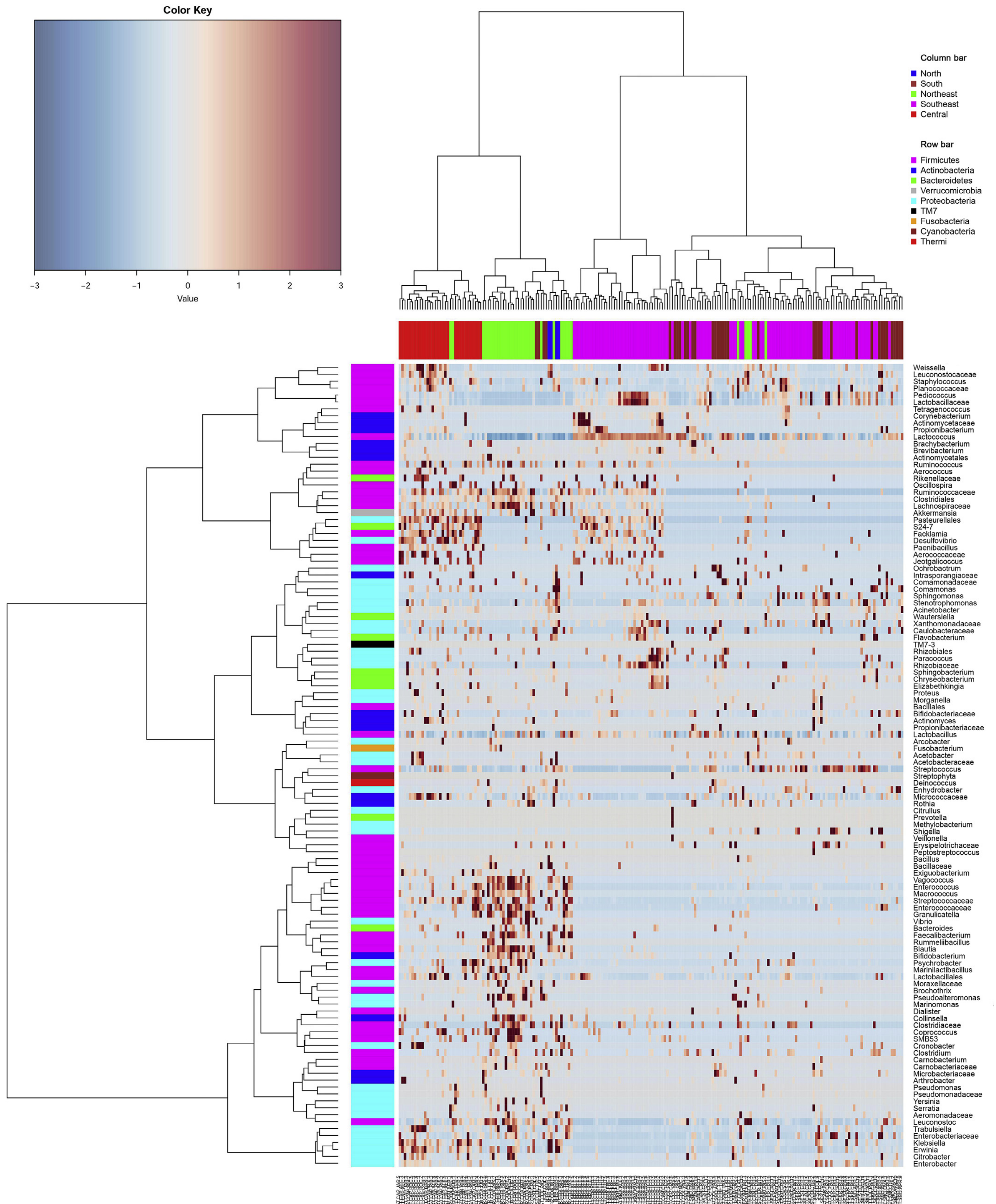

Fig. 2. Heat map showing microbial abundance in the cheese samples analysed. Hierarchical Ward linkage clustering of the samples based on the Pearson's correlation coefficient for the abundance of genera present in at least 10% of the samples. The color scale represents the scaled abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

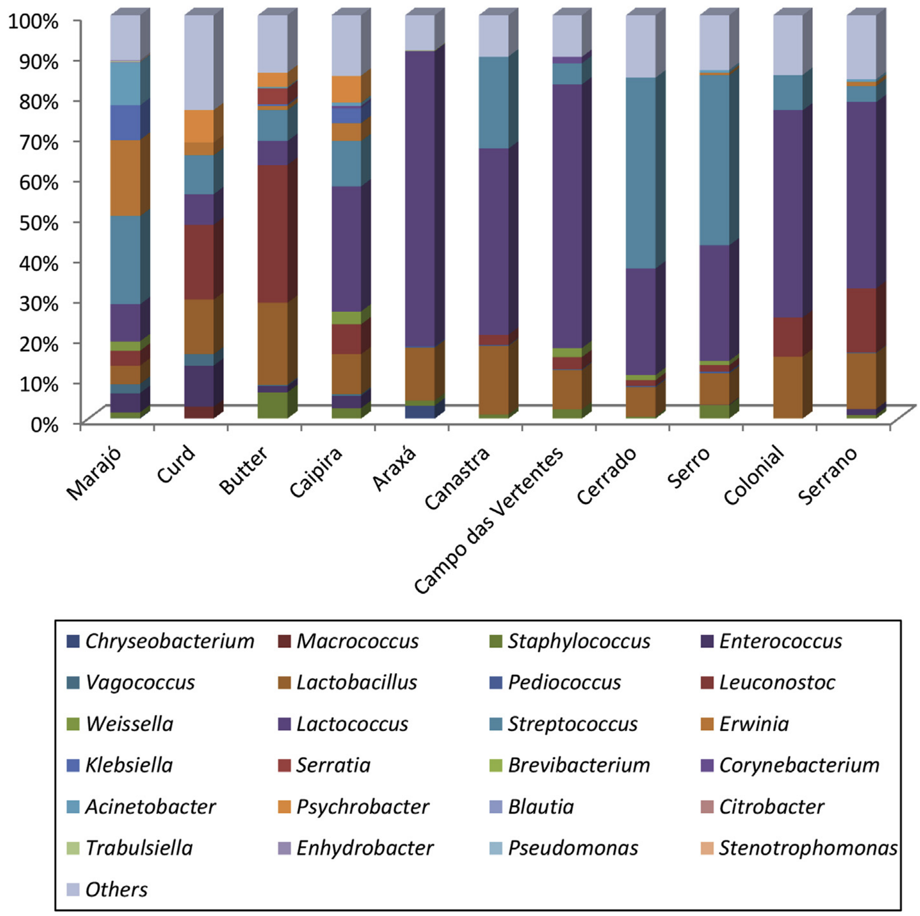

Fig. 3. Abundance of the main microbial genera found in the different types of Brazilian artisanal cheeses. The average value for each type of cheese is shown. Only the genera with abundance > 0.01% and present in at least 90% of the samples are shown, while sub-dominant genera were summed up as "others".

2.6. Statistical analysis and plotting of data

Statistical analysis and plotting were carried out in the R environment (http://www.r-project.org). Significant differences in microbial taxa were computed by using non-parametric Wilcoxon pairwise tests. Sequence data were made available in the Sequence Read Archive of the National Center of Biotechnology Information (accession number SRP165151).

3. Results

The values of pH and a_w of cheeses grouped according to the geographical origin are reported in Fig. S1. Cheeses from the North showed lower pH (5.33 ± 0.08) and higher a_w (0.987 ± 0.002), whereas the cheeses produced in the Southeast have a pH of 5.29 ± 0.28 and lower a_w (0.950 ± 0.014).

The bacterial richness and estimated diversity are shown in Fig. S2. Diversity was higher for cheeses from the North, Northeast and Central regions compared to Southern and Southeastern cheeses ($p < 0.05$). The highest number of OTUs (360) was observed in cheeses from the Northeast. On the other hand, Southeastern (91) and Southern (70) cheeses showed the lowest number of OTUs ($p < 0.05$).

Principal Component Analysis (PCA) (Fig. 1) showed a clear clustering of cheese microbiota according to the type of cheese (production technology) and region of production. Three major groups were observed: a group including un-ripened cheeses (Marajó, Curd and Butter), one with ripened Central cheese (Caipira) and another group of ripened cheeses from Southeast and South.

Regarding the production region, when samples from the South and Southeast were analyzed separately (Fig. S3) two major clusters were obtained. Campo das Vertentes and Araxá cheeses (from the Southeast) clustered apart from other cheeses from Southern or Southeast (Colonial, Serrano, Serro, Canastra and Cerrado cheeses).

Accordingly, hierarchical clustering based on the microbial diversity at genus level showed a clear separation of the samples driven by the production region (Fig. 2). In particular, higher levels of microbial genera from Firmicutes and Proteobacteria were observed in cheeses from the North, Northeast and Central Brazil. Indeed, these phyla showed significantly different abundance between cheeses from the different regions (Table S2).

The distribution of the most abundant genera in samples grouped according to the type of cheese and region of production is shown in Fig. 3. All the samples showed the dominance of lactic acid bacteria (LAB) and the main genera were *Streptococcus*, *Leuconostoc* and *Lactococcus* (Fig. 3). Genera including potential undesirable species were also observed in Marajó, Curd, Butter and Caipira. *Erwinia* was present in all the four types of cheeses, but with higher abundance in Marajó cheese (18.91%). In Curd and Caipira cheeses, *Psychrobacter* reached average abundance of 7.96% and 6.71%, respectively, while high levels of *Staphylococcus* were found in Butter cheese (6.53%). On the other hand, cheeses from Southeastern and Southern Brazil presented a low abundance of undesired microbial contaminants, mainly *Staphylococcus*, in Araxá, Campo das Vertentes and Serro cheeses. The average abundance of the main microbial genera in cheeses grouped by region of production is shown in Fig. S4, while microbial genera significantly different across cheeses produced in different regions are reported in Table S1.

A core of bacterial genera for each region of production, which were present in at least 95% of the cheese samples from a specific region, was

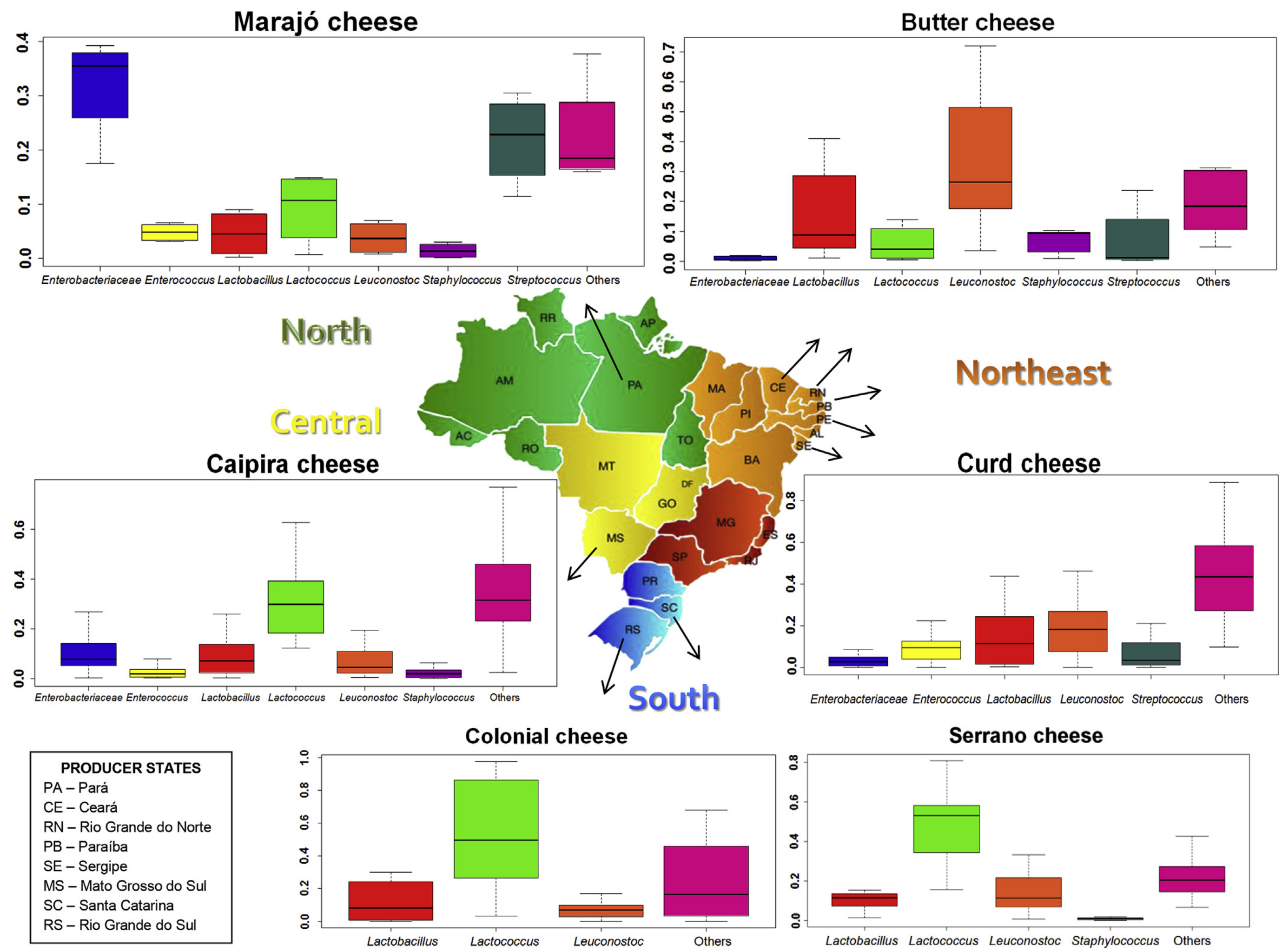

Fig. 4. Boxplots showing the core microbiota of Brazilian artisanal cheeses grouped according to the geographical area of production. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile). Whiskers denote the lowest and the highest values within 1.5 IQR from the first and third quartiles, respectively.

identified. The bio-geographical distribution of the core genera is shown in Figs. 4 and 5.

The core microbiota of Brazilian artisanal and Colonial cheeses included at least six different genera. Thermophilic lactic acid bacteria prevailed in cheeses from the North, Cerrado and Serro, with the dominance of *Streptococcus*. On the other hand, the incidence of mesophilic lactic acid bacteria was observed in cheeses from Northeast, in which *Leuconostoc* prevailed, and in cheeses from Central, South and Southeast (Araxá, Campo das Vertentes, Canastra), in which *Lactococcus* was the most abundant. In addition, the presence of potentially undesirable taxa (*Enterobacteriaceae*, *Staphylococcus*) was observed mainly in cheeses produced in the North, Northeast and Central regions. However, a low incidence of *Staphylococcus* was also found in the core microbiota of cheeses produced in the Southeast (Araxá, Canastra) and South (Serrano).

Different *Lactobacillus* species were part of the cheese microbiota in the different geographical areas (Fig. 6). Indeed, oligotyping allowed to identify 15 different *Lactobacillus* species (Table S3). Cheeses from the North showed high levels of *Lactobacillus fermentum* (oligotype 50). On the contrary, high abundance of *Lb. paracasei* (oligotype 3) and *Lb. plantarum* (oligotypes 1 and 2) was found in cheeses from Central/Southern Brazil, which also showed higher diversity within *Lactobacillus* spp.

4. Discussion

In this paper, a large-scale analysis of the microbiota of Brazilian artisanal cheeses, mapping the differences in microbiota composition associated with the specific production regions, as well as with the type of cheese were carried out. The microbiota involved in the production and ripening of cheeses, arising from raw materials, starter cultures or from the production environment, is responsible for the typical sensorial properties of the cheeses (De Filippis et al., 2014, 2016; Montel et al., 2014; Stellato et al., 2015). Herein, the “core” microbiota of Brazilian artisanal cheeses was characterized for the first time. This core microbiota mainly included LAB genera (*Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*), as well as microbial contaminants such as *Enterobacteriaceae* and *Staphylococcus.* Even though these genera were widespread in the cheeses analyzed, a differential abundance across the production regions was found. These differences might be related to the processing technology, the origin and storage conditions of the milk, the environmental conditions.

Microbial diversity was higher in cheeses from the North, Northeast and Central Brazil, where homofermentative and heterofermentative LAB were coupled with microbial contaminants and potential pathogens, whose presence can possibly arise from the use of raw milk and from the dairy processing environment (Stellato et al., 2015). Cheeses from the North region are hand-made, produced using a simple technology, as natural coagulation and manual pressing (Figueiredo et al.,

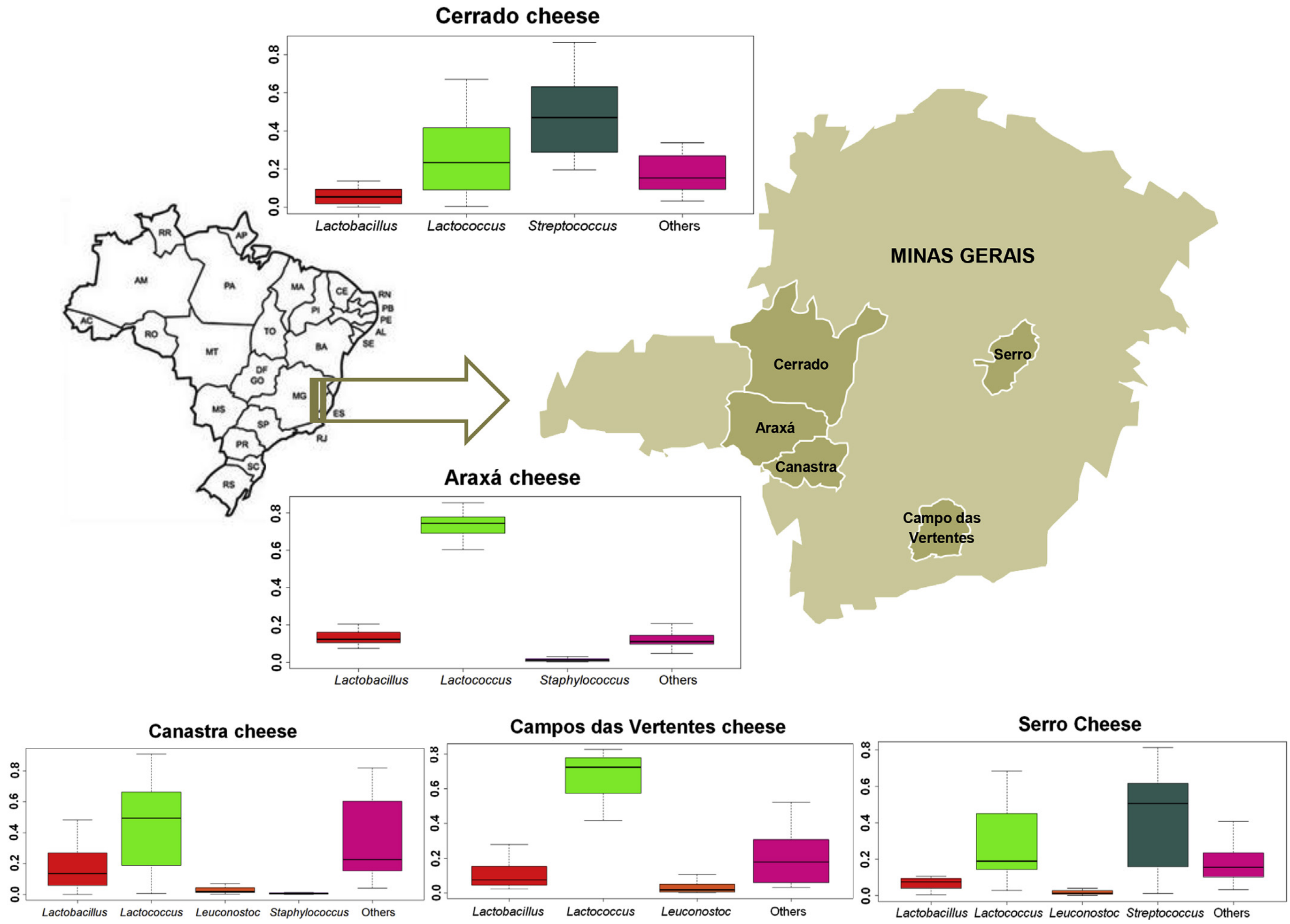

Fig. 5. Core microbiota in artisanal cheeses produced in different regions from Southeast Brazil (Adapted from EMATER-MG, (2018) and SertãoBras (2011)).

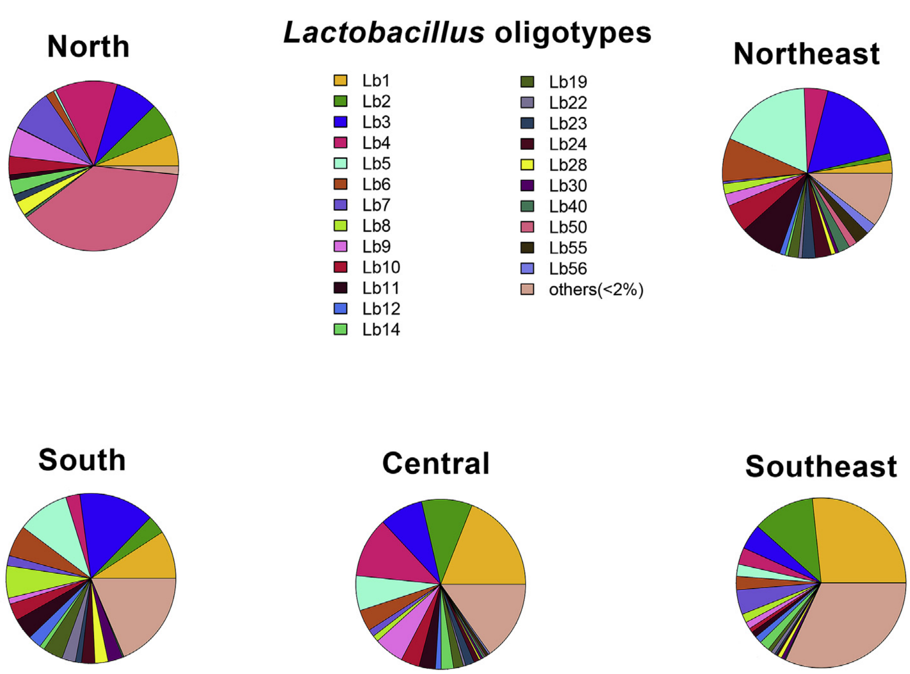

Fig. 6. Pie charts showing the abundance of *Lactobacillus* oligotypes in the different cheese production regions in Brazil. The identification of the oligotypes is reported in Supplementary Table S3.

2016). In Central region, the production of Caipira cheese is artisanal and its details are rarely reported (Mato Grosso do Sul, 2004). Marajó (from North) and Butter (from Northeast) cheeses are produced with a mass melting step at 80–90 °C, without ripening. Therefore, the presence of potential pathogenic microorganisms in Marajó cheese may be associated with post-processing contamination, as well as with the presence of DNA from dead microbial cells. Moreover, Marajó cheese is produced without addition of starter and the curd fermentation occurs only thanks to the autochthonous microbiota present in the raw milk and in the processing environment (Ferreira et al., 2017; Figueiredo et al., 2016). In agreement with previous reports, we also found microbial contaminants in Marajó cheese, such as *Staphylococcus* (Seixas et al., 2014). The pH (5.33) of Marajó cheese is related to the natural fermentation of milk and a pH value (5.26 in rainy season) similar to ours was previously reported (Simões et al., 2014). The high water activity (0.987) in this cheese may be associated with the practice of washing the cheese mass with water and milk to reduce the acidity.

Curd and Butter cheeses are often produced from raw milk, whose quality may influence that of the final product. Irregularities in physical and microbiological parameters were found in raw milk for Curd and Butter cheeses production (Medeiros et al., 2017). Nonetheless, most of Curd cheese samples (68%) collected in this study were produced with pasteurized milk and showed the presence of contaminants in the final product. Although pasteurization reduces some of the endogenous and pathogenic bacteria present in raw milk, thermoduric microorganisms survive to this process (D'Amico and Donelly, 2017). However, since this analysis was based on DNA, it is possible that it arose from dead or damaged cells. Despite the presence of contaminants, the Curd artisanal cheese showed a diversified microbiota dominated by LAB (*Enterococcus*, *Lactococcus, Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Weissella)*, as previously reported in a culture-dependent study (Medeiros et al., 2016).

Oligotyping was used to explore the sub-genus diversity within *Lactobacillus* spp. and revealed different patterns according to the geographical origin. Central/Southern cheeses were characterized by higher *Lactobacillus* diversity, with dominance of *Lb. casei/paracasei* and *Lb. plantarum*. *Lb. casei* group that is commonly found in ripened cheeses (De Filippis et al., 2018b, 2016) where it contributes to proteolysis and flavor development (De Filippis et al., 2016; De Pasquale et al., 2014a). Moreover, several *Lb. plantarum* strains are considered as potential probiotics and can produce antimicrobial compounds, possibly contributing to cheese safety (Kumar et al., 2016; Lin and Pan, 2017). In our study, the presence of *Lactococcus* has been observed in the cheeses produced in the Southeast region (Minas Gerais state). *Streptococcus* was dominant only in Cerrado and Serro cheese. An important characteristic of artisanal cheeses from Minas Gerais (Southeast region) is the use of natural starter culture, popularly known as "pingo". This culture is obtained from the whey of the previous cheese production (Perin et al., 2017). Nóbrega (2007) reported high counts of *Lactococcus/Streptococcus*, *Lactobacillus*, *Enterococcus* and *Leuconostoc* in "pingo" samples collected from eight artisanal cheese production facilities located in the region of "Serra da Canastra". According to Rafael (2017), the physico-chemical characteristics of "pingo" that may affect its microbiota vary considerably. In a survey of 20 samples of "pingo" from "Serra da Canastra" region (Minas Gerais), the pH, titratable acidity and salt content varied from 4.2 to 5.6, 0.2–1.2% of lactic acid and 1.9–4.9%, respectively. In this study, *Lactobacillus* and *Enterococcus* corresponded to the identified lactic acid bacteria isolated from "pingo" (Rafael, 2017). Even though 16S rRNA amplicon sequencing approaches were not used to gain insights on the microbiota composition of the "pingo" samples in the studies by Rafael (2017) and Nóbrega (2007), these data indicate that the core microbiota present in "pingo" may vary quantitatively and qualitatively from region to region. Besides, it has been found that the microbial community of the ripened cheeses is associated to the microbiota of "pingo" (Cruvinel et al., 2016). "Pingo" has also been found as the main source of novel bacteria to the "Serra da Canastra" artisanal cheeses (Cruvinel et al., 2016). Given these findings, it is clear that "pingo" core microbiota may vary from region to region and therefore, that may explain the abundances of *Lactococcus* and *Streptococcus* in the Southeast region cheeses and Cerrado and Serro cheeses, respectively. Several bacteria present in "pingo" have been found to present antagonistic activities against pathogens, such as *Listeria monocytogenes* (Campagnollo et al., 2018). Cheeses from the Southeast region showed acid pH and low a_w by decrease of moisture content, increase in solids-not-fat and NaCl concentration during cheese ripening, as well as in soluble nitrogen, non-protein nitrogen and free fatty acids (Dores, 2007). Each micro-region producing Minas Artesanal cheese has its peculiarities, as the use of the synthetic cheesecloth for the syneresis of Canastra and Cerrado cheeses (EMATER, 2003, 2004). In addition, factors such as climate, topography, pasture, dairy cattle breed and production characteristics, can affect the microbial diversity.

The abundance of *Lactobacillus spp.* was previously reported in artisanal Minas cheeses as well as in our study, thanks to its ability to grow under the highly selective conditions that exist in cheese, such as low pH and high salt concentrations (Perin et al., 2017). In addition, *Streptococcus* was reported as the dominant genus in Cerrado and Serro cheeses (Arcuri et al., 2013) and the genus *Lactobacillus, Lactococcus* and *Leuconostoc* were also reported in Canastra cheese (Nóbrega, 2012).

Discrepancies in the dominant microbiota found in Minas Gerais cheeses compared with others studies may be associated to the low number of samples previously analyzed, as well as to the higher sensibility of the method used here. In our study, we analyzed 259 Mina's cheese samples, while only 15 to 24 samples were analyzed in previous studies (Nóbrega, 2012; Perin et al., 2017).

Cheeses from the South are characterized by the use of raw milk and natural whey culture, which minimizes the variability during the ripening process and confers desirable organoleptic characteristics to the final product. In our study, the most abundant genera were *Lactococcus* and *Lactobacillus*.

In particular, *Lactococcus*, *Leuconostoc* and *Lactobacillus* were prevalent in Serrano cheese. Accordingly, *Lactobacillus* prevailed throughout the manufacturing and ripening process, especially in the late steps of the process, suggesting that this genus may have an important role in the production of this cheese (Rosa et al., 2008).

This is the first wide-range study of microbial diversity in Brazilian cheeses produced in different regions. We showed that, although LAB prevailed in all the types of cheeses, differences in the microbiota composition exist, possibly linked with the inter-regional diversity in the manufacturing practices and in the raw materials used. Results obtained here may support the achievement of quality certifications or specific regulation, helping the artisanal producers in standardizing the manufacturing process and spreading these traditional products worldwide.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2018.12.014.

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