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Artigo

In vitro assessment of the probiotic properties of lactic acid bacteria isolated from Minas artisanal cheese from the Campo das Vertentes region, Minas Gerais, Brazil, and preparation of fermented milk

Avaliação in vitro das propriedades probióticas de bactérias lácticas isoladas de queijo Minas artesanal da região do Campo das Vertentes, Minas Gerais, Brasil, e preparação de leite fermentado

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Abstract - The objective of this study was to evaluate the probiotic potential of lactic acid bacteria (LAB) isolated from Minas artisanal cheese produced in Campo das Vertentes, MG, and to select bacteria for the production of fermented milk. The following strains, including *Lactiplantibacillus plantarum* (LPL), *Levilactobacillus brevis* (LB), *Latilactobacillus curvatus* (LC), *Laticaseibacillus paracasei* (LPR), *Lactococcus garvieae* (LG), *Lactococcus lactis* (LL), *Leuconostoc mesenteroides* (LM001), and *Leuconostoc pseudomesenteroides* (LPS), were assessed for their tolerance to artificial gastric acid and bile salts, susceptibility to antimicrobials, and antagonistic properties. *Leuc. mesenteroides* (LM001) survived the simulation of gastrointestinal barriers, displayed susceptibility to ten of the 12 antimicrobials tested, and exhibited the ability to inhibit pathogenic microorganisms. Consequently, it was selected for production of fermented milk, which was submitted to physicochemical and microbiological analyses for up to 14 days of storage, yielding results in accordance with current Brazilian legislation. It is recommended that *in vivo* tests be conducted to assess the probiotic characteristics of *Leuc. mesenteroides* LM001 for its use in functional foods.

Index terms: functional foods; fermented milk; *Leuconostoc*; probiotic

Resumo - O objetivo deste estudo foi avaliar o potencial probiótico de bactérias láticas (BAL) isoladas de queijo Minas artesanal produzido em Campo das Vertentes, MG, e selecionar bactérias para produção de leite fermentado. As seguintes cepas, incluindo *Lactiplantibacillus plantarum* (LPL), *Levilactobacillus brevis* (LB), *Latilactobacillus curvatus* (LC), *Laticaseibacillus paracasei* (LPR), *Lactococcus garvieae* (LG), *Lactococcus lactis* (LL), *Leuconostoc mesenteroides* (LM001) e *Leuconostoc pseudomesenteroides* (LPS), foram avaliados quanto à tolerância ao ácido gástrico artificial e aos sais biliares, suscetibilidade a antimicrobianos e propriedades antagônicas. *Leuc. mesenteroides* (LM001) sobreviveu à simulação de barreiras gastrointestinais, apresentou suscetibilidade a dez dos 12 antimicrobianos testados e exibiu capacidade de inibir micro-organismos patogênicos. Consequentemente, foi selecionado para produção de leite fermentado, que foi submetido a análises físico-químicas e microbiológicas por até 14 dias de armazenamento, obtendo

resultados de acordo com a legislação brasileira vigente. Recomenda-se que sejam realizados testes *in vivo* para avaliar as características probióticas do *Leuc. mesenteroides* LM001 para seu uso em alimentos funcionais.

Palavras-chave: alimentos funcionais; leite fermentado; *Leuconostoc*; probióticos

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Introduction

Given the increasing interest of the consumers for healthy products, the demand for functional foods, such as those containing probiotics is growing. Probiotics are described as microorganisms that offer health benefits to those who consume them when provided in sufficient quantities (HILL et al. 2014). Benefits are attributed to probiotics, such as enhancing intestinal health, lowering total cholesterol levels, boosting the immune system, and reducing oxidative stress during vigorous physical exercise (SALOTTI-SOUZA et al. 2019).

For the selection of new probiotic strains, functional, technological and safety aspects must be evaluated, including the lack of pathogenicity of the strains and resistance to antibiotics (PARKER et al. 2018).

The majority of microorganisms with probiotic potential are lactic acid bacteria (LAB), which can be found in the microbiota of dairy products, including artisanal ones. These artisanal products often have diverse cultures depending on their regions of origin. Minas artisanal cheese is one of the most traditional dairy products sold in Brazil, produced from raw milk with the addition of endogenous yeast obtained from cheese whey, which is rich in LAB.

In Brazil, artisanal cheeses are produced in various regions and possess distinct flavor characteristics (FIRMO et

al. 2023). Considering the availability of raw materials, cheese production in the State of Minas Gerais represents the culture and history of the local population. It is the Brazilian federative unit with the greatest tradition in cheese manufacturing and a pioneer in the regulation of sanitary legislation for artisanal cheeses (CAMARGO et al. 2021; COSTA et al. 2022).

Thus, the present study aimed to evaluate the *in vitro* probiotic potential of LAB isolated from Minas artisanal cheeses produced in the Campo das Vertentes region, Minas Gerais, Brazil, and select LAB with functional food properties for the elaboration of fermented milk.

Material and methods

Characterization of lactic acid bacteria

The study evaluated the in vitro probiotic potential of eight LAB strains: *Lactiplantibacillus* (*Lp.*) *plantarum* (LPL) (formerly known as *Lactobacillus plantarum*), *Levilactobacillus* (*Lv.*) *brevis* (LB) (formerly known as *Lactobacillus brevis*), *Latilactobacillus* (*Lt.*) *curvatus* (LC) (formerly known as *Lactobacillus curvatus*), *Lacticaseibacillus* (*Lc.*) *paracasei* (LPR) (formerly known as *Lactobacillus paracasei*), *Lactococcus* (*Lact.*) *garvieae* (LG), *Lactococcus* (*Lact.*) *lactis* (LL), *Leuconostoc* (*Leuc*) *mesenteroides* (LM001) and *Leuconostoc* (*Leuc*) *pseudomesenteroides* (LPS). LAB were isolated from Minas artisanal cheeses produced in the Campo das Vertentes region and identified by the Matrix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF) mass spectrometry (SINGHAL et al. 2015). The bacteria were deposited at the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SISGEN (A56D9A9), in compliance with the legislation in force (Brazilian Federal Law number 13123 from May 20th, 2015). The isolated LAB were stored at -20°C in cryotubes with Man, Rogosa and Sharpe broth (MRS, Merck®, Darmstadt, Germany) with 20% sterilized glycerol (v/v) as a cryoprotector.

Tolerances of artificial gastric acid and bile salts

The *in vitro* tolerances of LAB to artificial gastric acid and bile salts were performed in duplicate, with three replications, using the adapted technique, described by Silva et al. (2013). The LAB were grown in MRS broth and then centrifuged (13000g for 5 min) to obtain the pellets, which were

washed with 0.9% saline solution three times. The pellets were transferred to tubes containing 1 mL of artificial gastric acid, consisting of 0.9% saline with pH 2.0 and 3 g/L pepsin and tubes with 0.9% saline and pH 7.0 (control). The tubes were incubated at 37ºC for 2 h and then centrifuged (13000g for 5 min) to remove gastric acid and saline. The supernatant was discarded, and the pellets were suspended in MRS broth. For cell viability analysis, 200 µL/well of control inoculum and samples treated with artificial gastric acid were placed in a 96-well microplate that incubated in a spectrophotometer (Microplate, Spectrophotometer System 47 SpectraMax 340 – Molecular Devices, Sunnyvale, California, USA) at 37°C. The absorbance was determined by reading the OD_{620NM} every one hour, during 24 h. The percentage of growth inhibition was calculated by the formula (1-SG/CT) x 100, in which SG and CT correspond to the area under the growth curve of the bacterium treated with artificial gastric acid and the control, respectively.

The bile salt tolerance was performed similarly to the former test, except that there was no previous incubation. The absorbance was determined by reading the OD_{620NM} at the same times of the former analysis and the percentage of growth inhibition was calculated by the formula (1-SB/CT) x 100, in which SB and CT correspond to the area under the growth curve of the bacterium treated with bile salts and the control, respectively. The interpretation of results was based on the percentage of inhibition, according to the criterion proposed by Acurcio et al. (2014). Samples were considered tolerant when they showed a percentage of inhibition lower than 40%, moderately tolerant from 40 to 80% and sensitive higher than 80%.

Antimicrobial susceptibility

The antimicrobial susceptibility was performed in duplicate, with three repetitions, according to the techniques adapted from Charteris et al. (1998) and the Clinical and Laboratory Standards Institute - CLSI (2017). The selected LAB were activated in MRS broth and incubated under aerobic conditions at 37ºC for 24 h. Subsequently, an aliquot of 10 µL was inoculated onto plates with MRS agar, which were incubated under aerobic conditions at 37ºC for 24 h. After incubation, colonies were transferred to tubes with 3.5 mL of 0.9% saline until the turbidity reached 0.5 on the McFarland scale (10^8 CFU/mL). Then, sterile swabs were immersed in the saline

and the liquid was distributed on the entire surface of 14 cm diameter Petri dishes containing MRS agar. Twelve disks containing antimicrobials (Oxoid®, Basingstoke, England) were distributed on the plates: amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin G (10 µg), sulfa/trimethoprim (25 µg), tetracycline (30 µg) and vancomycin (30 µg). These antimicrobials were selected due to their importance in animal health. The plates were incubated at 37°C for 48 h. Quality control of the disks was performed with *Escherichia coli* ATCC 25922. The diameters of the inhibition halos were measured using a Mitutoyo Digimatic Caliper® digital caliper (Mitutoyo Sul Americana Ltda., São Paulo, Brazil). The results were submitted to a qualitative classification as sensitive, moderately sensitive or resistant to antimicrobial drugs, according to the standard proposed by Charteris et al. (1998). The cutoff points for oxacillin were considered according to information reported by the CLSI (2017) for *Streptococcus pneumoniae*, as the classification for *Lactobacillus* was not found in the literature.

***In vitro* antagonism test**

The antagonism test of LAB against indicator microorganisms was performed using the spot-on-the-lawn technique, described by Tagg et al. (1976), in duplicate with three replications. The antagonism activity was verified against pathogenic bacteria (*Staphylococcus aureus* ATCC 33591, *Salmonella enterica* var. Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 15313 and *Escherichia coli* ATCC 25922) and a LAB from artisanal cheese produced in the Serra Geral region (*Lactobacillus rhamnosus* Q221). The pathogenic bacteria and the LAB strain were activated twice in BHI (Difco®, Detroit, Michigan, USA) and MRS broths, respectively, which were incubated at 37ºC for 24 h. The LAB selected as producers of antagonistic effect were growth twice in MRS broth, under aerobic conditions, at 37ºC for 24 h. Then, 5 µL of each broth were deposited in the center of Petri dishes with MRS agar to form the spots. The plates were incubated at 37ºC for 48 h under aerobiosis, and then 1 mL of chloroform was added to the plate lids to eliminate the producing bacteria and maintain only the antagonistic substances. The plates were kept inverted inside the laminar flow for 30

minutes. To complement the LAB inactivation, the plates were exposed to ultraviolet light for 30 minutes. Subsequently, 3.5 mL of the semi-solid BHI agar, containing 10 µL of the aforementioned pathogenic bacteria, were homogenized and poured onto the plates containing the spots. The plates were incubated at 37ºC for 48 h, in aerobiosis. Then, the diameter of inhibition halos around the spots were measured with a Mitutoyo Digimatic Caliper® digital. The results showed a non-parametric behavior and, therefore, the Kruskal-Wallis test was used to compare the means of inhibition halos.

Milk fermentation

To evaluate the fermentative capacity of the pre-selected LAB, it was inoculated in a 10 mL tube containing 10% heat-treated reconstituted skimmed milk. The tubes were then incubated at 37°C until clotting. The fermented milk with the selected LAB was prepared using skimmed milk powder (Molico, Nestlé®, Araçatuba, São Paulo, Brazil) from three different batches, consisting of three repetitions. The skimmed milk powder used was reconstituted at 10% in distilled water and was subjected to heat treatment at 110ºC for 10 min. Subsequently, the evaluation of the microbiological quality of the reconstituted milks was carried out in order to ensure the efficiency of the heat treatment. For this, counts of aerobic mesophilic microorganisms (CFU/mL), molds and yeasts, coliforms at 30/35ºC and at 45ºC (MPN/mL), *Staphylococcus* spp. and search of *Salmonella* spp. (APHA, 1992) were carried out. Each

container had 100 mL of fermented milk, which were destined for physicochemical analyses and LAB counts (IDF, 1997) at 1, 7 and 14 days of storage at 8-10ºC. To evaluate the physicochemical quality of the fermented milks, the following determinations were carried out in triplicate: titratable acidity , pH using a digital pH meter (Gehaka PG1800, São Paulo, Brazil) and contents of moisture by the drying oven method (Gehaka G4023D, São Paulo, Brazil) and balance (Shimadzu AY220, São Paulo, Brazil), proteins by the Micro-Kjedahl method and fat by the Gerber method (OFFICIAL METHODS OF ANALYSIS, 2019).

Statistical analyses

Graphpad Prism 7.0 software (Graphpad Software, San Diego,California, USA) was used for statistical analyses. All the results were submitted to the analysis of normality by

the Shapiro-Wilk test and variance by the Two-way ANOVA test. The comparison between the medians of the bacterial antagonism was performed using the Kruskall-Wällis test, since it was considered as non-parametric, while the means of the physical-chemical parameters were compared by the Tukey test, since they were parametric. All the comparisons were performed with a significance level of 5%.

Results and Discussion

A total of 25% (2/8) of the LAB were tolerant, 62.5% (5/8) moderately tolerant and only one (12.5%) sensitive to artificial gastric acid (Tabel 1). The percentages of inhibition of gastric acid varied from 27.70% (*Lt. curvatus*) to 81.96% (*Lact. lactis*). The percentage values of inhibition to artificial gastric acid presented by the studied LAB were satisfactory, since only *Lact. garvieae* showed sensitivity.

Tolerance to acidic environments shown by *Lactobacilli* occurs by the maintenance of intracellular pH homeostasis, cell membrane functionality and the upregulation of stress-response proteins (GUAN & LIU 2020).

Regarding resistance to bile salts, all LAB (100%) were tolerant (Table 1), with inhibition values ranging from - 10.47% (*Lv. brevis*) to 34.22% (*Lt. curvatus*). Bile salts can eliminate microorganisms by detergent action on the plasma membrane. However, *Lactobacilli* may adopt survival strategies, such as the presence of a bile salts hydrolase enzyme (BSH), which deconjugate bile salts and prevents their detergent action (VINDEROLA & REINHEIMER 2003). In addition, these microorganisms can carry out the active efflux of bile and changes in the architecture and composition of cell membranes.

According to Abriouel et al. (2017), resistance to antimicrobials that inhibit nucleic acid synthesis, such as trimethoprim and sulfonamides, has been reported as an intrinsic characteristic of LAB. According to the results, only *Leuc. pseudomesenteroides* showed resistance to this compound. The results found for sulfa/trimethoprim differ from those reported in the literature.

The four *Lactobacilli* analyzed were resistant to amikacin, ciprofloxacin, oxacycline, vancomycin and gentamicin. Amikacin resistance is generally intrinsic, meaning there is less likelihood of transfer to the pathogenic microbiota. Likewise, oxacycline and vancomycin are antimicrobials described

as providing intrinsic resistance to Lactobacilli (MAY-TORRUCO et al. 2020; SALOTTI-SOUZA et. al. 2019; VALENTE et al. 2019). As for ciprofloxacin, there is evidence that Lactobacilli present intrinsic resistance to this drug, due to characteristics related to the structure of the cell wall, membrane permeability and efflux mechanisms (ABRIOUEL et al. 2015).

Table 1. Inhibition percentages and classification regarding the level of tolerance to artificial gastric acid (pH 2.0) and bile salts of LAB strains isolated from Minas artisanal cheese produced in the region of Campo das Vertentes, Minas Gerais state, Brazil.

LAB	**Inhibition percentage (%)**	
	Artificial gastric acid (pH = 2.0)	Bile salts (0.3% Oxgall)
Lp. plantarum	69.77 (MT)	4.71 (T)
Lv. brevis	60.62 (MT)	-10.47 (T)
Lt. curvatus	27.70 (T)	34.22 (T)
Lc. paracasei	68.88 (MT)	22.87 (T)
Lact. garvieae	81.96 (S)	7.04 (T)
Lact. lactis	71.68 (MT)	5.70 (T)
Leuc. mesenteroides	29.91 (T)	28.50 (T)
Leuc. pseudomesenteroides	74.85 (MT)	18.66 (T)

Legend: Tolerant – T (<40%), moderately tolerant – (40-80%) and sensitive – S (>80%), according to the classification proposed by Acurcio *et al.* (2014).

Of the eight LAB tested, two strains showed resistance to only two antimicrobials: Lact, lactis resistant to amikacin and ciprofloxacin, and *Leuc. mesenteroides* resistant to amikacin and gentamine.

Lp. plantarum, *Lv. brevis*, *Lc. paracasei*, *Leuc. mesenteroides* and *Leuc. pseudomesenteroides* were able to form inhibition halos against the four pathogenic strains (*Staphylococcus aureus* ATCC 33591, *Listeria*

monocytogenes ATCC 15313, *Escherichia coli* ATCC 25922 and *Salmonella enterica* var. Typhimurium ATCC 14028) and the LAB strain (*Lactobacillus rhamnosus* Q221). All the studied LAB were able of forming inhibition halos against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*. These results are important because these bacteria are often associated with public health problems due to the occurrence of foodborne diseases.

However, *Lact. garvieae*, *Lact. lactis* and *Lt. curvatus* did not inhibit *Salmonella* Typhimurium ATCC 14028. Mahmoudi et al. (2021) demonstrated inhibitory activity of *Lt. curvatus* KMJC3 against several pathogens, including that pathogen. According to Haraguchi et al. (2019), the antagonistic activity of *Lact. lactis* against *S.* Typhimurium varied according to the strains, and several of them showed antagonistic effect against the pathogen. Until the present study was carried out, no data were found in the literature regarding the inhibitory activity of *Lact. garvieae* against *S.* Typhimurium.

The inhibition of other microorganisms by LAB is related to the production of lactic acid by the fermentation of carbohydrates carried out by these bacteria, which contributes to their survival in cheese and other dairy products. However, these microorganisms are also capable of producing other compounds with inhibitory activity, such as bacteriocins and H_2O_2 (VOIDAROU et al. 2020).

The studied LAB also did not inhibit *Lactobacillus rhamnosus* Q221. This suggests that LAB could act in harmony in the production of fermented milks and in the development of flavor, without self-inhibition.

The pre-selected BAL, according to the promising results in the in vitro evaluation of the probiotic potential, was the strain *Leuc. mesenteroides*. The results were satisfactory in all in vitro tests, in addition to *Leuc. mesenteroides* fermented the milk after 24 hours.

All analyzes for microbiological control after heat treatment of the three batches of reconstituted skimmed milk powder showed low counts (below the detection threshold) of aerobic mesophilic microorganisms, total coliforms at 35°C and coliforms at 45ºC, *Staphylococcus* spp., molds and yeasts and absence of *Salmonella* spp. Thus, all reconstituted skimmed milk powder met the requirements established by the

Brazilian legislation (BRAZIL 2007).

Fermented milks showed high moisture values, 91.83% (Time 1), 91.81% (Time 2) and 91.98% (Time 14).

The percentage results of the protein contents of the fermented milks at all storage times did not show significant interaction with the days of storage under refrigeration at 8-10ºC ($p>0.05$) and, therefore, did not vary significantly. At 1 and 7 days of storage, the fermented milks did not reach the reference values proposed by Brazilian legislation of at least 2.9 g of protein/100g (BRAZIL, 2007). However, after 14 days of storage, the fermented milk had a protein content of 3.33%, adapting to the proposed standards. In all fermented milks the percent of fat was 0% during all the storage time. This result was expected, since the powdered milk used in the elaboration of the products was skimmed.

The titratable acidity in fermented milk met the standards established by Brazilian legislation for fermented milk at all storage times, time 1: 1.08 ± 0.07; time 7: 1.16 ± 0.04 and time 14: 1.04 ± 0.14, remaining between the established values of 0.6 to 2.0 g of lactic acid/100g (BRAZIL, 2007). The pH values remained at 4.77 (Time 1), 4.70 (Time 7) and 4.76 (Time 14). Viegas et al. (2010) also found no difference in the decrease in pH between different fermented milks added with probiotic microorganisms during 40 days of storage ($p>0.05$) and the pH variations were not able to interfere with the viability of the potential probiotics. Likewise, the decrease in pH observed in fermented milks did not compromise the viability of *Leuc. mesenteroides*, as no significant difference was observed in total LAB counts during the entire storage period (Figure 1).

The inoculum of *Leuc. mesenteroides* LM001 used in the preparation of fermented milks had $1.3x10^9$ CFU/mL. Mean counts of *Leuc. mesenteroides* was always higher than 10^8 CFU/mL. The LAB maintained their viability up to 14 days of storage, reaching the minimum count of 10^6 CFU/mL during the entire shelf life. No significant difference ($p>0.05$) was observed between the mean values of three replicates.

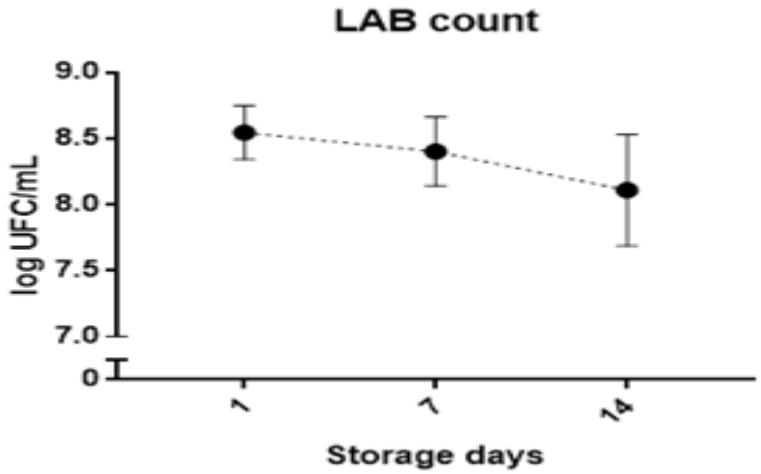

Figure 1. Means counts (*log* CFU/mL), from three replicates of *Leuc. mesenteroides* LM001 at 1, 7 and 14 days of storage of fermented milk under refrigeration at 8-10ºC.

Conclusion

LAB isolated from Minas artisanal cheeses produced in the Campo das Vertentes region, MG, presented promising results in the evaluation of probiotic potential *in vitro*, suggesting that these microorganisms can resist the natural challenges of the gastrointestinal tract. Among the screened bacteria, *Leuc. mesenteroides* showed probiotic potential and was capable of ferment milk. The fermented milk made with LAB indigenous Minas artisanal cheese from the region of Campo das Vertentes, MG, met the standards required by legislation and *Leuc. msesenteroides* LM001 remained viable for up to 14 days in fermented milk stored at a temperature from 8 to 10ºC. It is suggested to carry out *in vivo* tests to certify the probiotic characteristics of *Leuc. mesenteroides* LM001 to be used in the preparation of functional foods.

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References

ABRIOUEL H, KNAPP C W, GÁLVEZ A, BENOMAR N (2017) Antibiotic resistance profile of microbes from traditional fermented foods. In: Fermented Foods in Health and Disease Prevention. *Elsevier Science* 675–704. Doi: 10.1016/B978-0-12-802309-9.00029-7.

ABRIOUEL H, MUÑOZ M D C C, LERMA L L, MONTORO B P, BOCKELMANN W, PICHNER R, KABISCH J, CHO GS, FRANZ C M A P, GÁLVEZ A, BENOMAR N (2015) New insights in antibiotic

resistance of *Lactobacillus* species from fermented foods. *Food Res. Int.* 78 465-481. DOI: 10.1016/j.foodres.2015.09.016.

ACURCIO, L.B.; SOUZA, M. R.; NUNES, A. C.; OLIVEIRA, D. L. C.; SANDES, S. H. C, ALVIM, L.B. (2014) Isolation, enumeration, molecular identification and probiotic potential evaluation of lactic acid bacteria isolated from sheep milk. *Arq. Bras. Med. Vet. Zootec.* 66 940-948. DOI: 10.1590/1678-41625796.

(AOAC) ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis of the Association of Official Analytical Chemists**. 12. ed. Washington, 2019. 620p.

APHA - American Public Health Association (1992) Compedium of methods for the microbiological examination of foods. 3rd ed. Washington, DC.

BRAZIL, Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 46 de 23 de outubro de 2007 – Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. Diário Oficial da União, Brasília, 23 de outubro de 2007.

CAMARGO, A.C.; COSTA, E.A.; FUSIEGER, A.; FREITAS, R.; NERO, L.A.; CARVALHO, A.F. (2021) Microbiais shifts through the ripening of the "Entre Serras" Minas artisanal cheese monitored by high-throughput sequencing. *Food Res. Int.* 39 1-8. DOI: 10.1016/j.foodres.2020.109803.

CHARTERIS, A.; KELLY, P.M.; MORELLI, L.; COLLINS, J. K. (1998) Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *J. Food Prot.* 61 1636-1643. DOI: 10.4315/0362-028x-61.12.1636.

CLSI - Clinical and Laboratory Standards Institute (2017) Performance Standards for Antimicrobial Susceptibility Testing. 27ª ed. CLSI supplement M100. Wayne, PA.

COSTA J R, PEREIRA D A, PAULA I L, ABREU L R, PINTO S M, EDWARDS H G M, STEPHANI R, OLIVEIRA L F C (2022) The taste of a champion: Characterization of artisanal cheeses from the Minas Gerais region (Brazil) by Raman spectroscopy and microstructural analysis. *Journal of Food Composition and Analysis* 112 1-9. DOI: 10.1016/j.jfca.2022.104704.

FIRMO M J N, MENEZES L D M, SALES G A, CARVALHO A F, COSTA N M E P L, LEITE-JÚNIOR B R C, MARTINS M L (2023) Diagnosis of the microbiological quality of fiscal artisanal Minas cheese samples. *Food Control* 153 1-10. DOI: 10.1016/j.foodcont.2023.109887.

GUAN N, LIU L (2020) Microbial response to acid stress: mechanisms and applications. *Appl. Microbiol.* 104 51-56. DOI: 10.1007/s00253-019-10226-1.

HARAGUCHI Y, GOTO M, KUDA T, FUKUNAGA M, SHIKANO A, TAKAHASHI H, KIMURA B (2019) Inhibitory effect of *Lactobacillus plantarum* Tennozu-SU2 and *Lactococcus lactis* subsp. *lactis* BF1 on *Salmonella* Typhimurium and *Listeria monocytogenes* during and post fermentation of soymilk. *LWT-Food Sci Technol.* 102 379–384. DOI: 10.1016/j.lwt.2018.12.042.

HILL, C.; GUARNER, F.; REID, G.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.;

FLINT, H.J.; SALMINEN, S.; CALDER, P.C.; SANDERS, M.E. (2014) The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Consens statements* 11 506-514. https:// doi. org/ 10. 1038/ nrgas tro IDF - International Dairy Federation (1997) Yogurt: Enumeration of characteristic microorganism's colony count technique at 37º. Bulletin of the International Dairy Federation, n.117B, p.1 – 4.

MAHMOUDI, M.; KHOMEIRI, M.; SAEIDI, M.; DAVOODI, H. (2021) *Lactobacillus* species from iranian jug cheese: identification and selection of probiotic based on safety and functional properties. *Appl. Food Biotechol.* 8 47-56. DOI: 10.22037/afb. v8i1.29253.

MAY-TORRUCO, A.L.; CORONA-CRUZ, A.; JIMÉNEZ, A.L.L.; CORTÉZ, N.G.; VERA, R.J. (2020) Sensibilidad y resistencia a antibióticos de cepas probióticas empleadas en productos comerciales. *Eur. Sci. J.* 16 43. DOI: 10.19044/esj. 2020.v16n18p43.

OFFICIAL METHODS OF ANALYSIS (2019) Official Method 905.02-1973. International 21th Ed., Gaithersburg, MD, USA.

PARKER, E.A.; ROY, T.; D'ADAMO, C. R.; WIELAND, L.S. (2018) Probiotics and gastrointestinal conditions: an overiview of evidence from the Cochrane collaboration. *Nutr. Rev.* 45 125, 134. DOI: 10.1016/j.nut.2017.06.024.

SALOTTI-SOUZA, B.M.; BORGONOVI, T.F.; CASAROTTI, S.N.; TODOROV, S. D.; PENNA, A.L.B. (2019) *Lactobacillus casei* and *Lactobacillus fermentum* strains isolated from mozzarella cheese: probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. *Probiotics and Antimicrobial Proteins* 11 382-396. DOI: 10.007/s12602-018-9406-Y.

SILVA, B.C.; JUNG, L.R.C.; SANDES, S. H.C.; ALVIM, L.B.; BOMFIM, M.R.Q.; NEUMANN, E.; NUNES, A.C. (2013) *In vitro* assessment of functional properties of lactic acid bacteria isolated from faecal microbiota of healthy dogs for potential use as probiotics. *Benef. Microbes* 4 267-275. DOI: 10.3920/BM2012.0048.

SINGHAL, N.; KUMAR, M.; KANAUJIA, P. K.; VIRDI, J.S. (2015) MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front. Microbial* 6 1-16. DOI: 10.3389/fmicb.2015.00791.

TAGG, J.R.; DAJANI, A..S.; WANNAMAKER, L.W. (1976) Bacteriocins of Gram-positive bacteria. *Bacteriol. Rev.* 40 722-756. DOI: 10.1128/br.40.3.722-756.1976.

VALENTE, G.L.C.; ACÚRCIO, L.B.; FREITAS, L.P.V.; NICOLI, J.R.; SILVA, A. M.; SOUZA, M. R.; PENNA, C.F.A.M. (2019) Short communication: *In vitro* and *in vivo* probiotic potential of *Lactobacillus plantarum* B7 and *Lactobacillus rhamnosus* D1 isolated from Minas artisanal cheese. *Int. J. Dairy Sci.* 102 5957-5861. DOI: 10.3168/jds.2018-15938.

VIEGAS, R.P.;SOUZA, M.R.; FIGUEIREDO, T.C.; RESENDE, M. F. S.; PENNA, C. F. A. M.; CERQUEIRA, M. M O P (2010) Qualidade de leites fermentados funcionais elaborados a partir de bactérias ácido-lácticas isoladas de queijo coalho. *Arq. Bras. Med. Vet. Zootec.* 62. DOI: 10.1590/s0102-09352010000200028.

VINDEROLA, C.G.; REINHEIMER, J.A. (2003) Lactic acid starter and probiotic bacteria: a comparative *in vitro* study of

probiotic characteristics and biological barrier resistance. *Food Res. Int.* 36 895–904. DOI: 10.1016/S0963-9969(03)00098.

VOIDAROU, C.; ALEXOUPOULOS, A.; TSINAS, A.; ROZOS, G.; TZORA, A.; SKOUFOS, I.; VARZAKAS, T.; BERZITZOGLOU, E. (2020) Effectiveness of bacteriocin-producing lactic acid bacteria and Bifidobacterium isolated from honeycombs against spoilage microorganisms and pathogens isolated from fruits and vegetables. *Appl. Sci.* 10. DOI: 10.3390/app10207309.