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Article

The Bacteriocin-Producing Strain *Lacticaseibacillus paracasei* LPa 12/1 from Raw Goat Milk, a Potential Additive in Dairy Products

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Featured Application: Based on safety testing using BALB/c mice, bacteriocin-producing strain *Lacticaseibacillus paracasei* LPa 12/1 resulted as safe strain. Encapsulation of bacteriocin-producing LPa 12/1 strain seems to be suitable form for its potential application in dairy products. LPa 12/1 strain colonized better ewe-goat milk yoghurt than cow milk yoghurt; however, in both types of yoghurts sufficient counts of LPa 12/1 strain were counted and the pH of yoghurts was not negatively influenced.

Abstract: Goat milk starts to be gradually the preferred milk by consumers worldwide and also in Slovakia. It is also demanded as functional and/or nutraceutical drink as it is rich in bioactive components. Goat milk contains complex of microbiota among which the phylum Firmicutes have been occurred with abundance 20.5 %. Among individual representatives of this phylum are involved also lacticaseibacilli. This study has been focused on bacteriocin-producing, beneficial strain *Lacticaseibacillus paracasei* LPa 12/1 isolated from raw goat milk and its potential to be additive in dairy products. Based on safety testing of bacteriocin-producing LPa 12/1 strain using BALB/c mice, it looks as safe. Even increase tendency in phagocytic activity in blood of mice was noted after LPa 12/1 strain application. Encapsulated form of LPa 12/1 strain producing thermo-stable bacteriocin seems to be a suitable to supplement dairy products. The strain colonized better ewe-goat milk yoghurt (up to 6.1 cfu/g log 10) than cow milk yoghurt (almost 5.0 cfu/g log 10). Although specific organoleptic tests were not involved, cow milk yoghurts remained in better consistency after LPa 12/1 strain supplementation in comparison with ewe-goat milk yoghurts. LPa 12/1 strain may be supposed as a new potential additive in dairy products/yoghurts.

Keywords: lacticaseibacilli; benefit; yoghurt; supplement; functional food

1. Introduction

Goat milk provides multiple functions [1]. It starts to be gradually the preferred milk by consumers worldwide [1,2] and also in Slovakia [3]. Because of commonly described components which makes goat milk attractive for consumers [2,4], goat milk is also demanded as functional and/or nutraceutical drink as it is rich in bioactive components [5]. Milk contains beneficial microbiota that are important for development of flavor, taste, texture, technological and health-related perception but it also have contaminant bacteria that create risks associated with the consumption of raw milk and raw-derived products [5,6].

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Among useful microbiota, bacteria of the phylum Firmicutes have been occurred with 20.5 % abundance in tested Slovak raw goat milk using next-generation sequencing technique [5]. Although it has not been dominating phylum in raw goat milk, genera involved in the phylum Firmicutes and their individual representatives have been detected and isolated from raw goat milk using the standard microbiological method [7]. There are e.g. lactic acid -producing lactococci, lactobacilli, lacticaseibacilli but also the others such as enterococci [3,7]. Among lactococci, mostly the species *Lactococc*us *lactis* was identified [7]. Lactobacilli have been mostly represented by the species *Lacticaseibacillus paracasei* or *Lactiplantibacillus plantarum* [7,8]. When those species strains can produce bacteriocins (antimicrobial proteinaceous substances) and also posses additional beneficial properties (bile and low pH tolerance, sufficient adhesive ability, lactic acid production, diacetyl, hydrogen peroxide or useful enzymes, etc.), they can play important role in food fermentation process, in biopreservation and or in prevention of contamination [9]. Especially, bacteriocin production exerted by some of former mentioned species strains can be effective against food-borne pathogens. Therefore, this study has been focused on bacteriocin-producing, beneficial strain *Lacticaseibacillus paracasei* LPa 12/1 isolated from raw goat milk [7], its safety, stability and/or surviving in yoghurts for its further potential to be additive in dairy products. Besides former mentioned bacteriocin activity of LPa 12/1 strain, this strain is hemolysis-negative (ɣ-hemolysis), it tolerates sufficiently oxgall/bile. LPa 12/1 strain posses low-grade biofilm-formation and produces 10 nmol of useful enzyme β-galactosidase [7].

2. Materials and Methods

2.1. Safety control of LPa 12/1 strain using BALB/c mice

First, by rifampicin labeled variant of the strain LPa 12/1 (to differ it from the other lactic acid bacteria/lactobacilli) was prepared as previously described Strompfová et al. [10]. Then it was cultivated in MRS broth (pH 6.2, Merck, Darmstadt, Germany) overnight at 37 ˚C in incubator (with partial CO_2 atmosphere) to have absorbance (A_{600}) up to 1.0. Then it was centrifuged (10 000 x g) for 30 min. The cells were re-suspended in Ringer solution (Merck, Germany) and diluted to have final concentration 10^9 cfu/ml). The cells count was checked after dilutions spreading on MRS agar (Merck) and incubation at 37 ˚C for 24-48 h.

The experimental design was performed as previously described by Dvorožňáková et al. [11]. The pathogen-free male BALB/c mice aged 8 weeks (VELAZ, Prague, Czech Rebulic; *n= 27*) were used with weighting 18-20 g. Animals were kept under a 12-h light-dark regime at room temperature (22-24 ˚C). Humidity was 56%. Mice were on a commercial diet and water. They were divided in the control group (CG, *n=12*) and experimental group (EG, *n=15*). The strain LPa 12/1 was administered *per os* daily at a dose of 10^9 cfu/ml in 100 µl for 30 days. The experiment lasted 45 days. Mice were placed at Institute of Parasitology of the Slovak Academy of Sciences in Košice (Slovakia), approved by Slovak Veterinary Administration as well as Ethic Committee of the institute (Ro-1604/19-221/3). Sampling was provided at time 0/1 before application, on day 30 (application) and at the end (on day 45, meaning 15 days after cessation of the strain). Fecal, jejunal and liver samples were checked for selected microbiota. On day 0/1 (*n=8*, mixture fecal sample) was checked. LPa 12/1 strain was checked on MRS agar (Merck, Darmstadt, Germany) with rifampicin (100 µg/ml), LAB were counted on MRS agar (Merck), amylolytic streptococci were counted on M17 agar (Difco, Sparks, Maryland, 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). Moreover, phagocytic activity in blood (PA) was checked using modified method described by Větvička et al. [12]. The percentage of phagocytic cells was evaluated using an optical microscope by counting PMN (polymorphonuclear cells) up to 100. Blood was sampled from *vena auricularis*.

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

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2.2. LPa 12/1 strain encapsulation process (dry freezing)

Dry freezing is the most simple form of encapsulation [13]. For this process, the strain LPa 12/1 (by rifampicin labeled) was grown in 300 ml of MRS broth (pH 6.2 Merck, Germany) overnight at 37 ˚C in incubator (with partial CO_2 atmosphere) to reach A_{600} up to 1.0. Then broth culture was mixed with skim milk (Simandl company, Czech Republic) in small flasks (in ratio 1:1). They were placed for freezing at - 80 ˚C. Then dry freezing process was started using the device Micro Modulyo 230-freeze dryer (Thermo-electron corporation, Asheville NC 28804, USA). After sufficient dry freezing, powder was weighted. The cells count was checked as it has been formerly described; part of dry frozen strain was diluted in Ringer solution and dilutions were spread on MRS agar (Merck). 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 at commercial market network. They also contain yoghurt commercial 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. Encapsulated strain was checked for cells count before application and it was applied (0.5 g) in the experimental yoghurt- E. C- control yoghurt was not enriched with the strain. Before application, yoghurt samples were diluted in peptone water and spread on Mac Conkey agar (Difco) to control contamination-enterobacteriae (it was negative). Also LAB were counted on MRS agar (Merck) (5.1 cfu/g log 10) and control was provided also on M17 agar (Difco) because of commercial culture - *Streptococcus* spp. (count reached 5.1 cfu/g). Sampling was performed after 24 h, on day 7, on day 10 and on day 14 (which is also declared expiration time for this type of yoghurt). The LPa 12/1 strain was selected on MRS agar enriched with rifampicin (100 μg/ml), LAB were determined on MRS agar and also amylolytic streptococci were checked using M17 agar (Difco). Yoghurt samples (one g) were taken and mixed (Stomacher-Masticator, IUL, Spain) with peptone water (Merck, ratio 1:9), diluted and spread on the selected cultivation media (ISO) as formerly indicated. Moreover, pH was measured using Checker-pH tester (Hanna instruments Inc., Woonsocket, USA). Initial pH was 3.94. Yoghurts were maintained in the fridge during 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 commercial market network. They contain commercial culture. It was declared in 100 g of product energy 500 KJ, fat 9.0 g of which saturated fatty acids participated with 5.2 g, carbohydrates were involved in 4.5 g, sugar value of which was 4.1 g; proteins content formed 3.4 g, and salt 0.1 g. Encapsulated strain was checked for cells count before application and 0.5 g of it was applied in the experimental yoghurt- E. C- control yoghurt was not enriched with the strain. Before application, yoghurt samples were diluted in peptone water and spread on Mac Conkey agar (Difco) to control contaminant bacteria (enterobacteriae). They were absent. Control was also spread on MRS (Merck) (5.1 cfu/g log 10) and also on M17 agar (Difco) because of commercial culture (*Streptococcus* spp., 5.1 cfu/g). Sampling was performed after 24 h, on day 7, on day 10 and on day 14. The LPa 12/1 strain was selected on MRS agar enriched with rifampicin (100 μg/ml), LAB were determined on MRS agar and also amylolytic streptococci were checked using M17 agar (Difco). Yoghurt samples (one g) were taken and mixed (Stomacher-Masticator, IUL, Spain) with peptone water (Merck in ratio 1:9), diluted and spread on the selected cultivation media (ISO) as formerly indicated. Moreover, also pH was measured as formerly mentioned. The initial pH was 3.50. Yoghurts were maintained in the fridge during whole testing period.

2.5. Bacteriocin 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-*Enterococcus avium* EA5 (the most sensitive strain) was

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used in the test (our strain isolated from feces of piglet). Then a substance was precipitated using the following protocol: LPa 12/1 strain (0.1% inoculum) was grown in the volume 60 ml of MRS broth (Merck, Germany) overnight at 37° C. It was centrifuged (10 000 x g, 30 min). The cell-free supernatant was treated with Chelaton-EDTA III (Merck) and heated at 80 °C for 10 min to eliminate the other organic substances effect. To have concentrated bacteriocin substance, treated supernatant with pH 4.5 was filtrated (0.22 μm, Millipore corporation, Bedford, MA, USA) and precipitated with ammonium sulphate (70 % saturation) at 4 °C overnight. After precipitation followed again centrifugation (10 000 x g for 30 min). Supernatant was through away and precipitate was re-suspended in minimal volume of phosphate buffer (5 ml, pH 5). Inhibitory activity was checked against the indicator *E. avium* EA5 and expressed in arbitrary units per ml. It means, the highest dilution of precipitate which caused growth inhibition of indicator strain (AU/ml).

Precipitate was treated with enzymes such as α-chymotrypsin, trypsin, proteinase and pepsin, Merck) and again inhibitory activity was checked against EA5 strain but also against *Listeria monocytogenes* LM 7223 (Veterinary Institute in Olomouc, Czech Republic.)

Precipitate was also tested for its thermo-stability. It was exposed for 1 h at 60 °C and at -20 °C storage for 2 weeks. Remained 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 safety control experiment. On day 30, LPa 12/1 strain reached in feces the amount 3.64 ± 0.56 cfu/g (log 10), meaning almost 10^4 cfu/g from the initial application concentration (10^9 cfu/mL). This colonization was sufficient. On day 45 (15 days of LPa 12/1 strain cessation), its decrease was noted ($p<0.01$). LPa 12/1 strain contributed in the total LAB increase on day 30 in feces compared with day 0/1 (difference 0.59 log cycle) and on day 45 ($^{ab}p<0.001$). Fecal amylolytic streptococci were present in high counts (Table 1). Coliforms count was reduced significantly in 30EG comparing with 45EG ($^{ab}p<0.05$).

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

Faeces	LPa 12/1	LAB	Amyl. str.	Coliforms
Sampling 0/1 (*n=8*)	nt	5.48 ± 0.52^a	5.49 ± 0.07	3.27 ± 0.49
Sampling30EG (*n=5*)	3.64 ± 0.56^a	6.07 ± 0.07^d	5.16 ± 0.30^a	4.66 ± 0.59^a
Sampling 30 CG	nt	6.47 ± 0.51	5.04 ± 0.47	5.08 ± 0.42
Sampling 45 EG	2.62 ± 0.61^b	7.04 ± 0.15^b	5.59 ± 0.04	3.76 ± 0.44^b
Sampling 45 CG	nt	7.1 ± 0.0^c	6.05 ± 0.18^b	2.28 ± 0.33

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, ^{ab,ac}, $p<0.001$, $^{db}p<0.01$; Amylolytic streptococci, $^{ab}p<0.05$; coliforms, $^{ab}p<0.05$; sampling 0/1, before experiment, day 30-application of LPa 12/1, day 45, 15 days of LPa 12/1 cessation; nt-not tested;.

In jejunum, the count of LPa 12/1 strain was lower comparing with feces (Table 2). It reached up to 10^2 cfu/g on day 30. On day 45 it was reduced up to almost 10^1 cfu/g (Table 2). The total LAB were high and not reduced and the counts of amylolytic streptococci as well (Table 2). The counts of coliforms were in counts as normally could be detected in animals jejunum; however, they were slightly (mathematically reduced) with difference 0.74 log cycle.

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

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

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, day 30 (mathematical difference 0.74 log cycle); sampling 0/1, before experiment, day 30-application of LPa 12/1, day 45, 15 days of LPa 12/1 cessation, nt-not tested.

In liver, LPa 12/1 reached 1.30 ± 0.0 cfu/g (log 10) and on day 45 its count was decreased (less as 0.1 cfu/g). The count of LAB reached 4.23 ± 0.5 cfu/g which means the strain contributed to the total LAB count because on day 45 decrease was noted 2.42 ± 0.55 cfu/g Table 3). Amylolytic streptococci were counted up to 10^2 cfu/g and were mathematically reduced with differences 0.53 log cycle on day 30 and 1.55 on day 45. Coliforms were in low counts and not influenced.

Table 3. The LPa 12/1 counts and controlled selected microbiota in liver of mice BALB/c after strain application and cessation.

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

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 30EG comparing with 30CG (difference 0.53 log cycle); and on day 45 in 45EG (1.55 log cycles) in comparison with 45CG; sampling 0/1, before experiment, day 30-application of LPa 12/1, day 45, 15 days of LPa 12/1 cessation; nt-not tested.

Regarding the PA, its value on day 30 was higher in EG (61.0± 1.05) than in CG (60.0 ± 1.30). The same situation was in case of IPA (index of phagocytic activity) (Table 4). On day 45 even higher value of PA was measured in EG comparing with day 30 and also with day 45 in CG (62.0 ± 2.37 % to 61.0 ± 0.84 %) and the same situation was also in IPA. Significantly higher PA was noted in EG on day 30 comparing with day 45 in EG ($p<0.001$). It means, LPa 12/1 showed stimulating effect on parameter unspecific immunity with prolonging character.

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

n=6	PA	IPA
Sampling 30 EG	61.5 ± 1.05[a]	2.53 ± 0.21
Sampling 30 CG	60.0 ± 1.30	2.82 ± 0.11
Sampling 45 EG	62.0 ±2.37[b]	2.93 ± 0.10
Sampling 45 CG	61.0 ± 0.84	2.82 ± 0.11

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; sampling 0/1, before experiment, day 30-application of LPa 12/1, day 45, 15 days of LPa 12/1.

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3.2. Stability and surviving of LPa 12/1 strain in ewe-goat milk yoghurts

The count of LPa 12/1 strain in dry frozen form immediately before its addition in yoghurts reached 6.6 x 10^7 cfu/g respectively 7. 82 cfu/g (log 10). The strain LPa 12/1 showed sufficient stability and surviving in the ewe-goat milk yoghurt. Its count after 24 h reached 5.1 cfu/g (log 10) from the initial count 10^7 cfu/g in dry frozen dose at the moment of application (Table 5). When checking LPa 12/1 strain count on day 7 (1 week), its count was almost the same (5.15 cfu/g (log 10); however, on day 10, its increase almost about 1 log cycle (0.95 log cycle) was noted (6.1 cfu/g (log 10) and this count was stable also on day 14. It also looks, that LPa 12/1 strain contributed in the total LAB count on day 7, 10 and 14. Their count was higher and stable. LAB also reached higher counts in E yoghurts comparing with their counts in control (C- yoghurt). After 24 h, difference 0.46 log cycle was found, on day 7 it was already difference 1.56 log cycle and then 1.15 respectively 1.0 log cycle differences were noted comparing E and C yoghurts. It seems that commercial culture count was not influenced by LPa 12/1 strain addition from the start of experiment. On day 10, LPa12/1 count was increased almost about 1 log cycle (Table 5) but on day 14 the count of LPa 12/1 strain was decreased to the initial level. As already mentioned, the initial pH of yoghurt reached 3.94. After 24 h it was almost the same in both, E and C yoghurts (Table 5). On day 7, in both yoghurts, pH was increased up to 4.55 in E and 4.89 in C. On day 10, in C yoghurt was pH stable; however, in E yoghurt decrease of pH was noted. Finally, on day 14 in both yoghurts almost the same pH 4. 0 and 4.05 was measured. It was not negatively influenced by LPa 12/1 strain addition.

Table 5. Stability and surviving of LPa 12/1 strain in ewe-goat milk yoghurts (expressed in cfu per g (cfu/g, log 10).

	pH	LPa12/1	LAB	Amyl. str.
E/24 h	3.90	5.1	5.1	5.1
C/24 h	3.91	nt	4.61	4.41
E/ Day 7	4.55	5.15	6.1	5.1
C/ Day 7	4.89	nt	4.54	5.30
E/Day 10	3.70	6.1	6.1	6.1
C/Day 10	4.85	nt	4.95	6.1
E/ Day 14	4.0	6.1	6.1	5.1
C/ Day 14	4.05	nt	5.1	5.1

LAB-lactic acid bacteria, amylolytic streptococci; nt-not tested; E-experiment, C-control.

3.3. Stability and surviving of LPa 12/1 strain in cow milk yoghurts

After 24 h LPa 12/1 strain count in cow milk yoghurt reached 2. 1 cfu/g (log 10, Table 6) from initial count 6.5 x 10^7 cfu/g. On day 7, slight increase was noted (2.36 cfu/g); however, it was less (mathematical difference 3. 0 respectively 2.81 log cycles) than in the same time LPa 12/1 strain colonized ewe-goat milk yoghurt. The LPa 12/1 increase was also noted on day 10 (4.72 cfu/g) and it was almost stable on day 14 (4.72 cfu/g); but it was again less (difference 1. 32 respectively 1.38 log cycles) than in case of ewe-goat milk yoghurt. It is interesting, that LAB count in cow milk yoghurts and in ewe-goat milk yoghurts were almost the same. The count of amylolytic streptococci were lower in cow milk yoghurts in comparison with ewe-goat milk yoghurts; however, they were increased from day 7 (Table 6). The value of pH in cow milk yoghurt was slightly lower than in ewe-goat milk yoghurts and it was not negatively influenced.

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

	pH	LPa12/1	LAB	Amyl. str.
E/24 h	3.59	2.1	4.76	3.69
C/24 h	nt	nt	nt	nt
E/Day 7	3.70	2.36	6.1	2.56
C/Day 7	3.77	nt	4.54	2.75
E/Day 10	3.80	4.78	6.1	5.1
C/Day 10	3.90	nt	4.95	5.1
E/Day 14	3.60	4.72	5.1	4.92
C/Day 14	3.60	nt	4.65	4.70

LAB-lactic acid bacteria, amylolytic streptococci; nt-not tested; E-experiment, C-control.

3.4. Bacteriocin activity and stability

Using the qualitative method, inhibitory zone size due to substance produced by LPa 12/1 strain measured 30 mm in average. Inhibitory activity of precipitate LPa 12/1 against EA5 indicator reached 100 AU/ml. Inhibitory activity was retained after precipitate treatment with α-chymotrypsin against the indicator EA5 (100 AU/ml). Regarding the thermo-stability test, precipitate remained active after 2 weeks storage at -20 ˚C (100 AU/ml) testing against EA5 strain as well as after treatment at 60 ˚C for 1 h (against EA5 strain- 100 AU/ml). LM7223 was not inhibited.

4. Discussion

The species *Lacticaseibacillus paracasei* is bacterium that operates by commensalism. It is the most frequently isolated species from dairy environment among the other lacticaseibacilli [15]. Some of these species strains could be labeled as beneficial because of their probiotic properties among which also a bacteriocin-production can be involved [16]. Tolinacki et al. [9] reported bacteriocin - producing substance from the *L. paracasei* BGUB 9 strain isolated from homemade hard cheese. Its bacteriocin substance UB9 retained the antimicrobial activity within the pH range 1-10 and after treatment at 100 ˚C for 30 min. Similarly, bacteriocin substance produced by *L. paracasei* LPa 12/1 strain retained activity at 60 ˚C and -20 ˚C and up to now it showed narrow antimicrobial spectrum as was also noted in UB9 substance. The production of bacteriocins by beneficial/probiotic strains can provide additional benefits concerning their application potential in food industry [9]. Colonization of products by beneficial strains can be influenced by their adhesive ability. E.g. Lauková et al. [17] described different adhesive ability in lactobacilli under *in vitro* condition in relation with autochthonous character of its source. Very important is also application form of the beneficial strain. As formerly mentioned, the most simple way/form of encapsulation is dry freezing [18]. Encapsulated LPa 12/1 strain reached sufficient colonization in both types of yoghurts, although its higher counts were determined in ewe-goat milk yoghurt in comparison with cow milk yoghurts. Probably because of autochthonous strain. On the other hand, cow milk yoghurts retained better consistency during 14 days in comparison with ewe-goat milk yoghurts. When ewe-goat milk yoghurt was supplemented with the strain *Lactiplantibacillus plantarum* LP17/1 (10^9 cfu/ml) and maintained at 4 ˚C it colonized sufficiently yoghurt with stability during 10 days without changes of the product quality [18]. Speranza et al. [19] reported functional cream cheese with *L. reuteri* DSM 20016 strain (and *Bifidobacterium animalis* subsp. *lactis* DSM 10140). The cheese resulted in favorable viability of both strains during 28 days of storage at 4 ˚C with good sensory characteristics. Also Patrovský et al. [20] 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 anti-listerial effect. Besides lactobacilli, in yoghurt also antimicrobial effect of bacteriocin (enterocin, Ent 4231) produced by *Enterococcus faecium* CCM 4231 was reported [21]. There yoghurt was experimentally contaminated with *Listeria monocytogenes* Oxford 209P strain. A retardation in the

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Oxford 209P count was detected in yoghurt after 1 day storage in comparison with the control (10^3 vs. 10^0 cfu/ml/g), a decrease of 3 orders of magnitude. Taking into account 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 such as e.g. inhibitory substances-bacteriocins and/or competitive exclusion [2022]. Together with animal products consumption, health safety has been associated. As formerly mentioned the beneficial strain safety has to be assessed/confirmed. In this study, experimental model using BALB/c mice was used. No mortality of mice was assessed and coliforms were significantly reduced in feces, jejunum and mathematically also in liver after LPa 12/1 strain application. The highest count of LPa 12/1 strain was in feces, then in jejunum and almost the same also in liver. Moreover, increase in PA was mentioned. It means that safety aspect of LPa 12/1 strain was fulfilled. Moreover, some representatives of lactobacilli can be successfully used as additives to biotherapy in case of trichinelosis [11]. E.g. when mice BALB/c were infected with *Trichinella spiralis* (400 larvae) on day 7 of treatment with 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 lymphocytes subpopulations from 5 to 25 day post-infection (dpi). The strain LP17L/1 stimulated the CD8+ T cells numbers in infected mice, which were restored in lamina propria on 11 dpi and in the epithelium on day 32. The immunomodulatory effect of LP17L/1 strain was confirmed. It is supposed also in case of LPa 12/1 strain. Studies are in processing. Th safe, beneficial strain LPa 12/1 has shown promising application potential in yoghurts. Based on already published immunomodulatory effect of the other strain of dairy origin (LP17L/1), understanding immunological mechanism allows risk reduction of parasitic infection or allows to enrich anti-parasitic therapy and/or to reduce anti-helmintics dosage. The promosing way is indicate via dairy products.

5. Conclusions

Based on safety testing using BALB/c mice, bacteriocin-producing strain *Lacticaseibacillus paracasei* LPa 12/1 looks as safe strain. In addition, inhibitory activity against coliforms in feces and jejunum of mice BALB/c was noted. Moreover, after its application increase tendency in phagocytic activity in blood of mice was noted. In spite of the fact, that its bacteriocin (precipitate) has shown low inhibitory activity, encapsulated form of LPa 12/1 strain seems to be a suitable to supplement dairy products. LPa 12/1 strain colonized better ewe-goat milk yoghurt than cow milk yoghurt, although in both types of yoghurts sufficient counts of LPa 12/1 strain were counted. The value of pH was not negatively influenced. In spite of the fact, that specific organoleptic tests were not performed, cow milk yoghurts remained in better status quality with LPa 12/1 strain addition comparing with ewe-goat milk yoghurts. The other tests are in processing to increase additive potential of this bacteriocin-producing strain LPa 12/1 for dairy products.

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