

Article

The Bacteriocin-like Inhibitory Substance Producing *Lactacaseibacillus paracasei* LPa 12/1 from Raw Goat Milk, a Potential Additive in Dairy Products

Andrea Lauková ^{1,*} , Emília Dvorožňáková ² , Miroslava Vargová ², Jana Ščerbová ¹ , Valentína Focková ¹ , Iveta Plachá ¹  and Monika Pogány Simonová ¹ 

¹ Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia; scerbova@saske.sk (J.Š.); fockova@saske.sk (V.F.); placha@saske.sk (I.P.); simonova@saske.sk (M.P.S.)

² Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovakia; dvoroz@saske.sk (E.D.); vargovam@saske.sk (M.V.)

* Correspondence: laukova@saske.sk; Tel.: +42-19-0586-1083

Featured Application: Based on the safety testing results of the LPa 12/1 strain using Balb/c mice, the bacteriocin-like inhibitory substance-producing *Lactacaseibacillus paracasei* LPa 12/1 was deemed safe. The encapsulation of the LPa 12/1 strain seems suitable due to its potential application in dairy products. The LPa 12/1 strain has survived in higher counts in ewe-goat milk yoghurt than in cow milk yoghurt. However, sufficient amounts of the LPa 12/1 strain were counted in both types of yoghurts. The pH of yoghurts was not negatively influenced.



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Abstract: Goat milk is gradually becoming the preferred milk by consumers worldwide, including Slovakia. It is also demanded as a functional and/or nutraceutical drink as it is rich in bioactive components. However, another new development is the research into the beneficial autochthonous strains used to enrich goat dairy products. Among individual species, representatives in raw goat milk are also involved in lacticaseibacilli. Bacteriocin-like inhibitory substance-producing *Lactacaseibacillus paracasei* LPa 12/1 was isolated from raw goat milk. This study focuses on its potential as a new additive in dairy products. No mortality was found when checking the safety of the LPa 12/1 strain using Balb/c mice. The strain reached higher counts in ewe-goat milk yoghurt (up to 6.1 cfu/g log 10) than in cow milk yoghurt (almost 5.0 cfu/g log 10). Cow milk yoghurts remained in more stable consistency after LPa 12/1 supplementation compared with ewe-goat milk yoghurts, although specific organoleptic tests were not performed. However, LPa 12/1 has survived sufficiently in both types of yoghurts. This bacteriocin-like inhibitory substance-producing strain LPa 12/1 in its encapsulated form applied in yoghurts seems suitable to supplement dairy products.

Keywords: postbiotic; enrichment; yoghurt; supplement; functional food

1. Introduction

Goat milk has multiple functions [1]. It is, apparently, the preferred milk by consumers worldwide [1,2], including in Slovakia [3]. Goat milk is attractive to consumers [2,4] because of its content components. It is also demanded as a functional and/or nutraceutical drink as it is rich in bioactive components [5]. Generally, milk contains beneficial microbiota crucial for developing flavour, taste, texture, and technological and health-related perceptions. However, contaminant bacteria can also introduce risks associated with raw milk and raw-derived product consumption [5,6].

As previously reported [5], using a next-generation sequencing method, useful bacteria of the phylum Firmicutes occurred in tested Slovak raw goat milk with an abundance of 20.5% [5]. The phylum Actinobacteria reached an abundance of 62.8% [5]. Although

Firmicutes was not the dominating phylum in tested raw goat milk, the genera involved in the phylum Firmicutes were detected there [5]. Moreover, individual representatives of the detected genera were isolated from raw goat milk using the standard microbiological method [7]. Lactic acid-producing lactococci, lactobacilli, lactocaseibacilli, and others, such as enterococci, are present in raw goat milk [3,7]. Among lactococci, the species *Lactococcus lactis* was primarily identified [7]. Lactobacilli have been mostly represented by the species *Lactocaseibacillus paracasei* or *Lactiplantibacillus plantarum* [7,8]. When those species strains produce bacteriocins (antimicrobial proteinaceous substances) and have additional beneficial properties such as low bile and pH tolerance, sufficient adhesive ability, lactic acid production, diacetyl, hydrogen peroxide, or useful enzyme production; moreover, they can play an important role in the food fermentation process, biopreservation, and/or contamination prevention [9]. In particular, bacteriocin production exerted by some of the above-mentioned species strains can be effective against food-borne pathogens. Therefore, this study has been focused on the bacteriocin-like inhibitory substance-producing beneficial strain *Lactocaseibacillus paracasei* LPa 12/1 isolated from raw goat milk [7]; its safety, stability, and survival in yoghurts to determine its potential use as an additive in dairy products. Besides the above-mentioned bacteriocin-like inhibitory substance activity of LPa 12/1, this strain is hemolysis negative (α -hemolysis); it sufficiently tolerates oxgall/bile. The strain LPa 12/1 showed low-grade biofilm formation and produced 10 nmol of useful enzyme β -galactosidase [7]. As reported in our previous study [7], its taxonomical species allocation was performed based on MALDI-TOF mass spectrometry identification score evaluation (2.004).

2. Materials and Methods

2.1. Safety Control of LPa 12/1 Strain Using Balb/c Mice

The strain LPa 12/1 (by a rifampicin-labelled variant of LPa 12/1 strain to differentiate it from the other lactic acid bacteria/lactobacilli) was prepared as previously described by Stropfová et al. [10]. It was grown in an MRS broth (pH 6.2, Merck, Darmstadt, Germany) overnight at 37 °C in an incubator within a partial CO₂ atmosphere to increase the absorbance (A_{600}) to 1.0. After centrifuging (10,000 × g) for 30 min, the cells were resuspended in the Ringer solution (Merck, Germany) and diluted to a final concentration of 10⁹ cfu/mL. The cell count was checked after dilutions were spread on MRS agar with rifampicin (Merck) and incubated at 37 °C for 24–48 h.

The experimental design was performed as previously described by Dvorožňáková et al. [11]. The animal study protocol was conducted in accordance with current European and Slovak national legislative requirements for animal use, optimality of animal use, and cruelty of the procedures. The pathogen-free male Balb/c mice aged 8 weeks (VELAZ, Prague, Czech Republic; $n = 27$) were used, weighing 18–20 g. The animals were acclimatized for 15 days after the stress of transport, and when they did not show signs of illness, they were used in the experiment. Experiments with laboratory mice were carried out in the accredited vivarium at the Institute of Parasitology of the Slovak Academy of Sciences in Košice (Slovakia). The animals were handled in accordance with the “Requirements for the handling of animals” specified in the Slovak Law no. 377/2012 on veterinary care. Animals were kept under a 12 h light–dark regime at room temperature (22–24 °C). Humidity was 56%. They were on a commercial diet and given water. Mice were divided into the control group (CG, $n = 12$) and the experimental group (EG, $n = 15$). The experiment lasted 45 days. It was approved by the Animal Care Ethics Committee at the Institute of Parasitology SAS (No. EK-PaU-2/2019, 2nd of April 2019) and the State Veterinary and Food Administration of the Slovak Republic (No. Ro-1604/19-221/3, 13th of June 2019). The strain LPa 12/1 (100 μ L) was administered to mice per os daily at a dose of 10⁹ cfu/mL for 30 days. Sampling was provided at time 0/1, i.e., before application, on day 30 (after strain application) and at the end (on day 45, i.e., 15 days after the strain cessation). Faecal, jejunal, and liver samples were checked for selected microbiota. On day 0/1, ($n = 8$) the faecal mixture sample was checked. The strain LPa 12/1 was counted on an

MRS agar (Merck, Darmstadt, Germany) with rifampicin (100 µg/mL). LAB was counted on MRS agar (Merck), (amylolytic) streptococci were counted on M17 agar (Difco, Sparks, MD, USA), and coliforms on Mac Conkey agar (Difco) according to ISO (International Organization for Standardization). Bacterial counts were expressed in colony-forming units per mL/gram (cfu/mL and/or g). Based on this type of experiment, phagocytic activity in the blood (PA) was checked using a modified method described by Větrovická et al. [12]. The percentage of phagocytic cells was evaluated using an optical microscope by counting PMN (polymorphonuclear cells) up to 100.

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post test. The results are quoted as means $\pm$ SD; the level of significance is set at $p < 0.05$.

2.2. LPa 12/1 Strain Encapsulation Process (Freeze Drying)

Freeze drying is the simplest form of encapsulation [13]. For this process, the strain LPa 12/1 (via a rifampicin-labelled variant) was grown in 300 mL of MRS broth (pH 6.2 Merck, Germany) overnight at 37 °C in an incubator with partial CO₂ atmosphere to reach an A₆₀₀ of 1.0. The appropriate broth culture of LPa 12/1 strain was mixed with skim milk (Simandl company, Petrovice u Karviné, Czech Republic, in small flasks in a ratio of 1:1). Flasks were placed to freeze at −80 °C. Then, they were processed for the freeze-drying process using a Micro Modulyo 230 freeze dryer (Thermo-electron corporation, Asheville, NC 28804, USA). The powder was weighed after sufficient freeze drying. The cell count was checked as it was formerly described; part of the frozen dried product was diluted in the Ringer solution, and dilutions were spread on an MRS agar enriched with rifampicin (Merck) to check the LPa 12/1 strain count. Plates were incubated at 37 °C for 24–48 h.

2.3. Surviving and Stability of Encapsulated Strain LPa 12/1 in Ewe-Goat Milk Yoghurt

Fresh ewe (75%)–goat (25%) milk white yoghurts (145 g) used in the application experiment were bought from the commercial market network. According to the product label, they contain commercial yoghurt culture; energy 309 KJ/74 kcal, fat 4.6 g of which saturated fatty acids participated with 3.6 g, carbohydrates were involved in 4.1 g, sugar value of which was 3.4 g, proteins content formed 4.1 g, and salt 0.07 g. The encapsulated strain was checked for cell count before application, and it was applied (0.5 g) in the experimental yoghurt-E. The LPa 12/1 strain was absent in control yoghurt-C. Before LPa 12/1 strain application, yoghurt samples were diluted in peptone water and controlled for possible contamination by spreading dilutions on Mac Conkey agar (Difco) to control Enterobacteriaceae. Yoghurts had no traces of Enterobacteriaceae. LAB count was checked on MRS agar (Merck), and the control was provided on the M17 agar (Difco) from the commercial culture *Streptococcus* spp. (both counts reached 5.1 cfu/g). Then, yoghurt sampling was performed after 24 h, on days 7, 10, and 14 (the declared expiration time for this type of yoghurt). The cells of the applied LPa 12/1 strain were selected on an MRS agar enriched with rifampicin (100 µg/mL). The LAB count was determined on the MRS agar. Amylolytic streptococci were checked using M17 agar (Difco). The commercial culture contained amylolytic *Streptococcus* spp., and the dairy streptococci are amylolytic. It was an indication to check for amylolytic streptococci. Yoghurts (one g) were sampled and mixed (Stomacher-Masticator, IUL, Barcelona, Spain) with peptone water (Merck, ratio 1:9). Then, they were diluted and spread on the selected cultivation media (ISO) as formerly indicated. Moreover, pH was measured using a Checker-pH tester (Hanna instruments Inc., Woonsocket, RI, USA). The initial pH was 3.94. Yoghurts were maintained in the fridge during the whole testing period.

2.4. Surviving and Stability of Encapsulated Strain LPa 12/1 in Cow Milk Yoghurt

Fresh cow milk yoghurts (145 g) were supplied from a commercial market network. Component content involving commercial culture was declared for 100 g of product: energy 500 KJ and fat 9.0 g, of which saturated fatty acids formed 5.2 g. The carbohydrate content

was 4.5 g, the sugar value of which was 4.1 g. Protein content was 3.4 g, and salt was 0.1 g. The schedule processing, sampling, and controlling were the same as ewe–goat yoghurt. Again, 0.5 g of LPa 12/1 strain (via a rifampicin-labelled variant) in its encapsulated form was applied in the experimental yoghurt-E. Control-C yoghurt was not enriched with the strain.

2.5. Bacteriocin-Like Inhibitory Substance Preparing and Inhibitory Activity Testing

Firstly, bacteriocin activity was checked using the qualitative method as previously described by Lauková et al. [14]. The principal indicator strain *Enterococcus avium* EA5 (the most sensitive faecal strain isolated from piglets from our collection) was used in the test [14]. A partially purified bacteriocin substance (precipitate) was prepared according to the following protocol: LPa 12/1 strain (0.1% inoculum) was grown overnight in 60 mL of MRS broth (Merck, Germany) at 37 °C. Then, it was centrifuged at $10,000 \times g$ for 30 min. The cell-free supernatant (pH 4.5) was treated with Chelaton-EDTA III (Merck) and heated at 80 °C for 10 min to eliminate the other organic substance effects. Then, the supernatant was filtrated using a 0.22 µm filter (Millipore corporation, Bedford, MA, USA). The filtrate was precipitated with ammonium sulphate (70% saturation) overnight at 4 °C. After precipitation, it was centrifuged ($10,000 \times g$) for 30 min. The precipitate was resuspended in a minimal volume of phosphate buffer (5 mL, pH 5.0). Its inhibitory activity was checked against the indicator *E. avium* EA5 and expressed in arbitrary units per ml (AU/mL). In other words, this is the highest dilution of bacteriocin-like inhibitory substance (precipitate) that caused the growth inhibition of indicator strain. In addition, the following indicator bacteria were used: 10 vancomycin-resistant strains of *Enterococcus faecium* (VRE2, VRE3, VRE5, VRE 9, VRE 10, VRE 11, VRE 12, VRE 13, VRE15, VRE16, kindly provided by Dr. Bírošová from Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Slovakia), 11 faecal strains of *E. faecalis* from dogs (EE211-EE221), and 5 faecal strains of *Staphylococcus chromogenes* from cows (kindly provided from Dr. Troscianczyk Aleksandra, University of Life Sciences in Lublin, Faculty of Veterinary Medicine, Poland). Moreover, *Listeria monocytogenes* LM 7223 (Veterinary Institute, Olomouc, Czech Republic) was used as an indicator (as a frequent contaminant in milk). Altogether, 28 indicator bacteria were tested.

The enzymes such as α-chymotrypsin, trypsin, proteinase K and pepsin (Merck) were used to treat precipitate. The inhibitory activity of the precipitate was checked again against the EA5 strain.

The precipitate was also tested for its thermostability. It was exposed for 1 h at 60 °C and at −20 °C storage for 2 weeks. The remaining activity was tested against the strains EA5 and LM 7223 and expressed as formerly mentioned.

3. Results

3.1. Safety Control of LPa 12/1 Strain

No mortality was noted in mice during the safety control experiment. On day 30, the count of LPa 12/1 strain in faeces reached 3.64 ± 0.56 cfu/g (log 10). This colonization was sufficient. On day 45 (15 days of LPa 12/1 strain cessation), a decrease in LPa12/1 strain was noted ($p < 0.01$). The LPa 12/1 strain contributed to the total LAB increase in faeces on day 30 (difference 0.59 log cycle) and day 45 ($^{ab} p < 0.001$) compared with day 0/1. Faecal (amylolytic) streptococci were present in high counts (Table 1). The coliform count was significantly reduced in the experimental group on day 30 (30EG) compared with the experimental group on day 45—45EG ($^{ab} p < 0.05$).

Table 1. The LPa 12/1 counts and controlled selected microbiota in faeces of Balb/c mice after strain application and its cessation.

Faeces	LPa 12/1	LAB	Amyl. Str.	Coliforms
Sampling 0/1 (<i>n</i> = 8)	nt	5.48 ± 0.52 ^a	5.49 ± 0.07	3.27 ± 0.49
Sampling 30EG (<i>n</i> = 5)	3.64 ± 0.56 ^a	6.07 ± 0.07 ^d	5.16 ± 0.30 ^a	4.66 ± 0.59 ^a
Sampling 30CG	nt	6.47 ± 0.51	5.04 ± 0.47	5.08 ± 0.42
Sampling 45EG	2.62 ± 0.61 ^b	7.04 ± 0.15 ^b	5.59 ± 0.04	3.76 ± 0.44 ^b
Sampling 45CG	nt	7.1 ± 0.0 ^c	6.05 ± 0.18 ^b	2.28 ± 0.33

Sampling 0/1, sampling before LPa 12/1 strain application; sampling 30EG and 30CG, sampling at day 30 in the experimental and control groups; Sampling 45EG and 45CG, sampling at day 45 in the experimental and control groups; LPa 12/1, ^{ab} *p* < 0.01; LAB-lactic acid bacteria, ^{ab,ac} *p* < 0.001, ^{db} *p* < 0.01; amylolytic streptococci, ^{ab} *p* < 0.05; coliforms, ^{ab} *p* < 0.05; day 30—30 day application of LPa 12/1, day 45, 15 days of LPa 12/1 cessation; nt—not tested.

In the jejunum, the LPa 12/1 strain count was lower than in the faeces (Table 2). It reached up to 10² cfu/g on day 30. On day 45, it reduced by almost up to 10¹ cfu/g (Table 2). The total LAB count in the jejunum was high and not reduced, as well as the counts of streptococci (Table 2). The coliforms were detected in the jejunum in counts commonly determined in animal jejunum; however, they were slightly mathematically reduced, with a difference of 0.74 log cycles.

Table 2. The LPa 12/1 counts and controlled selected microbiota in jejunum of Balb/c mice after strain application and its cessation.

<i>n</i> = 5	LPa 12/1	LAB	Amyl. Str.	Coliforms
Sampling 30EG	1.70 ± 1.30	6.03 ± 0.07	4.54 ± 0.11	5.33 ± 0.53
Sampling 30CG	nt	6.04 ± 0.07	4.15 ± 0.52	6.07 ± 0.06
Sampling 45EG	0.72 ± 0.08	6.04 ± 0.10	5.51 ± 0.3	5.96 ± 0.50
Sampling 45CG	nt	6.47 ± 0.58	5.13 ± 0.21	5.71 ± 0.33

Sampling 0/1, sampling before LPa 12/1 strain application; Sampling 30EG and 30CG, sampling at day 30 in the experimental and control groups; Sampling 45EG and 45CG, sampling at day 45 in the experimental and control groups; coliforms, on day 30 (mathematical difference 0.74 log cycle); day 30—30 days application of LPa 12/1, day 45, 15 days of LPa 12/1 cessation, nt—not tested; LAB—lactic acid bacteria, amylolytic streptococci, coliforms—coliform bacteria.

In the liver, LPa 12/1 reached 1.30 ± 0.0 cfu/g (log 10). On day 45, its count decreased (less than 0.1 cfu/g). The LAB count reached 4.23 ± 0.5 cfu/g, which means the strain probably contributed to the total LAB count because a decrease was noted on day 45 (2.42 ± 0.55 cfu/g Table 3). Streptococci were counted up to 10² cfu/g, and they were mathematically reduced with a difference of 0.58 log cycles comparing day 30 and day 45. In the liver, the counts of coliforms were low and not influenced.

Table 3. The LPa 12/1 counts and controlled selected microbiota in liver (tissue) of Balb/c mice after strain application and its cessation.

<i>n</i> = 5	LPa 12/1	LAB	Amyl. Str.	Coliforms
Sampling 30EG	1.30 ± 0.0	4.23 ± 0.5	1.98 ± 0.40	2.93 ± 0.40
Sampling 30CG	nt	4.25 ± 0.06	2.51 ± 0.58	1.98 ± 0.41
Sampling 45EG	<0.1	2.42 ± 0.55	1.30 ± 0.14	1.7 ± 0.30
Sampling 45CG	nt	2.58 ± 1.60	2.85 ± 0.65	0.95 ± 0.0

Sampling 0/1, before experiment, Sampling 30EG and 30CG, sampling at day 30 in the experimental. and control groups; Sampling 45EG and 45CG, sampling at day 45 in the experimental and control groups; amylolytic streptococci were mathematically decreased in the group 30EG on day 30 compared with day 45 (difference 0.58 log cycle); day 30—30 days of LPa12/1 strain application, day 45, 15 days of LPa. 12/1 cessation; nt—not tested.

On day 30, the PA value was higher in mice of EG (61.0 ± 1.05) than in CG (60.0 ± 1.30). The same situation was seen in the case of IPA (index of phagocytic activity) (Table 4). On day 45, an even higher value of PA was measured in EG than on days 30 and 45 in CG ($62.0 \pm 2.37\%$ to $61.0 \pm 0.84\%$), and the same situation was seen in the IPA value. On day 30, higher PA was noted in EG than on day 45 ($^{ab} p < 0.001$), which was anticipated.

Table 4. Phagocytic activity (PA) and index of PA (IPA) after LPa 12/1 strain application and cessation in blood of Balb/c mice in percentage (%).

<i>n</i> = 6	PA	IPA
Sampling 30EG	61.5 ± 1.05^a	2.53 ± 0.21
Sampling 30CG	60.0 ± 1.30	2.82 ± 0.11
Sampling 45EG	62.0 ± 2.37^b	2.93 ± 0.10
Sampling 45CG	61.0 ± 0.84	2.82 ± 0.11

Sampling 0/1, before experiment; Sampling 30EG and 30CG, sampling on day 30 in the experimental and control groups; Sampling 45EG and 45CG, sampling on day 45 in the experimental and control groups; experiment, day 30—application of LPa 12/1, day 45, 15 days of LPa 12/1. Significantly higher PA was noted in EG on day 30 compared with day 45 ($^{ab} p < 0.001$).

3.2. Stability and Surviving of LPa 12/1 Strain in Ewe–Goat Milk Yoghurts

The count of the LPa 12/1 strain in its freeze-dried form reached 6.6×10^7 cfu/g, i.e., 7.82 cfu/g in log 10. After application, the strain LPa 12/1 showed sufficient stability and survival in the ewe–goat milk yoghurt. Its count reached 5.1 cfu/g (log 10) (Table 5) after 24 h. On day 7 (one week), the LPa 12/1 strain count was almost the same (5.15 cfu/g (log 10)). However, on day 10, its increase of almost about one log cycle (0.95 log cycle) was noted (6.1 cfu/g, log 10). It was also stable on day 14. The LPa 12/1 strain contributed to the total LAB count on days 7, 10, and 14. On those days, the LAB count was higher and stable. LAB counts were also higher in E yoghurts than their counts in control (C-yoghurts). A difference of 0.46 log cycle was noted when checking after 24 h compared with LAB counts in E and C yoghurts. On day 7, a difference of 1.56 log cycles was found; then, the difference of 1.15 and 1.0 log cycles was noted when comparing E and C yoghurts, respectively. The commercial culture count was seemingly not influenced by the LPa 12/1 strain addition from the start of the experiment. On day 10, the LPa12/1 count was increased by almost 1 log cycle (Table 5). On day 14, the LPa 12/1 strain count decreased to the initial level. As mentioned, the initial pH of yoghurt reached the value of 3.94. It was almost the same in both E and C yoghurts after 24 h (Table 5). On day 7, the pH was increased in both experimental and control yoghurts; in E yoghurt, the pH reached 4.55. The pH was 4.89 in control-C yoghurt. On day 10, the pH value was stable in C yoghurt. However, in E yoghurt, a decrease in pH was noted. Finally, on day 14, in both yoghurts, almost the same pH was measured (4.0 and 4.05). It was not negatively influenced by the LPa 12/1 strain addition.

Table 5. Stability and survival of LPa 12/1 strain in ewe–goat milk yoghurts (expressed in cfu per g (cfu/g, log 10) $\pm$ SD).

	pH	LPa12/1	LAB	Amyl. Str.
E/24 h	3.90 ± 0.0	5.1 ± 0.0	5.1 ± 0.0	5.1 ± 0.0
C/24 h	3.91 ± 0.0	nt	4.61 ± 0.2	4.41 ± 0.1
E/Day 7	4.55 ± 0.2	5.15 ± 0.1	6.1 ± 0.2	5.1 ± 0.0
C/Day 7	4.89 ± 0.1	nt	4.54 ± 0.5	5.30 ± 0.1
E/Day 10	3.70 ± 0.1	6.1 ± 0.1	6.1 ± 0.3	6.1 ± 0.0
C/Day 10	4.85 ± 0.2	nt	4.95 ± 0.1	6.1 ± 0.0

Table 5. *Cont.*

	pH	LPa12/1	LAB	Amyl. Str.
E/Day 14	4.0 ± 0.2	6.1 ± 0.1	6.1 ± 0.2	5.1 ± 0.0
C/Day 14	4.05 ± 0.20	nt	5.1 ± 0.0	5.1 ± 0.0

LAB—lactic acid bacteria, amylolytic streptococci; nt—not tested; E—experimental yoghurts, C—control yoghurts. It was double tested.

3.3. Stability and Surviving of LPa 12/1 Strain in Cow Milk Yoghurts

In cow milk yoghurt, the LPa 12/1 strain count reached 2.1 cfu/g (log 10, Table 6) after 24 h. Its count in encapsulated form was 6.5×10^7 cfu/g (7.81 cfu/g, log 10). A slight increase in the LPa 12/1 count was noted (2.36 cfu/g) on day 7; it was lower (mathematical difference 2.79 log cycles) than the values observed in ewe–goat milk yoghurt at the same time. An increase in the LPa 12/1 strain was also noted on day 10 (4.78 cfu/g), and it was almost stable on day 14 (4.72 cfu/g). However, that count was lower (difference of 1.32 and 1.38 log cycles) than for ewe–goat milk yoghurt. Interestingly, the LAB counts in cow milk yoghurts and ewe–goat milk yoghurts were almost the same. The total streptococci count was lower in cow milk yoghurts than ewe–goat milk yoghurts. However, the streptococcal count was increased from day 7 (Table 6). The pH value in cow milk yoghurts was slightly lower than in ewe–goat milk yoghurts, and it was not negatively influenced.

Table 6. Stability and survival of LPa 12/1 strain in cow goat milk yoghurts (expressed in cfu per g (cfu/g, log 10) ± SD.

	pH	LPa 12/1	LAB	Amyl. Str.
E/24 h	3.59 ± 0.1	2.1 ± 0.0	4.76 ± 0.3	3.69 ± 0.2
C/24 h	nt	nt	nt	nt
E/Day 7	3.70 ± 0.0	2.36 ± 0.2	6.1 ± 0.1	2.56 ± 0.1
C/Day 7	3.77 ± 0.2	nt	4.54 ± 0.1	2.75 ± 0.1
E/Day 10	3.80 ± 0.1	4.78 ± 0.3	6.1 ± 0.0	5.1 ± 0.0
C/Day 10	3.90 ± 0.3	nt	4.95 ± 0.1	5.1 ± 0.0
E/Day 14	3.60 ± 0.1	4.72 ± 0.1	5.1 ± 0.0	4.92 ± 0.2
C/Day 14	3.60 ± 0.1	nt	4.65 ± 0.2	4.70 ± 0.1

LAB—lactic acid bacteria, amylolytic streptococci; nt—not tested; E—experimental yoghurt, C—control yoghurt. It was double tested.

3.4. Bacteriocin Activity and Stability

Using the qualitative method, the inhibitory zone size due to the bacteriocin-like inhibitory substance (BLIS) produced by the LPa 12/1 strain measured 30 mm on average. The inhibitory activity of BLIS (precipitate) LPa 12/1 was indicated against 13 out of 28 indicator strains, reaching 100 AU/mL. The growth of the EA5 indicator had an inhibitory activity of 100 AU/mL. Vancomycin-resistant *E. faecium* strains of food-origin VRE9, VRE3, VRE 2, VRE10, and VRE12 were inhibited with an activity of 100 AU/mL. Moreover, *E. faecalis* strains EE211, EE215, EE220, and EE221 were inhibited using BLIS LPa 12/1 (100 AU/mL). Three out of five *Staph. chromogenes* strains (Sch141, Sch143, and Sch 147) were inhibited (100 AU/mL). The inhibitory activity of BLIS was lost (inactivated) after precipitate treatment with trypsin, proteinase K, pepsin and α -chymotrypsin (tested against the indicator *E. avium* EA5), which confirms its proteinaceous nature. Regarding the thermostability test, the precipitate remained active after 2 weeks of storage at -20°C (100 AU/mL) testing against the EA5 strain, as well as after treatment at 60°C for 1 h (testing against EA5 strain- 100 AU/mL). The indicator LM7223 was not inhibited.

4. Discussion

The species *Lacticaseibacillus paracasei* is a species that operates via commensalism [15]. Commensal lactobacilli are good candidates for development as probiotics [15]. Some species show beneficial/probiotic properties, among which bacteriocin production can be involved [14]. The species *Lacticaseibacillus paracasei* is the most frequently isolated species from the dairy environment among the other lacticaseibacilli [16]. Tolinacki et al. [9] reported a bacteriocin-producing substance from the *L. paracasei* BGUB 9 strain isolated from homemade hard cheese. Its bacteriocin substance UB9 retained an antimicrobial activity within the pH range of 1–10 and after treatment at 100 °C for 30 min. Similarly, a bacteriocin-like inhibitory substance produced by the *L. paracasei* LPa 12/1 strain retained activity at 60 °C and –20 °C, and it showed a narrow antimicrobial spectrum up to now. A narrow antimicrobial spectrum was noted in the formerly mentioned bacteriocin substance UB9. The production of bacteriocins and/or bacteriocin-like substances via beneficial/probiotic strains can provide additional benefits for their application potential in the food industry [9], e.g., antimicrobial, antibiofilm, and cytotoxic effects of bacteriocin lactococcin produced by the dairy origin strain *Lactococcus lactis* CH3 reported by Krishnamoorthi et al. [17]. Biadala et al. [18] outlined the antibacterial activity of selected LAB for the bioconversion of milk and whey from goat milk. Enriching products using beneficial strains can be influenced by their adhesive ability, e.g., Lauková et al. [19] described different adhesive abilities in lactobacilli under in vitro conditions in relation to the autochthonous character of its source. The application form of beneficial strains is also very important. As previously mentioned, the simplest form of encapsulation is freeze drying [20]. The LPa 12/1 strain in encapsulated form sufficiently survived in both types of yoghurts, although its higher count was determined in ewe–goat milk yoghurts compared with cow milk yoghurts, likely from its autochthonous strain. On the other hand, cow milk yoghurts retained better consistency over 14 days than ewe–goat milk yoghurts. When ewe–goat milk yoghurt was supplemented with the strain *Lactiplantibacillus plantarum* LP17L/1 (10^9 cfu/mL) and maintained at 4 °C, it sufficiently survived in yoghurts with stability for 10 days without changes in the product quality [20]. Speranza et al. [21] reported functional cream cheese with the *L. reuteri* DSM 20016 strain (and *Bifidobacterium animalis* subsp. *lactis* DSM 10140). The cheese resulted in favourable viability in both strains during 28 days of storage at 4 °C with good sensory characteristics. Patrovský et al. [22] reported that bacteriocins introduced into foodstuffs via protective cultures in situ offer new perspectives on enhancing food quality and safety. They used freeze-dried preparations of bacterial strains producing particular bacteriocins. Plantaricin was found to exhibit the highest antilisterial effect. Besides lactobacilli, the antimicrobial effect of bacteriocin (enterocin, Ent 4231) produced by *Enterococcus faecium* CCM 4231 was reported in yoghurt [23]. The yoghurt was experimentally contaminated with the *Listeria monocytogenes* Oxford 209P strain. A retardation in the Oxford 209P count was detected in yoghurt after one-day storage compared with the control (10^3 vs. 10^0 cfu/mL/g); a decrease of three orders of magnitude was noted. Some bacteria can influence the proteolysis of casein differently and thus influence the processing of product/yoghurt [24]. In the case of the LPa 12/1 strain, its optimal technological properties, e.g., sufficient clotting capacity in milk were previously described [25]. Taking into account the present and previous results obtained and/or reported, LAB, in general, or their individual representatives seem to be the most suitable as beneficial strains because of their ability to modify the microenvironment, in which they have been delivered, via producing various metabolites, e.g., inhibitory substances-bacteriocins and/or competitive exclusion [26,27]. Together with animal products consumption, health safety has been associated. As previously mentioned, the beneficial strain safety must be assessed/confirmed. In this study, an experimental model using Balb/c mice was used. No mortality of mice was assessed, and coliforms were significantly reduced in the faeces and jejunum of mice and mathematically decreased in the liver after LPa 12/1 strain application. The highest count of the LPa 12/1 strain was detected in the faeces of mice and then in the jejunum; almost the same count was present

in the liver. Finding LPa 12/1 and/or microbiota in the liver can be explained: the gut microbiota and liver have a bidirectional relationship. As a result, bacterial products and metabolites from microbiota can pass through the intestinal barrier and reach the liver via the portal circulatory system. In the case of beneficial bacteria, they can provide a beneficial influence on the liver [28]. In our case, the safety of LPa 12/1 strain was fulfilled. Moreover, some lactobacilli representatives can be successfully used as additives to biotherapy in the case of trichinelosis [11], e.g., when Balb/c mice were infected with *Trichinella spiralis* (400 larvae) on day 7 of treatment with the beneficial strain *L. plantarum* LP17L/1; the strain restored the CD4+ T cell numbers in the epithelium and *lamina propria* at the control level from 11 dpi. *T. spiralis* infection significantly inhibited lymphocyte subpopulations from 5 to 25 days postinfection (dpi). The strain LP17L/1 stimulated the CD8+ T cell numbers in infected mice, which were restored in *lamina propria* on 11 dpi and in the epithelium on day 32. The immune-modulatory effect of the LP17L/1 strain was confirmed. This is also assumed for LPa 12/1 strains. Related studies are in progress. The safe beneficial strain LPa 12/1 has shown promising application potential in yoghurts. Based on the already published immune-modulatory effect of the other strain of dairy origin (LP17L/1), understanding the immunological mechanism allows risk reductions in parasitic infection or allows for the enrichment of antiparasitic therapy and/or reducing antihelminthic dosage. A promising way to obtain this information is indicated via the dairy products.

5. Conclusions

Although LPa 12/1 strain safety testing was conducted using the Balb/c mice model, the bacteriocin-like inhibitory substance-producing strain *Lacticaseibacillus paracasei* LPa 12/1 did not cause mortality in Balb/c mice. In addition, the inhibitory activity against coliforms in the faeces and jejunum of Balb/c mice was noted after its application. Moreover, LPa 12/1 strain application stimulated non-specific immunity parameters in the blood of mice. Bacteriocin-like inhibitory substance (precipitate) has shown low inhibitory activity. Encapsulating the LPa 12/1 strain seems to be a suitable form to supplement dairy products. The LPa 12/1 strain has survived in higher counts in ewe–goat milk yoghurt than in cow milk yoghurt. However, sufficient counts were determined in both types of yoghurts. The pH value of yoghurts was not negatively influenced. Although organoleptic tests were not performed, cow milk yoghurts remained in more stable consistency with LPa 12/1 strain addition compared with ewe–goat milk yoghurts. Other tests are in the processing to increase the additive potential of bacteriocin-like inhibitory substance-producing strain LPa 12/1 and its bacteriocin-like inhibitory substance.

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References

1. Yami, A.; Merkel, R.C. Compositional and technological properties of goat milk and milk products A review. *Con. Dairy Vet.Sci.* **2020**, *3*, 295–300.
2. Vaquil, R.R. A review on Health promoting aspects of goat milk. *Pharma Innov.* **2017**, *6*, 5–8.
3. Lauková, A.; Focková, V.; Pogány Simonová, M. *Enterococcus mundtii* isolated from Slovak raw goat milk and its bacteriocinogenic potential. *Int. J. Environ. Res. Pub. Health* **2020**, *17*, 9504. [\[CrossRef\]](#)
4. Lad, S.S.; Aparnathi, K.D.; Mehta, B.; Velpula, S. Goat milk in human nutrition and health-a review. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 1781–1792. [\[CrossRef\]](#)
5. Lauková, A.; Micenková, L.; Grešáková, L.; Mad'arová, M.; Pogány Simonová, M.; Focková, V.; Ščerbová, J. Microbiome associated with Slovak raw goat milk, trace minerals, and vitamin E content. *Int. J. Food Sci.* **2022**, *8*, 4595473. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Quigley, L.; O' Sullivan, O.; Stanton, C.; Beresford, T.P.; Ross, R.P.; Fitzgerald, G.F.; Cotter, P.D. The complex microbiota of raw milk. *FEMS Microbiol. Rev.* **2013**, *37*, 664–698. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Lauková, A.; Pogány Simonová, M.; Tomáška, M.; Kološta, M.; Drončovský, M.; Dvorožňáková, E. Lacticaseibacilli and lactococci from Slovak raw goat milk and their potential. *Sci. Agri. Bohem.* **2021**, *52*, 19–28. [\[CrossRef\]](#)
8. Klapáčová, L.; Greif, G.; Greifová, M.; Tomáška, M.; Hanuš, O.; Dudříková, E. Antimicrobially active lactobacilli from goats milk that do not produce biogenic amines. *J. Food Nutr. Res.* **2015**, *54*, 270–274.
9. Tolinački, M.; Kojič, M.; Lozo, J.; Teržič-Vidojevič, A.; Topisarovič, L.; Fira, D. Characterization of the bacteriocin-producing strain *Lactobacillus paracasei* subsp. *paracasei* BGUB9. *Arch. Biol. Sci. Belgrade* **2010**, *262*, 889–899. [\[CrossRef\]](#)
10. Strompfová, V.; Marciňáková, M.; Simonová, M.; Bogovič-Matijašič, B.; Lauková, A. Application of potential probiotic *Lactobacillus fermentum* AD1 strain in healthy dogs. *Anaerobe* **2006**, *12*, 75–79. [\[CrossRef\]](#)
11. Dvorožňáková, E.; Vargová, M.; Hurníková, Z.; Lauková, A.; Revajová, V. Modulation of lymphocyte subpopulations in the small intestine of mice treated with probiotic bacterial strains and infected with *Trichinella spiralis*. *J. Appl. Microbiol.* **2022**, *132*, 4430–4439. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Větvička, V.; Fornousek, L.; Kopeček, J.; Kamínková, J.; Kašpárek, L.; Vránová, M. Phagocytosis of human blood leucocytes, a simple micro-method. *Immunol. Lett.* **1982**, *5*, 97–100. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Nataraj, B.H.; Azmal Ali, S.; Betore, P.V.; Yadav, H. Postbiotics-parabiotics:the new horizon in microbial biotherapy and functional food. *Microb. Cell Fact.* **2020**, *19*, 168. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Lauková, A.; Tomáška, M.; Fraqueza, M.J.; Szabóová, R.; Bino, E.; Ščerbová, J.; Pogány Simonová, M.; Dvorožňáková, E. Bacteriocin-producing strain *Lactiplantibacillus plantarum* LP17L/1 isolated from traditional stored ewes milk cheese and its beneficial potential. *Foods* **2022**, *11*, 959. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Fang, F.; O' Toole, P.W. Commensal lactobacilli are good candidates for development as probiotics. *Front. Biosci. (Landmark Ed.)* **2009**, *14*, 3111–3127. [\[CrossRef\]](#)
16. Smokvina, T.; Wels, M.; Polka, J.; Chervaux, C.; Brisse, S.; Boekhorst, J.; Vlieg, J.E.; van Hylckama, T.; Siezen, R.J.; Highlander, S.K. *Lactobacillus paracasei* comparative genomics: Towards species pan-genome definition and exploitation of diversity. *PLoS ONE* **2013**, *8*, e68731. [\[CrossRef\]](#)
17. Krishnamoorthi, R.; Srinivash, M.; Mahalingam, P.U.; Malaikozhundan, B.; Suganya, P.; Gurushanakar, K. Antimicrobial, antibiofilm, antioxidant, and cytotoxic effects of bacteriocin by *Lactococcus lactis* strain CH3 isolated from fermented dairy products-An *in vitro* and *in silico* approach. *Int. J. Biol. Macromol.* **2022**, *220*, 291–306. [\[CrossRef\]](#)
18. Biadala, A.; Szablewski, T.; Cegielska-Radziejewska, R.; Lasik-Kurdys, M.; Adzahan, N.M. The evaluation of activity of selected lactic acid bacteria for bioconversion of milk and whey from goat milk to release biomolecules with antibacterial activity. *Molecules* **2023**, *28*, 3696. [\[CrossRef\]](#)
19. Lauková, A.; Strompfová, V.; Ouwehand, A. Adhesion properties of enterococci to intestinal mucus of different hosts. *Vet Res. Com.* **2004**, *28*, 647–655. [\[CrossRef\]](#)
20. Lauková, A.; Tomáška, M.; Drončovský, M.; Mad'ar, M.; Bino, E.; Dvorožňáková, E.; Chrastinová, L.; Pogány Simonová, M.; Kološta, M. *Lactiplantibacillus plantarum* LP17L/1 from ewes stored cheese, promising strain for use not only in dairy products. In Proceedings of the Scientific Works from the Conference Food Safety and Quality, Piešťany-Nitra, Slovakia, 29–31 March 2023; pp. 34–36. ISBN 978-80-8266-028-2. [\[CrossRef\]](#)
21. Speranza, B.; Campaniello, D.; Monacis, N.; Bevilacqua, A.; Sinigaglia, M.; Corbo, M.R. Functional cream cheese supplemented with *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and *Lactobacillus reuteri* DSM 20016 and prebiotics.. *Food Microbiol.* **2016**, *72*, 16–22. [\[CrossRef\]](#)
22. Patrovský, M.; Kouřimská, L.; Havlíková, Š.; Marková, J.; Pechar, R.; Rada, V. Utilization of bacteriocin-producing bacteria in dairy products. *Mljekarstvo* **2016**, *66*, 215–224. [\[CrossRef\]](#)
23. Lauková, A.; Czikková, S.; Burdová, O. Anti-staphylococcal effect of enterocin in Sunar and yoghurt. *Folia Microbiol.* **1999**, *44*, 707–711. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Leitner, G.; Kriechus, O.; Merin, U.; Lavi, Y.; Silamihove, N. Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bacteriocin. *Int. Dairy J.* **2006**, *16*, 648–659. [\[CrossRef\]](#)
25. Tomáška, M.; Drončovský, M.; Klapáčová, L.; Slottová, A.; Kološta, M. Potential probiotic properties of lactobacilli isolated from goats milk. *Sci. J. Food Ind.* **2015**, *9*, 66–71. [\[CrossRef\]](#)

26. Zommiti, M.; Chikindas, M.L.; Ferchichi, M. Probiotics-live biotherapeutics: A story of success, limitations, and future prospects—not only for humans. *Prob. Antimicrob. Prot.* **2020**, *12*, 1266–1289. [[CrossRef](#)]
27. Orlando, A.; Refolo, M.G.; Messa, C.; Amati, L.; Lavermicocca, P.; Guerra, V.; Russo, F. Antiproliferative and proapoptotic effects of viable or heat-killed IMPC2.1 and GG in HGC-27 gastric and DLD-1 colon cell lines. *Nutr. Cancer* **2012**, *64*, 1103–1111. [[CrossRef](#)]
28. Hsu, L.C.; Schnabel, B. The gut-liver axis and gut microbiota in health and liver disease. *Nat. Rev. Microbiol.* **2023**, *21*, 719–733. [[CrossRef](#)]

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