Acta Alimentaria 52 (2023) 2, 199–208
DOI: 10.1556/066.2022.00237

AKADÉMIAI KIADÓ

Physical and microbiological characterisation of artisanal cheese and isolation, identification, and *in vitro* evaluation of *Lactobacillus* strains with probiotic potential and antibiotic susceptibility

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ORIGINAL RESEARCH PAPER

Received: November 28, 2022 • Accepted: May 3, 2023
Published online: June 6, 2023

ABSTRACT

The aim of this study was to isolate lactic acid bacteria (LAB) from artisanal cheeses and evaluate their probiotic potential and antibiotic susceptibility under *in vitro* conditions. Cheeses obtained at different maturation times were analysed for moisture and lipid contents, as well as for the presence of various microorganisms, including coagulase positive staphylococci, *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, filamentous fungi, yeasts, total mesophilic bacteria, and LAB. After identification, the selected LAB were subjected to human gastrointestinal tract (HGT) conditions to evaluate their survival rates. Of the 18 *Lactobacillus* strains isolated, 11 survived the HGT test and presented γ-haemolysis. No resistance was observed against antibiotics. *Lactobacillus fermentum* C1a, C1b, C1c, and C1f, as well as *Lactobacillus paracasei* C1d, C1e, and C1g, were identified as potential starter cultures for the food industry.

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KEYWORDS

lactic acid bacteria, microbial diversity, food safety, gut microbiota, starter cultures

1. INTRODUCTION

In recent years, the demand for artisanal products has increased due to their perceived higher quality and potential contribution to local economies. Artisanal cheeses, in particular, depend on the maturation process to develop desirable sensory characteristics and to reduce undesirable microbiota (Bemfeito et al., 2016). The MS *caipira* is a traditional artisanal cheese from Mato Grosso do Sul, Brazil, obtained by the enzymatic coagulation of raw milk using industrial curds. At the end of the maturation process, the cheeses have a viable microbiota, consisting primarily of LAB from either raw milk or the dairy environment. LAB are important components of cheese microbiota and contribute to desirable characteristics such as flavour and texture. They are also the predominant microorganisms in artisanal cheeses and are active and determinant at various stages of production and ripening (Nero et al., 2021). Isolated LAB can be utilised as starter cultures in cheese processing, accelerating maturation. Furthermore, LAB are considered the most suitable probiotics due to their natural presence in the healthy human intestine and in various dairy and fermented foods. Therefore, the aim of this study was to isolate LAB from artisanal cheeses, evaluate their *in vitro* probiotic potential and antibiotic susceptibility, and identify potential starter cultures for the food industry.

2. MATERIALS AND METHODS

2.1. Artisanal cheese sampling and composition

Artisanal cheese MS *caipira* samples ($n = 9$) were aseptically collected at different maturation times: 3 of fresh cheese (F) (up to 7 days), 3 of half-cured cheese (H) (from 8 to 14 days), and 3 of cured cheese (C) (from 15 to 21 days) from three different producers (1, 2, 3) (Dourados, MS, Brazil).

Moisture was determined by oven drying a mixture of sample and sand at 102 °C until constant weight, and fat by digesting the samples with a hydrochloric acid - ethanol solution, extraction with diethyl ether and light petroleum before removing solvents by evaporation until constant weight (IDF, 2004). All analyses were conducted in triplicate and the statistical results were evaluated through analysis of variance (ANOVA) and the Tukey test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 7.0.

Microbiological determinations were carried out for yeasts and moulds, total mesophilic bacteria (TMB), and lactic acid bacteria (LAB) according to the methodology for milk and dairy products (ISO, 2004). For filamentous fungi and yeasts, 0.1 mL from each appropriate dilution step was spread in duplicate on the surface of Petri dishes containing Potato Dextrose Agar (PDA) before incubation (30 °C, 120 h). For TMB, the pour plate inoculation technique (35 °C, 48 h) in Plate Count Agar (PCA) was utilised. For LAB, the decimal dilutions were deep plated on Man, Rogosa and Sharpe (MRS) agar before incubated (37 °C, 48 h). Enumeration of

coagulase-positive staphylococci, *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* was carried out to assess the hygienic-sanitary conditions.

2.2. Isolation of lactic acid bacteria (LAB)

Colonies were successively transferred on MRS agar and incubated (37 °C, 48–72 h) to select strains according to their morphological characteristics. Gram-positive strains were evaluated for catalase and fermentation (Zanini et al., 2012).

2.3. Identification of the LAB

The genomic DNA of the isolates was extracted using a bacterial genomic DNA isolation kit 17900 (Norgen Biotek, Canada) The samples were quantified by the BioDrop μLITE equipment (BioDrop, UK) and their integrity was analysed by electrophoretic run on a 0.5 × 1% Tris-Borate-EDTA agarose gel (Merck, Germany) stained with GelRed (Biotium, USA) 100×. The PCR reaction for the 16S gene was performed in a T100 thermocycler (Unicycler UNI-E251, Uniscience, Brazil) under the following conditions: 3 min at 94 °C; 45 s at 94 °C; 30 s at 60 °C; 90 s at 72 °C (25 cycles); 10 min at 72 °C. The PCR products were electrophoresed in a 2% 0.5× TBE agarose gel stained with GelRed, purified using a purification kit 14400 (Norgen Biotek, Canada) and sequenced (GoGenetic, Brazil). Sequences were analysed using Sequencher 5.4 software (Gene Codes Corporation, Ann Arbor, USA) and compared to the GenBank public database (http://www.ncbi.nlm.nih.gov) using the BLASTn tool (Altschul, 1997).

2.4. Characterisation of LAB

For the *in vitro* evaluation of probiotic potential, a survival test under conditions that simulate the human gastrointestinal tract (HGT), as well as a haemolytic activity test were carried out. In these tests, only LAB with rod morphology were selected, which is characteristic of the *Lactobacillus* genus (Nogueira and Gonçalves, 2011).

The selected strains were reactivated in MRS broth (37 °C, 48 h), spiked in Petri dishes containing MRS agar (37 °C, 48 h) and resuspended in saline solution buffered with phosphate (PBS) (pH 7.2) up to an initial amount of around 10^7 CFU mL^{-1}. The saline solution was prepared with 2M sodium hydroxide solution and the pH was adjusted with 0.1M hydrochloric acid solution. The pH was measured using a digital potentiometer. The LAB isolate cells were cultured overnight (18 h) in MRS broth, centrifuged at 10,000 *g* (4 °C, 5 min), and washed twice with PBS (pH 7.2). Determination of resistances to low pH, pepsin, pancreatin, and bile salts were carried out according to Maragkoudakis et al. (2006).

The evaluation of the haemolytic activity was adapted from Zoumpopoulou et al. (2008). The selected strains were reactivated in MRS broth (48 h, 37 °C) and then seeded on Mueller-Hinton Agar, containing 5% (v/v) of lamb blood and incubated (37 °C, 48 h). The agar plates were examined in duplicate for signs of β-haemolysis or total haemolysis, α-haemolysis or partial haemolysis, and γ-haemolysis or absence of haemolysis. Only total haemolysis was classified as positive for haemolysis, as partial haemolysis is not considered harmful to health (Adimpong et al., 2012).

The antibiotic susceptibility test was performed by using the disk diffusion technique (EUCAST, 2013) for the antibiotics meropenem, inipenem, ertapenam, aztreonam, cefepime, amoxicillin, ceftazidime, gentamicin, amicanin, polymixin B, chloramphenicol, tetracycline, penicillin, and neomycin. The inhibition zone diameters were assessed by linear regression analysis.

3. RESULTS AND DISCUSSION

The composition and microbiological determinations of artisanal cheese samples are presented in Table 1. According to legislation, fresh cheese from artisan producer 1 (F1) was classified as a medium-moisture cheese (42.78 g/100 g of moisture content), and fresh cheese from artisan producer 2 (F2) and fresh cheese from artisan producer 3 (F3) were classified as high-moisture cheeses (above 46.0 g/100 g). Half-cured cheeses (H) and cured cheeses (C) were classified as medium-moisture (from 36.0 to 45.9 g/100 g) and low-moisture (up to 35.9 g/100 g) cheeses, respectively. F2 and F3, and H2 and H3, did not statistically differ ($P > 0.05$) for the moisture content. F2, F3, H2, H3, and all C cheeses were classified as fat (lipids in dry basis between 45.0 and 59.9 g/100 g), while F1 and H1 cheeses were classified as semi-fat (lipids in dry basis between 25.0 and 44.9 g/100 g). F2 and F3, and C2 and C3, did not statistically differ ($P > 0.05$) for the fat content.

MS *caipira* cheese presented moisture and fat contents similar to other artisanal cheeses. For example, moisture contents ranged from 38.8 to 52.0 g/100 g, and the lipid contents in dry basis from 45.0 to 49.5% were previously observed for fresh Serra da Canastra cheese, after the second day of maturation (Costa Júnior et al., 2009), indicating a fat cheese with medium to high moisture content. Results were also close to those reported for half-cured Canastra cheeses (43.6 g/100 g moisture: medium moisture cheese; 49.86 g/100 g fat: fat cheese) (Silva et al., 2011). Other researchers observed average moisture contents of 37.4% for Serro and 37.3% for Canastra cured cheese samples after 17 and 22 days, respectively (Galinari et al., 2014). These values were close to those observed for the cured cheese (C) (from 15 to 21 days) (Table 1).

Here, it was verified that all the cheese samples evaluated had low filamentous fungi and yeasts counts ($\leq$0.5 log CFU g^{-1}), except for F3 (2.7 log CFU g^{-1}) (Table 1), indicating satisfactory processing conditions. In comparison, literature reports fungal counts ranging from 2.7 log CFU g^{-1} to >6.7 log CFU g^{-1} in artisanal Minas "Frescal" cheeses (Pinto et al., 2011) and averages of 5.2 log CFU g^{-1} in Canastra cheese and 5.0 log CFU g^{-1} in Serro cheese (Galinari et al., 2014). Cheeses had high counts of TMB, ranging from 4.3 (C1) to 5.9 log CFU g^{-1} (H2). However, these values were lower in comparison with the averages of 8.1 log CFU g^{-1} found on Canastra cheese and 8.0 log CFU g^{-1} in Serro cheese (Galinari et al., 2014). Except for the samples from the dairy producer 1 (F1, H1, and C1) that presented counts ranging from 2.6 to 4.8 log CFU g^{-1} of cheese, the cheeses presented a high LAB count, ranging from log 6.7 CFU g^{-1} (F2) to 7.8 log CFU g^{-1} (H2). These values are close to that reported for *Lactobacillus* spp. in Canastra and Serro cheeses (both 8.2 log CFU g^{-1}) (Galinari et al., 2014).

Coagulase-positive staphylococci, *Salmonella* spp., *L. monocytogenes*, and *E. coli* were found within the limits established by legislation (Table 1), indicating that these products produced from raw milk are suitable for consumption.

A total of 93 isolates were submitted to Gram staining, of which a total of 56 isolates (60.22%) were classified as Gram-negative and discarded. The remaining 37 isolates (39.78%) were classified as Gram-positive. These isolates were all characterised as catalase negative and positive for glucose fermentation. Among them, 19 (51.35%) were classified as cocci and the other 18 (48.65%) as rods. For comparison, the isolation of 406 LAB from cheeses produced with raw milk was reported elsewhere, where 309 (76.1%) were cocci and 97 (23.9%) were rods

Table 1. Composition and microbiological determinations for artisanal cheese samples

Sample	Composition		Microbiological determinations						
	Moisture (g/100 g)	Crude fat* (g/100 g)	LAB Log CFU g^{-1}	TMB Log CFU g^{-1}	Fungi Log CFU g^{-1}	Coagulase + *Staphylococcus* CFU g^{-1}	*Escherichia coli* CFU g^{-1}	*Salmonella* spp.	*Listeria monocytogenes*
F1	42.78 ± 1.23[b]	41.12 ± 1.94[b]	2.6	5.1	0	$<1 \times 10^2$	<10	Absence	Absence
F2	48.16 ± 1.39[a]	48.84 ± 0.95[a]	6.7	4.8	0.5	$<1 \times 10^2$	92	Absence	Absence
F3	49.01 ± 0.19[a]	49.65 ± 1.85[a]	7.0	4.8	2.7	$<1 \times 10^2$	<10	Absence	Absence
H1	37.50 ± 1.59[b]	44.37 ± 1.77[c]	4.6	4.7	0	$<1 \times 10^2$	<10	Absence	Absence
H2	45.38 ± 0.97[a]	54.93 ± 1.04[a]	7.8	5.9	0.5	$<1 \times 10^2$	60	Absence	Absence
H3	43.99 ± 1.86[a]	50.56 ± 1.91[b]	7.2	6.0	0.5	$<1 \times 10^2$	36	Absence	Absence
C1	33.28 ± 1.14[a,b]	47.22 ± 1.12[b]	4.8	4.3	0	$<1 \times 10^2$	<10	Absence	Absence
C2	30.77 ± 1.43[b]	58.26 ± 0.64[a]	7.0	5.7	0.5	$<1 \times 10^2$	36	Absence	Absence
C3	35.66 ± 1.23[a]	55.93 ± 1.13[a]	7.3	5.8	0	$<1 \times 10^2$	<10	Absence	Absence

*: dry basis; F: fresh cheese; H: half-cured cheese; C: cured cheese; (1, 2, 3): dairy producers. Values of moisture and crude fat (means ± standard deviations) for F, H, or C samples with the same superscript letter in the same column do not differ statistically at $P > 0.05$.

(Begovic et al., 2011), while of 36 LAB isolated from cheese made from raw goat's milk, 5 (13.9%) were identified as cocci and 31 (86.1%) as rods (Serhan et al., 2009).

The 16S rRNA gene sequencing revealed 5 different species belonging to the *Lactobacillus* genus; including *L. bulgaricus* (2 strains) and *L. brevis* (2 strains), both isolated from fresh cheese; *L. casei* (3 strains), *Lactobacillus paracasei* (2 strains), and *Lactobacillus fermentum* (1 strain) isolated from half-cured cheese; *L. fermentum* (4 strains) and *L. paracasei* (4 strains) isolated from cured cheese (Tables 1 and 2).

The lactic acid bacterium species found here at the different stages of ripening were related to those found in Buffalo Mozzarella cheese at different storage times. In Buffalo Mozzarella, *L. bulgaricus* strains were found in the three cheeses immediately after preparation, while *L. fermentum* and *L. casei* strains were found in two and one of the evaluated cheeses, respectively, after 14 days of storage, and *L. fermentum* strains after 28 days of storage (Silva et al., 2021). There is a group of LAB that includes the species *L. rhamnosus*, *L. paracasei*, and *L. casei* that are among the main LAB found in cured and hard cheeses. They can use other energy sources beside lactose, which favours their development and predominance in cured cheeses.

Among the identified species in cured cheese, *L. fermentum* is commonly used as a probiotic and has several health benefits, acting as preventive agent for colorectal cancer and lowering cholesterol levels (Naghmouchi et al., 2019), while *L. paracasei* is related to anticytotoxic and antiproliferative effects, in addition to stimulating the development of beneficial microorganisms

Table 2. Bacterial counts (log CFU g^{-1}) at low pH values of the lactic acid bacterium strains isolated from artisanal cheese samples

Strain	0 h			3 h		
	pH 2	pH 3	pH 4	pH 2	pH 3	pH 4
L. bulgaricus F1a	8.5 ± 1.37	8.8 ± 1.74	>7.0 ± 0.00	7.3 ± 0.00	7.7 ± 0.30	>7.0 ± 0.00
L. bulgaricus F1b	8.7 ± 1.69	8.7 ± 1.65	>7.0 ± 0.00	8.6 ± 1.47	8.9 ± 1.20	>7.0 ± 0.00
L. brevis F2a	8.4 ± 1.16	>7.0 ± 0.00	>7.0 ± 0.00	7.6 ± 0.00	8.3 ± 1.26	>7.0 ± 0.00
L. brevis F3a	8.7 ± 1.65	8.6 ± 1.59	>7.0 ± 0.00	7.9 ± 0.30	7.9 ± 0.30	8.3 ± 0.85
L. casei H1a	8.9 ± 0.30	>7.0 ± 0.00	>7.0 ± 0.00	8.5 ± 1.45	7.2 ± 0.18	>7.0 ± 0.00
L. casei H1b	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei H1c	9.1 ± 1.87	>7.0 ± 0.00	>7.0 ± 0.00	9.1 ± 0.70	7.7 ± 1.43	>7.0 ± 0.00
L. paracasei H1d	8.1 ± 0.90	7.9 ± 0.93	>7.0 ± 0.00	0.0 ± 0.00	6.7 ± 0.30	8.7 ± 1.72
L. casei H1e	8.6 ± 1.61	>7.0 ± 0.00	>7.0 ± 0.00	8.7 ± 1.28	8.7 ± 0.78	>7.0 ± 0.00
L. plantarum H2a	8.7 ± 1.73	>7.0 ± 0.00	>7.0 ± 0.00	7.8 ± 0.70	>7.0 ± 0.00	>7.0 ± 0.00
L. fermentum C1a	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	7.9 ± 0.90	7.7 ± 0.48	8.2 ± 1.20
L. fermentum C1b	7.6 ± 1.63	>7.0 ± 0.00	>7.0 ± 0.00	8.0 ± 1.00	6.7 ± 0.30	7.6 ± 1.63
L. fermentum C1c	8.5 ± 0.85	>7.0 ± 0.00	>7.0 ± 0.00	8.4 ± 1.04	8.7 ± 0.00	>7.0 ± 0.00
L. paracasei C1d	8.8 ± 1.79	>7.0 ± 0.00	>7.0 ± 0.00	0.0 ± 0.00	7.0 ± 0.18	7.0 ± 0.18
L. paracasei C1e	8.4 ± 1.35	>7.0 ± 0.00	>7.0 ± 0.00	8.4 ± 0.18	8.6 ± 0.60	8.9 ± 0.30
L. fermentum C1f	9.0 ± 1.97	>7.0 ± 0.00	>7.0 ± 0.00	6.7 ± 0.30	8.6 ± 0.60	>7.0 ± 0.00
L. paracasei C1g	8.8 ± 0.74	>7.0 ± 0.00	>7.0 ± 0.00	8.7 ± 0.30	8.8 ± 0.54	>7.0 ± 0.00
L. paracasei C1h	8.8 ± 1.77	8.5 ± 1.53	>7.0 ± 0.00	8.1 ± 0.81	7.9 ± 0.30	8.02 ± 0.98

Log CFU g^{-1} > 7.0 indicated uncountable LAB.

in the intestine and inhibiting the growth of pathogens (Okina et al., 2018). The addition of *L. paracasei* as starter culture in the production of cheese increases the acidity of the cheese, reducing the counts of coliforms, yeasts, and fungi, in addition to improving the quality of the cheese compared to those produced without starter culture (Mantzourani et al., 2018).

All 18 rod-shaped LAB were submitted to the survival tests. It was verified that all *Lactobacillus* strains submitted to the low pH resistance test survived both at 0 and 3 h, indicating that the strains tested (except *L. paracasei* H1d and *L. paracasei* C1d for 3 h at pH 2) could remain viable during the stomach's digestion process (Table 2). The survival of the LAB in the pH range 2–4 is considered an important requirement for a good probiotic performance (Plessas et al., 2017).

All strains were evaluated for their resistance to pepsin. Inhibition of some *Lactobacillus* strains after 3 h in contact with pepsin was observed. In comparison, reductions >2.5 log CFU mL^{-1} (Maragkoudakis et al., 2006) and >3.0 log CFU mL^{-1} (Plessas et al., 2017) of LAB submitted to the pepsin resistance test were reported in the literature. Considering that one of the main characteristics of probiotics is the ability to resist the gastric juice, LAB that did not resist the pepsin (*L. bulgaricus* F1a, *L. bulgaricus* F1b, *L. brevis* F2a, *L. brevis* F3a, *L. paracasei* H1d, *L. plantarum* H2a, and *L. paracasei* C1h; Table 3) were not used in the other tests.

In the following test, all *Lactobacillus* strains that resisted pepsin also showed resistance to pancreatin (Table 3). This behaviour seems to be strain-related, because while some authors observed that most of the strains could survive the action of pancreatin (Maragkoudakis et al., 2006), others reported a reduction in the survival of LAB in the presence of pancreatin (Plessas et al., 2017).

Table 3. Bacterial counts (log CFU g^{-1}) of the lactic acid bacterium strains isolated from artisanal cheese samples that were subjected to pepsin, pancreatin, and bile salts

Strain	Pepsin		Pancreatin		Bile salts	
	0 h	3 h	0 h	4 h	0 h	4 h
L. bulgaricus F1a	8.4 ± 0.60	0.0 ± 0.00	nd	nd	nd	nd
L. bulgaricus F1b	8.8 ± 1.41	0.0 ± 0.00	nd	nd	nd	nd
L. brevis F2a	8.5 ± 1.48	0.0 ± 0.00	nd	nd	nd	nd
L. brevis F3a	>7.0 ± 0.00	0.0 ± 0.00	nd	nd	nd	nd
L. casei H1a	8.8 ± 1.68	8.3 ± 0.85	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. casei H1b	9.1 ± 0.18	8.8 ± 0.98	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei H1c	>7.0 ± 0.0	8.9 ± 1.13	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei H1d	7.9 ± 0.81	0.0 ± 0.00	nd	nd	nd	nd
L. casei H1e	8.3 ± 1.27	8.4 ± 0.48	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. plantarum H2a	>7.0 ± 0.00	0.0 ± 0.00	nd	nd	nd	nd
L. fermentum C1a	8.6 ± 1.55	8.4 ± 0.54	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. fermentum C1b	8.8 ± 1.57	8.2 ± 0.65	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. fermentum C1c	8.5 ± 0.81	8.3 ± 0.70	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei C1d	8.8 ± 1.53	8.0 ± 0.30	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei C1e	8.9 ± 1.89	8.2 ± 0.30	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. fermentum C1f	>7.0 ± 0.00	8.4 ± 1.20	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei C1g	>7.0 ± 0.00	8.5 ± 1.45	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00	>7.0 ± 0.00
L. paracasei C1h	8.8 ± 1.76	0.0 ± 0.00	nd	nd	nd	nd

nd: not determined; Log CFU g^{-1} > 7.0 indicated uncountable LAB.

Finally, all *Lactobacillus* strains were tested for bile salt tolerance and presented resistance. These results are closely related to those reported elsewhere for the resistance to bile salts after 4 h of exposure, where the strains showed little or no loss of viability (Maragkoudakis et al., 2006; Zoumpopoulou et al., 2008; Plessas et al., 2017). Considering that all tested strains managed to survive in the bile salts solution, some authors suggested a possible recovery of the initial levels of LAB during the passage through the small intestine (Zoumpopoulou et al., 2008). The high tolerance to bile salts observed for 11 of the 18 *Lactobacillus* strains evaluated was an important result, because it is necessary that LAB survive the action of bile during gastrointestinal transit to promote beneficial effects (Plessas et al., 2017).

Regarding haemolytic activity, the 11 *Lactobacillus* strains that passed the HGT survival tests showed γ-haemolysis, *i.e.*, they do not undergo haemolysis. The absence of haemolytic activity is considered a safety requirement for choosing a strain with probiotic potential (Zoumpopoulou et al., 2008), since it indicates that the strains do not produce virulence factors.

These 11 strains were also considered sensitive to the antibiotics assessed. It was reported in other studies that the LAB strains were resistant depending on the antibiotic (Costa et al., 2013; Giazzi et al., 2020), *e.g.*, some authors reported strains resistant to ceftazidime, oxacillin, streptomycin, and vancomycin (Costa et al., 2013), while others isolated resistant ones to streptomycin, with some of them resistant to gentamicin, tetracycline, ciprofloxacin, and erythromycin (Giazzi et al., 2020).

4. CONCLUSIONS

The study confirms that MS *caipira* artisanal cheese, made from raw milk, is a safe food product. The cheese samples exhibited characteristics similar to other artisanal cheeses, such as Canastra and Serro. Additionally, the study isolated, identified, and characterised 18 strains of *Lactobacillus* spp. from fresh, half-cured, and cured cheese samples. Of these, 11 strains survived the HGT *in vitro* resistance test and displayed γ-haemolysis, while no resistance was observed against antibiotics. Seven strains were identified as potential culture starters for this type of cheese, or other dairy foods, since MS *caipira* cheese is typically consumed cured. These strains were named *L. fermentum* C1a, C1b, C1c, and C1f, as well as *L. paracasei* C1d, C1e, and C1g.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Brazilian research funding agencies CNPq, CAPES and FUNDECT for the financial support.

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