



Lactic acid bacteria with antimicrobial, proteolytic and lipolytic activities isolated from ovine dairy products

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Abstract

This study aimed to isolate lactic acid bacteria from sheep milk products and to characterize these microorganisms with a focus on their antimicrobial, proteolytic and lipolytic activities. Raw milk, pasteurized milk, pasteurized cream, and butter samples were collected, lactic acid bacteria were isolated and their proteolytic, lipolytic and antimicrobial activities were evaluated. Lactic acid bacteria counts were higher in raw milk collected at the farm number 4 ($8.91 \pm 0.05 \log \text{CFU/mL}$), in which adequate hygienic practices were observed for pre- and post-milking. A total of 253 isolates were obtained, and among them 37 were lactic acid bacteria, where 19 showed some type of activity, most of which from raw milk. Among the isolates of lactic acid bacteria, 48.65% (n=18) showed proteolytic activity, 13.51% (n=5) lipolytic activity, 10.81% (n=4) showed both proteolytic and lipolytic activities, and only 2.70% (n=1) showed antimicrobial activity. Isolation of lactic acid bacteria with technological properties demonstrated their potential use as starter cultures in processing of fermented dairy products.

Keywords: technological properties; Brazil; lactic acid bacteria; sheep milk.

Practical Application: The use of sheep dairy milk produced in Brazil for the isolation of indigenous lactic acid bacteria with proteolytic, lipolytic and antimicrobial activities indicates a technological potential of these isolates as starter cultures for production of matured sheep cheese.

1 Introduction

Milk is a source of lipids, proteins and carbohydrates despite their variable composition depending on the animal species (Food and Agriculture Organization of the United Nations, 2013). Sheep milk has higher total solids, fat, and protein contents than cow and goat milk, obtaining greater yield in the production of cheese, yogurt, ice cream and dairy beverages (Balthazar et al., 2017, 2018a, b; Food and Agriculture Organization of the United Nations, 2013; Merlin et al., 2015). The American continent produces 42,095 tons of the world's production of sheep milk, and Brazil contributes to 21.72% of this total (Food and Agriculture Organization of the United Nations, 2017).

In the Southern and South-eastern regions of Brazil, there is a large production of sheep milk that is processed in registered dairy plants or even with homemade processing (Santos et al., 2016). This activity increased its participation in the Brazilian agribusiness, with great potential for cheese production, which are highly valued in the Brazilian market (Santos et al., 2016). Sheep dairy farming is considered a productive system well adapted to small farms, but this economic activity has not yet reached its full capacity in Brazil, especially regarding dairy products such as cheese and yogurt (Balthazar et al., 2017; Nespolo et al., 2010; Santos et al., 2016).

From the microbiological point of view, raw milk kept under refrigeration may have many different species of bacteria, some with pathogenic or deteriorating capacity (Campagnollo et al., 2018; Forsythe, 2013). Raw milk is considered a good source of lactic acid bacteria (LAB), which can compose cultures of starter bacteria, usually added to raw milk to produce fermented foods (Luiz et al., 2017; Nespolo et al., 2010). LAB is a group of Gram-positive, non-spore-forming, catalase-negative microorganisms that can grow under microaerophilic or strictly anaerobic conditions (Bruno, 2011). The positive influence of LAB on cheeses is due to the development of sensorial characteristics, through biochemical reactions such as proteolysis and lipolysis during maturation (Asensio-Vegas et al., 2016; Bruno & Carvalho, 2009; Luiz et al., 2017; Farahani et al., 2017). The proteolytic enzymes are the main ones found in LAB and its lipolytic activity is also widely reported (Bruno & Carvalho, 2009; Farahani et al., 2017). In addition, some strains of LAB can synthesize antimicrobial compounds (Balthazar et al., 2018b; Campagnollo et al., 2018; Mechai et al., 2014), and these characteristics make them useful to ferment milk, as primary agents or as culture starters (Bruno & Carvalho, 2009; Campagnollo et al., 2018; Nespolo et al., 2010).

Considering the importance and the expansion of Brazilian dairy sheep, this study aimed to isolate LAB from raw sheep milk

Received 15 Apr., 2019

Accepted 12 Sept., 2019

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and derivatives from Southern Brazil and to characterize these microorganisms with a focus on their antimicrobial, proteolytic and lipolytic activities.

2 Materials and methods

Samples were obtained from a dairy company specialized in the processing of sheep milk located in the South of Brazil (29° 10' 17" S and 51° 31' 09" W) from June to September 2016. Cooled raw milk with a fat content of 6.5%, pasteurized milk at 72-75 °C/15-20s, pasteurized cream and butter with 1.5% of salt were collected. The samples of pasteurized milk and cream were collected at the outlet of the pasteurizer and butter was collected immediately after processing. Raw milk was collected in the dairy refrigeration tank and in four sheep dairy farms that supply milk to the dairy company, identified as farms 1 to 4. It was verified whether farmers followed milking good practices as recommended (Rosa et al., 2014). All samples were packed in sterile containers and transported under refrigeration to the laboratory.

Initial dilution was prepared for each sample using 25 mL or 25 g and 225 mL of 0.1% peptone water followed by homogenization in a Stomacher-type equipment. Serial dilutions were made up to 10⁻⁶ and 100 µL of each dilution was inoculated into Man Rogosa and Sharpe agar (MRS, Merck®). The incubation was carried out in an anaerobic tank containing anaerobic generator at 37 ± 1 °C for 48 to 72 hours (Silva et al., 2013). After this period, the colonies were counted. Colonies present in the MRS agar were transferred by scoring to MRS agar plates and, subsequently, catalase and Gram staining tests were performed. The catalase test was performed on a glass slide with the addition of one drop of 3% hydrogen peroxide solution to each of the collected colonies, being considered positive when bubbles were formed (Brasil, 2018; Silva et al., 2013). Gram staining was performed according to the standard procedure (Brasil, 2018; Bruno, 2011). Selected isolates were transferred to MRS agar and submitted to tests to verify the fermentative profile and the proteolytic, lipolytic and antimicrobial activities.

The fermentative profile was evaluated in Gram-positive and catalase-negative isolates to determine the production of carbon dioxide (CO₂) from the use of glucose. The isolates were cultured in MRS agar at 37 ± 1 °C for 24 hours and then re-inoculated in MRS broth (Merck®) supplemented with 3% glucose (10% v/v) in test tubes containing inverted Durham's tube, followed by the incubation at 37 °C for the 48 hours (Sehn, 2015; Silva et al., 2013). The tubes showing carbon dioxide production by the formation of gas and turbidity in the medium were classified as heterofermentative, and those with turbidity due to the presence of lactic acid were

The proteolytic activity of the isolates was evaluated in formulated Milk Agar, with subsequent incubation at 28 °C for 24 to 48 hours. This medium was supplemented with 10% skimmed milk powder (Nestlé®) (Nespolo & Brandelli, 2010; Tebaldi et al., 2008). The lipolytic activity was evaluated using the Tributyrin agar (Biolog®) incubated at 28 °C for five days. The presence of clear zones around the colonies in Milk Agar using 1% (v/v) of HCl for 1 min was indicative of proteolytic

activity. The presence of clear zones around the colonies in Tributyrin Agar was considered lipolytic activity. The proteolytic and lipolytic activities were classified according to halo sizes: very high activity for halo greater than 10 mm, high activity for halo of 3 to 10 mm, and low activity for halo lower than 3 mm (Alapont et al., 2015). All tests were performed in duplicate.

The antimicrobial activity of the isolates was evaluated using the agar diffusion method (Biscola et al., 2013). The isolates were cultured in MRS broth at 37 °C for 18 hours and then 5 µL of the culture was added in Brain Heart Infusion (BHI, Himedia®) previously inoculated with the 5 log CFU/mL of the indicator microorganisms: *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O157:H7 ATCC 43895 and *Staphylococcus aureus* ATCC 25923, followed by incubation at 37 °C for 18 hours. These strains were obtained from the collection of the Department of Microbiology, Immunology and Parasitology, Institute of Basic Health Sciences, UFRGS. After this time, the plates were observed for the presence/absence of inhibition halos, and the diameters of them were measured. Results were classified based on halo diameter: strong inhibition for halos greater than 8 mm, moderate inhibition with halos between 4 and 8 mm, and weak inhibition for halos of 1 and 4 mm (Akabanda et al., 2014). The assay was performed in triplicate.

3 Results

Twenty-five samples of sheep milk products were collected including raw milk (n=10), pasteurized milk (n=3), pasteurized milk cream (n=8), and butter (n=4). The average count in MRS agar for sheep milk obtained from the refrigerated storage tank and dairy farms are shown in Figure 1. After 72 hours of growth, the milk of the tank showed LAB counts of 7.35 ± 0.11 log CFU/mL, while the mean for milk from the farms was 6.50 ± 1.56 log CFU/mL. In Figure 1, it is possible to observe the quantification of LAB in the milk collected in each of the dairy farms that supplied milk to the dairy plant, with emphasis on counts of farm 4 (8.91 ± 0.05 log CFU/mL). As observed during sampling of raw milk, farm 4 was the only one performing pre-dipping and post-dipping treatments, with the use of lactic acid foam and iodine gel, respectively. The other producers performed only one, or none of these steps.

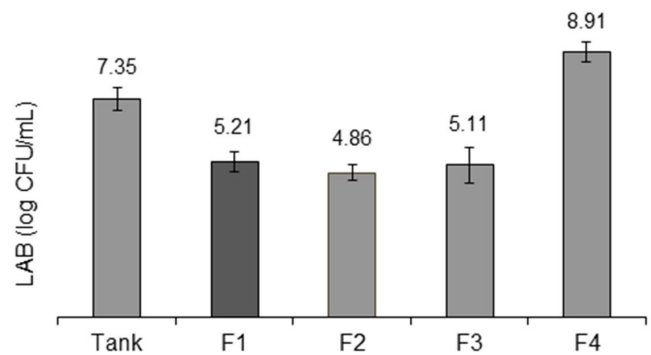


Figure 1. Lactic acid bacteria (LAB) counts in raw sheep milk from the dairy tank and dairy farms of the South of Brazil (2019).

The LAB counts per processed sheep milk and derivatives is shown in Figure 2 where butter (n=4) showed LAB counts of 5.85 ± 0.01 log CFU/mL, higher than that of milk cream (n=8) with 3.58 ± 0.60 log CFU/mL, which is the raw material for this product.

Table 1 shows the selection of LAB isolates in dairy products of ovine origin, in each of the isolation steps. Among the 253 colonies selected from MRS agar, 37 (14.62%) were catalase-negative and Gram-positive and the main source was raw milk, with 30 of these isolates. This fact is expected since this product is unpasteurized and a larger number of sample (n=10) were collected. Fermentation profile was evaluated to characterize 37 isolates catalase-negative and Gram-positive, and 86.48% were homofermentative type, with the highest number of isolates obtained from raw refrigerated milk.

In Table 1, the proteolytic, lipolytic and antimicrobial activities of selected isolates can also be verified. Antimicrobial activity was observed against only one microorganism tested (*Listeria monocytogenes* ATCC 7644). Raw milk was the source of most isolates, where 13 LAB showed proteolytic activity, three with lipolytic activity, and one with antimicrobial activity. Proteolytic activity was also observed in three isolates from the cream and in two isolates of pasteurized milk. Considering the values of initial LAB screening in this study (n=37), 48.65% (n=18) showed proteolytic activity, 13.51% (n=5) lipolytic, 10.81% both proteolytic and (n=4) and 2.70% (n=1) antimicrobial.

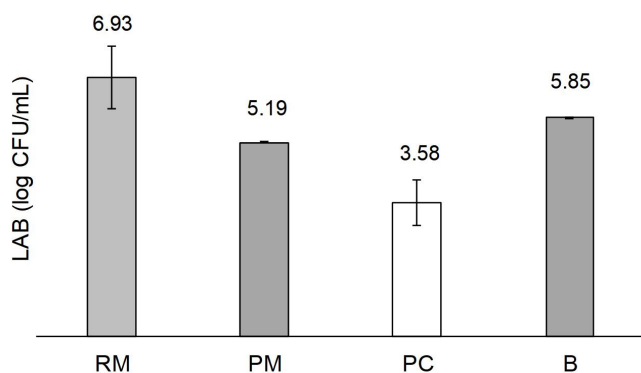


Figure 2. Lactic acid bacteria (LAB) counts in sheep's raw milk (RM), pasteurized milk (PM), pasteurized milk cream (PC) and butter with salt (B) from South of Brazil (2019).

Table 1. Number of lactic acid bacteria obtained from each stage of isolation from dairy sheep products South of Brazil (2019).

Isolation time	Number of isolates*				
	Total	RM	PM	PC	B
Growth in Man Rogosa and Sharpe agar	253	196	5	38	14
Catalase test (catalase-negative)	210	159	5	38	8
Gram staining (Gram-positive)	37	30	2	4	1
Proteolytic activity (presence of halo)	18	13	2	3	0
Lipolytic activity (presence of halo)	5	3	2	0	0
Antimicrobial activity** (presence of halo)	1	1	0	0	0

*RM (raw milk); PM (pasteurized milk); PC (pasteurized milk cream); B (butter); **activity against *Listeria monocytogenes* ATCC 7644.

Table 2 shows the proteolytic, lipolytic and antimicrobial activities of each LAB of sheep milk and derivatives of Southern Brazil. Of the total LAB isolated from raw milk, pasteurized milk, pasteurized cream, and butter, only 51.35% (n=19) had any of proteolytic, lipolytic and/or antimicrobial activity against *Listeria monocytogenes* ATCC 7644. Proteolytic activity ranged from 1.5 to 23 mm representing 48.65% (n=18) of the LAB isolates, where 37.84% (n=14) showed very high proteolytic activity (halo size > 10mm). Lipolytic activity varied from 1 to 23 mm in 13.51% (n=5) of the isolated LAB, and among them 8.11% (n=3) were classified with very high lipolytic activity (halo size > 10mm).

Among the LAB obtained in the present study with proteolytic and lipolytic activities, we highlight LAB LSE 05-21 obtained from raw milk of farm number 4, and LAB LSE 01-1 isolated from the pasteurized milk both with average halos above 10 mm and coccus morphology. Only LAB LSE 08-23 showed strong antimicrobial activity against *Listeria monocytogenes* ATCC 7644, with a mean halo size of 13.2 mm, in addition to high lipolytic and very high proteolytic actions.

4 Discussion

LAB counts obtained in this study were higher than those obtained in raw sheep milk, produced in the metropolitan region of Porto Alegre city, South of Brazil with values of 5.65 ± 0.53 log CFU/mL (Nespolo & Brandelli, 2010). In a study carried out in Tunisia with bovine and camel milk, mean values were 2 log CFU/mL (Zeineb et al., 2013). Goat milk produced in the Centre-West region of Brazil, 52.5% (n=21) did not have LAB, and the highest values were 3 to 4 log CFU/mL, quantified in only 7.5% (n=3) of the samples (Pádua, 2013). A study performed in Iran investigated LAB levels in bovine raw milk and showed average counts of 4.88 log CFU/mL in MRS agar at 30 °C (Farahani et al., 2017). In raw bovine milk used to produce handmade Brazilian Minas cheese, the average count was 6.4 log CFU/mL (Luiz et al., 2017). In Myzithra cheese, produced with a mixture of whey and raw ovine milk, counts of non-starter lactic acid bacteria ranged from undetected on the first day to 2.31 log CFU/g on 15th day storage at 4°C and varied from 4.07 to 3.31 log CFU/g for thermophilic cocci in the same periods (Kaminarides et al., 2018).

LAB counts might be affected, among other factors, by the treatment of the herd with antimicrobials. In this case, the withdrawal period should be observed to avoid antimicrobial residue, which could affect milk microbiota and inhibit the presence of LAB during cheese maturation, in addition to causing

Table 2. Quantification of proteolytic, lipolytic and antimicrobial activities in the lactic acid bacteria of sheep milk and derivatives of South of Brazil (2019).

Source	Isolate ID	Activity zone (mm)*		
		PA	LA	AA
Raw milk	LSE 01-15	23.7 ± 3.20	-	-
Raw milk	LSE 01-17	8.60 ± 2.80	-	-
Raw milk	LSE 01-18	22.6 ± 1.00	-	-
Raw milk	LSE 01-22	12.1 ± 6.70	-	-
Raw milk	LSE 01-23	22.6 ± 1.10	-	-
Raw milk	LSE 01-29	17.3 ± 0.50	-	-
Raw milk	LSE 01-30	17.4 ± 3.60	-	-
Raw milk	LSE 05-21	16.6 ± 0.50	14.1 ± 3.40	-
Raw milk	LSE 07-21	17.8 ± 8.00	-	-
Raw milk	LSE 07-24	14.0 ± 6.20	-	-
Raw milk	LSE 08-15	18.0 ± 1.20	-	-
Raw milk	LSE 08-23	23.3 ± 4.30	8.30 ± 1.80	13.2 ± 0.90
Raw milk	LSE 08-5	17.2 ± 0.40	7.30 ± 1.30	-
Pasteurized milk	LSE 01-3	-	15.4 ± 2.60	-
Pasteurized milk	LSE 01-5	14.2 ± 2.20	-	-
Pasteurized milk	LSE 01-1	16.5 ± 1.20	12.1 ± 2.10	-
Milk cream	LSE 06-28	1.70 ± 0.50	-	-
Milk cream	LSE 06-27	1.50 ± 0.50	-	-
Milk cream	LSE 06-18	3.00 ± 0.00	-	-

*PA (proteolytic activity) and LA (lipolytic activity): Halo size >10 mm (very high), 3-10 mm (high) and <3 mm (low) for proteolytic and lipolytic activities (Alapont et al., 2015); AA (antimicrobial activity): Halo size >8 mm (strong), 4-8 mm (moderate) and 1-4 mm (weak) for antimicrobial activity (Akabanda et al., 2014).

allergies to consumers (Beltrán et al., 2014). Milking under strict hygienic conditions leads to less contamination by pathogenic microorganisms and are considered a preventive measure for environmental mastitis (Gelasakis et al., 2015; Rosa et al., 2014). The major cause of subclinical mastitis is coagulase-negative staphylococci and *S. aureus* is mainly involved in clinical mastitis of dairy herds (Gelasakis et al., 2015). *S. aureus* is the most frequently bacterium diagnosed in sheep, in addition to *S. hyicus*, *S. intermedius*, *S. schleiferi*, *Mannheimia* spp. and *Streptococcus* spp., among others (Contreras et al., 2007; Gelasakis et al., 2015). Animal health is very important since it guarantees safety, and the appropriate characteristics of milk derivatives (Gelasakis et al., 2015), considering that contaminating pathogenic microorganisms of the raw milk may have faecal origin or through contaminated udder (Forsythe, 2013; Kaminarides et al., 2018). The high contamination of milk by unwanted microorganisms negatively influences LAB growth (Gelasakis et al., 2015; Kaminarides et al., 2018; Luiz et al., 2017), in the cheese maturation process, since they will compete for nutrients (Nespolo & Brandelli, 2010). A higher LAB count correlates with better sanitary conditions of the herds (Forsythe, 2013), or that the animals are not being treated for mastitis (Merlin et al., 2015), especially those from farm 4 as observed in this study. In the butter processing, a stage of separation of the liquid phase of the cream, called buttermilk (Forsythe, 2013) occurs, a fact that may explain the higher LAB count in this product. In pasteurized milk (n=3), the mean LAB value was 5.19 ± 0.01 log CFU/mL, lower than the value for raw milk (6.93 ± 0.84 log CFU/mL), possibly due to partial destruction of microorganisms (Forsythe, 2013) during milk pasteurization. The absence of heat treatment is permitted for milk used for cheeses that have undergone a maturation process

for a time not less than 60 days and at a temperature above 5 °C (Brasil, 1996). In the evaluated dairy, sheep milk always goes through the process of pasteurization prior to the processing of derivatives.

From the MRS agar plates obtained from the samples of raw milk and sheep milk products, 253 isolates were selected. Gram staining and catalase assay indicated 37 Gram-positive and catalase-negative isolates, of which 78.38% (n=29) were cocci. These results may be related to a higher prevalence of genera such as *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* (Bruno, 2011) in sheep milk isolates from the South of Brazil. Already in traditional Algerian butter samples (n=76) 66% of the LAB obtained were coccus (Bettache et al., 2012).

The homofermentative group of LAB produces lactic acid as the only or main product of glucose fermentation, while the group of heterofermentative produces the same molar amount of lactate, carbon dioxide and ethanol from the hexoses (Widyastuti et al., 2014). The latter group produces a series of antimicrobial substances, including lactic acid, hydrogen peroxide, diacetyl, and other organic acids (Farahani et al., 2017). The prevalence of homofermentative profile in LAB isolates obtained from dairy products was also observed in other studies of this Brazilian region. In samples from sliced mozzarella cheese, 91.04% (n=61) of the isolates were homofermentative (Sehn, 2015), and all 21 LAB isolated from milk samples and homemade cheeses from the Northwest region of Rio Grande do Sul were classified as homofermentatives (Hermanns, 2013). A greater number of homofermentative LAB is used in the industry for the processing of fermented products to improve texture and sensorial characteristics of the dairy products

(Asensio-Vegas et al., 2016; Balthazar et al., 2018b; Sehn, 2015; Widyastuti et al., 2014).

In a study carried out in Tunisia, 351 isolates obtained from camel and bovine milk 14.24% (n=50) were Gram-positive and catalase-negative (Zeineb et al., 2013). Another study in the same region of Brazil using raw sheep milk and cheese found 52.68% (n=59) of the catalase-negative and Gram-positive isolates (Nespolo & Brandelli, 2010).

In a study that evaluated sheep milk and cheese collected in Southern Brazil, 20.34% (n=12) of the LAB showed proteolytic, lipolytic and antimicrobial activities (Nespolo & Brandelli, 2010). Six isolates of LAB (23.08%) isolated from bovine raw milk from Iran demonstrated proteolytic activity (Farahani et al., 2017). LAB isolated from traditional Algerian dairy samples showed 3.95% (n=3) and 2.63% (n=2) with proteolytic and lipolytic activity, respectively in butter (Bettache et al., 2012), while in fermented milk and cheese no isolates with lipolytic activity were observed (Mechai et al., 2014). The importance of proteolytic and lipolytic activities in LAB is linked to biochemical processes that occur during the maturation of cheeses (Bruno & Carvalho, 2009). Proteolysis is considered an essential process in all types of cheeses with internal and superficial maturation, and the main responsible agents are enzymes, such as plasmin or those derived from the coagulant, or the proteolytic enzymes of starter bacteria or secondary inocula (Balthazar et al., 2017; Farahani et al., 2017; Pereira et al., 2008). The proteolytic agents act in combination to hydrolyse casein to peptides and amino acids (Balthazar et al., 2017; Farahani et al., 2017), which can be quantified by extension and depth indices of maturation (Pereira et al., 2008). The maturation depth is related to secondary proteolysis from the native milk microbiota or cultures added during cheese production, as observed for homofermentative LAB, *Lactobacillus* present in starter cultures (Widyastuti et al., 2014). In a study to develop yoghurt from ovine milk, a reduction in fermentation time was observed due to the high proteolytic activity of *Lactobacillus delbrueckii* sp. *bulgaricus* (Asensio-Vegas et al., 2016). In another study with fermented milk and traditional cheeses produced in Algeria, it was observed that two isolates of the genus *Lactococcus* showed the highest proteolytic activity (Mechai et al., 2014). However, lipolysis is a critical process in some varieties of cheeses, because it promotes the softening of the dough and makes the texture softer, as in blue cheeses, Italian hard dough, Swiss type cheese (Farahani et al., 2017), Scamorza cheese, and Pecorino cheese (Balthazar et al., 2017). Thus, the selection of LAB with both activities may be of interest for the development of starter cultures for regional sheep cheeses and for the characterization of origin of these dairy products.

The presence of an isolate with antimicrobial activity against *Listeria monocytogenes* ATCC 7644 (Table 1) indicates the possibility for food applications. The ability to inhibit the presence of this bacterium was also observed for one isolate from Brazilian mozzarella cheese, identified as *Lactobacillus curvatus* (Sehn, 2015). Samples of fermented milk and traditional Algerian cheeses presented 25% (n=52) of LAB isolates with anti-*Listeria monocytogenes* activity (Mechai et al., 2014), while the isolation in butter from the same site identified LAB with activity against *Listeria innocua* (Bettache et al., 2012). One study tested the use

of starter cultures with proteolytic, lipolytic and anti-*Listeria monocytogenes* activities ATCC 7644, isolated from raw sheep milk, and a beneficial effect was observed in the decrease of the unwanted microbiota in raw milk sheep cheeses matured for more than 60 days (Nespolo et al., 2010). Although there is a low incidence of this bacterium causing clinical mastitis in sheep, the presence of *Listeria monocytogenes* in raw sheep milk has been reported (Gelasakis et al., 2015), as well its relationship with contamination in dairy products (Buchanan et al., 2017; Forsythe, 2013). The verification of the presence of this pathogenic microorganism is required by Brazilian legislation in cheeses with humidity above 36% (6), although listeriosis is a disease of low incidence, it represents an important risk to public health, due to the degree of severity and high mortality (Buchanan et al., 2017). These data demonstrate the importance of this pathogen for the dairy industry and the feasibility of testing the application of this isolate in a derivative as a preventive measure against listeriosis.

In spontaneously fermented milk produced in Ghana from bovine milk, 373 LAB were isolated, obtaining a percentage of proteolytic activity of 80% and 76.41% of lipolytic activity (Akabanda et al., 2014). In Viamão (South of Brazil), 112 LAB were isolated from samples of sheep milk and derivatives, of these 51% (n=30) with proteolytic activity and 32% (n=19) with lipolytic activity (Nespolo & Brandelli, 2010). In a study with traditional Brazilian cheeses, 466 LAB were isolated in MRS Agar proteolytic and 30.7% of them showed proteolytic capacity (Campagnollo et al., 2018). Most of the isolates showed proteolytic and lipolytic profiles, which demonstrates the possibility of their use in the processing of cheeses that undergo proteolysis and lipolysis during maturation.

Isolate LAB LSE 08-23 isolated from sheep's milk from farm 4 showed a higher initial count for these bacteria, emphasizing the importance of adequate management of the sheep herd to maintain the desired microbiota in raw milk. This isolate was characterized as a coccus and showed homofermentative profile. In another study, the zone of inhibition against *Listeria monocytogenes* ATCC 7644 in LAB of sheep milk products ranged from 6.5 to 10.5 mm (Nespolo & Brandelli, 2010), while 15.01% (n=56) of isolates spontaneously fermented bovine milk showed some inhibition halo against *Listeria monocytogenes*, half of them from 1 to 4 mm (Akabanda et al., 2014). Tests in the LAB isolated from Brazilian Minas handmade cheese samples showed that 73.0% had activity against *Listeria monocytogenes* at 37 °C (Campagnollo et al., 2018). LAB LSE 08-23 was characterized as a Gram-positive, coccus type, with homofermentative profile, which may have potential for food preservation and/or production of sheep dairy products. However, more studies are needed to verify the viability and efficiency of this isolate during the processing of dairy products, as well as during its shelf life.

5 Conclusion

The raw sheep milk was the source of the largest number of LAB isolates with lipolytic and proteolytic activities, especially those collected at farm number 4. LAB LSE 08-23, a coccus type with homofermentative profile, was highlighted due to its proteolytic, lipolytic and anti-*Listeria monocytogenes* ATCC 7644 activities. Thus, sheep dairy samples from the South of Brazil

are viable sources of lactic acid bacteria with potential use as starter cultures for fermented dairy products.

Acknowledgements

We thank the National Council for Scientific and Technological Development (CNPq), Brazil, and Research and Innovation Support Foundation of Santa Catarina State (FAPESC), Santa Catarina, Brazil.

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