



Autochthonous lactic acid bacteria—presentation of potential probiotics application

Mirjana Ž. Grujović · Katarina G. Mladenović · Danijela D. Nikodijević · Ljiljana R. Čomić

Received: 4 June 2019 / Accepted: 5 September 2019 / Published online: 12 September 2019
© Springer Nature B.V. 2019

Abstract

Objective The objective of this study was to evaluate the probiotic potential as well as the ability of adhesion and aggregation of natural and autochthonous lactic acid bacteria, isolated from traditionally made cheese.

Results Lactic acid bacteria from natural food sources can be promising probiotic candidates and they can be used in natural food preservation or like starter cultures. Tested autochthonous isolates showed tolerance to the simulated gastrointestinal condition as well as the sensitivity to clinically relevant antibiotics, especially to ampicillin (MIC at $0.195 \mu\text{g mL}^{-1}$ for lactobacilli and from 0.195 to $3.125 \mu\text{g mL}^{-1}$ for lactococci). Among isolates, the highest percentage of adhesion was detected with chloroform, while the adhesion ability of selected isolates to pig intestinal epithelium was in the correlation with the results of adhesion ability with solvents. The auto-aggregation ability of isolates was demonstrated, while co-aggregation with *Escherichia coli* was strain specific.

Conclusion The results indicated the potential probiotic properties of the isolates and give evidence for

further investigation and potential application in the dairy industry.

Keywords Antibiotic · Adhesion · Aggregation ability · Lactic acid bacteria · Probiotics · Pig intestinal epithelium

Introduction

Probiotics are defined as viable microorganisms which have a positive impact on medical condition of their host (FAO/WHO 2006). Lactic acid bacteria (LAB) are the most commonly used microorganisms as probiotics, because they have “Generally Recognized as Safe” (GRAS) status and because they are desirable members of the intestinal microflora (Shokryazdan et al. 2014). The tolerance to gastrointestinal conditions (Hernandez-Hernandez et al. 2012) as well as the safety aspect of LABs, which include antimicrobial resistance (Vesković-Moračanin et al. 2017) and haemolysis on blood agar (Kaktcham et al. 2012), are the major criteria for selection of probiotics bacteria.

A high number of in vitro models have been used for studying the adhesion of probiotic to epithelial cells. Many difficulties were found, which have led to the development of in vitro model systems for the preliminary selection of potentially adherent strains (Kos et al. 2003; Carasi et al. 2014; Garriga et al. 2014;

M. Ž. Grujović (✉) · K. G. Mladenović · D. D. Nikodijević · L. R. Čomić
Department of Biology and Ecology, Faculty of Science,
University of Kragujevac, Radoja Domanovića 12,
Kragujevac 34000, Republic of Serbia
e-mail: mirjana.grujovic@pmf.kg.ac.rs

Sitepu et al. 2016). It is known that *Lactobacillus reuteri* could inhibit the adherence of enteropathogenic *E. coli* to human intestinal epithelium (Walsham et al. 2016). Some authors indicated that auto-aggregation ability of LAB was necessary for adhesion to intestinal epithelial cells. They also indicated that LAB, with co-aggregation ability, could form a barrier that prevent colonization by pathogenic microorganisms (Kos et al. 2003; Younes et al. 2012).

The increase of interest regarding the commercial utilization of *Lactobacillus* strains isolated from traditionally and naturally fermented dairy products, was noticed (Magdoub et al. 2015). But, Solieri et al. (2014) indicated that there is no universal strain that would provide all probiotic benefits, not even strain of the same species.

The aims of this study were to evaluate the tolerance of selected LABs to different gastrointestinal conditions; their sensitivity to clinically relevant antibiotics; their ability to synthesize biogenic amines and ability to grow on media with phenol as well as detection of haemolysis on blood agar. Also, the aims were to evaluate the ability of auto-aggregation, co-aggregation and adhesion of LABs, as well as the investigation of the adhesive properties of selected LAB to pig ileal epithelial cells, by using the in vitro model.

Material and methods

Bacteria used in study

Four isolates of *Lactobacillus* genera (*Lb. fermentum* KGPMF28, *Lb. fermentum* KGPMF29, *Lb. brevis* KGPMF35, *Lb. plantarum* KGPMF62) and six isolates of *Lactococcus* genera (*L. lactis* subsp. *lactis* KGPMF23, *L. lactis* subsp. *lactis* biovar. *diacetyllactis* KGPMF50, *L. lactis* subsp. *lactis* biovar. *diacetyllactis* KGPMF54, *L. lactis* subsp. *lactis* biovar. *diacetyllactis* KGPMF55, *L. lactis* subsp. *lactis* biovar. *diacetyllactis* KGPMF57 and *L. lactis* subsp. *lactis* biovar. *diacetyllactis* KGPMF59) were used in this study. All tested bacteria were isolated from Sokobanja's cheese (Southeastern Serbia) and provided by the Microbiology Laboratory, Faculty of Science, University of Kragujevac, Serbia. The cheese was made on traditional way, without adding any bacterial starter culture, so the isolates tested in this study presents

the natural and autochthonous microflora. These isolates were chosen according to the previously investigated biochemical characteristics and antagonistic potential against enterobacteria isolated from the same cheese (Muruzović et al. 2018a, b). The bacterial strains were kept in glycerol stock at $-80\text{ }^{\circ}\text{C}$. *Lactobacillus plantarum* LP299v was used as reference strain. *Escherichia coli*, a clinical isolate, was a generous gift from the Institute of Public Health, Kragujevac, Serbia. Before experimental use, working cultures were revitalized by two consecutives in MRS broth (for LAB) and Nutrient broth (for *E. coli*).

In vitro gastrointestinal transit tolerance assay

The acid tolerance of LAB was studied in different pH solutions, which were prepared by adjusting the hydrochloric acid (HCl) (Zorka Šabac, Šabac, Serbia) to pH levels of 3, 4 and 5. Sterile MRS broth (pH 6.5) served as a control. Inoculum was prepared from overnight culture of LABs (turbidity of initial suspension 10^8 CFU mL^{-1}). Inoculum of each bacterial strain (10 μL) was added to the standard or modified MRS broth. The incubation was carried out at $37\text{ }^{\circ}\text{C}/48\text{ h}$. Optical densities of bacterial growth were determined with an ELISA plate reader at 600 nm. Results were performed in triplicate.

Simulated gastric and small intestinal juice tolerance assays were performed according to the method described in Huang and Adams (2004), with some modifications. Overnight cultures were inoculated in ratio 1:10. Simulated gastric juice were prepared by suspending 0.22% (w/v) pepsin (Merck, New Jersey, USA) in sterile filtered 0.5% (w/v) NaCl solution with the pH adjusted to 2. 96-well microtiter plates were incubated at $37\text{ }^{\circ}\text{C}/3\text{ h}$, because this is the time of staying food in the stomach (Zoumpopoulou et al. 2008). The results were determined with ELISA plate reader at 600 nm, in triplicate (Bassyouni et al. 2012).

Simulated small intestinal juice were prepared by suspending 0.2% (w/v) of pancreatin (Sigma-Aldrich, St. Louis, USA) in filter sterile 0.5% NaCl (w/v) solution with 0.4% of bile salts (Sigma-Aldrich, St. Louis, USA) and adjusting pH to 8 with adding sterile 0.1 M NaOH. 96-well microtiter plates were incubated at $37\text{ }^{\circ}\text{C}/4\text{ h}$, because this is the time of staying food in small intestine (Kumar and Murugalatha 2012). Results were performed in triplicate. The number of viable LABs was determined by

transferring the appropriate samples onto the MRS agar plates. The percentage of survival was calculated using the following formula:

$$\% \text{ Survival} = (\beta/\alpha) \times 100$$

α —CFU mL⁻¹ of the assayed strain (uninoculated MRS (pH 6.5), at 37 °C/48 h); β —CFU mL⁻¹ of the same strain after incubation with the different gastrointestinal conditions.

Antibiotic sensitivity

The antibiotics sensitivity of LABs was investigated by using the microdilution method with resazurin and the minimum inhibitory concentration (MIC) was determined (Sarker et al. 2007). Tetracycline, ampicillin, gentamicin, vancomycin and polymyxin B (Sigma Chemicals Co., USA), in concentration range from 0.05–4000 $\mu\text{g mL}^{-1}$, were used for this study. The method was described in detail in Muruzović et al. (2016).

Synthesis of biogenic amines and haemolysis on blood agar

The ability of isolates to synthesize biogenic amines (histamine and tyramine) from histidine and tyrosine, as well as haemolysis on blood agar plates, were analysed by method described in Jeong and Lee (2015).

Growth in the presence of phenol

Growth of isolates in the presence of phenol was determined by method described in Šušković et al. (2001).

Investigation of auto-aggregation and co-aggregation ability

The auto-aggregation ability of LABs isolates, as well as co-aggregation ability with *E. coli* was monitored by a modified method described in Ocaña and Nader-Macías (2002) and Tuo et al. (2013).

The auto-aggregation ability of examined isolates was monitored in PBS buffer (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, pH 7.5). The cells of overnight culture were settled down by centrifugation at 5000 rpm for 15 min, after which they were

twice washed in PBS buffer, and then re-suspended in 4 mL of the same buffer, so the number of cells was approximately 10⁸ CFU mL⁻¹. The suspension was well mixed in Vortex; 200 μL from the suspension surface was transferred into the micro tube with 1800 μL of PBS and the optical density was monitored at 600 nm (A_0). The same procedure was repeated after 1, 2, 3, 4, 5 and 24 h (A_t). The percent of auto-aggregation was calculated by following formula:

$$\text{Auto-aggregation } \% = (A_0 - A_t)/A_0 \times 100$$

where A_t represents the absorbance of supernatant after 1, 2, 3, 4, 5 and 24 h.

For the investigation of co-aggregation ability, the cells of examined LABs and *E. coli* were prepared identically as in the previous method, and re-suspended in PBS buffer, per 2 mL of each suspension of both types of bacteria for which the co-aggregation ability was monitored. Suspensions were mixed well in Vortex, 200 μL from the suspension surface was transferred into the micro tube with 1800 μL of PBS buffer and optical density was monitored at 600 nm (A_0). The same procedure was repeated after 2 h (A_t). The percent of co-aggregation was calculated by following formula:

$$\text{Co-aggregation } \% = (A_0 - A_t)/A_0 \times 100$$

where A_t represents the absorbance of supernatant after 2 h.

Microbial adhesion to solvents

Microbial adhesion to solvents (MATS) was measured according to the method described in Rosenberg et al. (1980), with some modifications (Kos et al. 2003; Palomares et al. 2007; Collado et al. 2008). After 24 h of incubation, the bacteria, which grew in the MRS broth, were harvested in the stationary phase by centrifugation at 5000 rpm for 15 min, washed twice, and resuspended in 0.1 M KNO₃ (pH 6.2), so the number of cells was approximately 10⁸ CFU mL⁻¹. The optical density of the cell suspension was measured at 600 nm (A_0). 1 mL of the solvent was added to 3 mL of cell suspension. After 10 min of incubation at room temperature, the two-phase system was mixed in Vortex, for 2 min. The aqueous phase was removed after 20 min of incubation at room temperature, and its optical density was measured at

600 nm (A_1). The percentage of bacterial adhesion to solvent was calculated as:

$$\text{Adhesion \%} = (1 - A_1/A_0) \times 100.$$

Three different solvents were tested in this study: xylene (Sineks, Belgrade, Serbia), which is an apolar solvent; chloroform (Alkaloid, Skoplje, Macedonia), a monopolar and acidic solvent; and ethyl acetate (Zorka Šabac, Šabac, Serbia) a monopolar and basic solvent. Only bacterial adhesion to xylene reflects the cell surface hydrophobicity or hydrophilicity. The values of MATS obtained with the two other solvents, chloroform and ethyl acetate, were regarded as a measure of electron donor (basic) and electron acceptor (acidic) characteristics of bacteria, respectively. According to Ocaña and Nader-Macías (2002), the percent of hydrophobicity was expressed as: 0–35%—low hydrophobicity; 36–70%—middle hydrophobicity; 71–100%—high hydrophobicity.

In vitro test for determination of adhesion ability of LAB to pig intestinal epithelium

The adhesion ability of *Lb. brevis* KGPMF35, *Lb. fermentum* KGPMF29 and *L. lactis* subsp. *lactis* biovar. *diacetylactis* KGPMF57 to the pig intestinal epithelium was tested according to the method described in Kos et al. (2003), with modifications. Pig ileal intestinal epithelium was used because of the similarity of porcine and human intestinal tracts. Ileal samples were collected from 9-month-old pig. Immediately after sacrificing the animal, the intestinal epithelium was stored at 4 °C in a refrigerator. Before the experiment, the intestinal epithelium, was cut to an appropriate length (1 cm²) and held for 30 min in PBS buffer (Alfa Aesar GmbH & Co, Karlsruhe, Germany) at 4 °C in a refrigerator, in order to loosen surface mucus. Furthermore, epithelium was washed three times in PBS buffer, mixing on a rotary shaker, in order to remove surplus of fat. Prepared samples were aseptically transferred to the Erlenmeyer flasks, which contained 20 mL of MRS broth (Torlak, Belgrade, Serbia), previously inoculated with 200 µL of with overnight culture of bacteria. Flasks were incubated for 24 h at 37 °C. After incubation, the ileal samples were washed with sterile saline, in order to remove free-floating bacteria, and fixed with methanol. After drying, the samples were staining with a florescent color, acridine orange, for 2 min. Excess color was

removed by washing with distilled water. The samples were examined and photographed by florescent microscope (Nikon, Ti-Eclipse, 400x, Austria). Intestinal epithelium in uninoculated MRS broth and in PBS buffer served as sterility control.

Statistical analysis

All data were presented as means ± standard deviations, by using Microsoft Excel (Redmond, Washington, DC, USA). Differences between bacterial tolerance to gastrointestinal conditions, as well as sensitivity on tested antibiotic were tested by using one-way ANOVA and Paired-T test. Paired T-test was used for statistical processing of the results of adhesion to different solvents. Spearman's correlation coefficient was used for determination of correlation between auto-aggregation and microbial adhesion to solvents. All statistical analyses were performed with SPSS (IBM SPSS Statistics 20).

Results

In vitro gastrointestinal transit tolerance assay

Probiotic strains need to be resistant to low pH values, since they need to pass through from the conditions of stomach. Although the optical densities of bacterial growth were reduced, all the isolates showed the growth ability at low pH (Table 1).

Among lactobacilli, only KGPMF35 isolate showed more than 100% of growth after 1 h of incubation in simulated gastric juice tolerance. The percentage of survival, after 3 h of incubation, obtained from 79.5 to 88.9%. Among lactococci, only KGPMF23 showed more than 100% of growth after 1 h of incubation, while KGPMF55 isolate showed decrease of growth. The percentage of survival was in range from 58.2 to 86.6% (Table 2).

The growth of all isolates after 4 h of incubation in stimulated small intestinal juice lead to further decrease. The percentage of survival of lactobacilli was in range from 26.2 to 63% and for lactococci was from 43.9 to 52.8% (Table 3).

Table 1 Resistance to low pH

Isolates	Growth control	pH 5	pH 4	pH 3
<i>Lb. fermentum</i> KGPMF28	1.07 ± 0.04	0.99 ± 0.02 (92.52)*	0.87 ± 0.02 (81.31)*	0.75 ± 0.04 (70.09)*
<i>Lb. fermentum</i> KGPMF29	1.02 ± 0.04	0.96 ± 0.02 (94.12)*	0.83 ± 0.02 (81.37)*	0.74 ± 0.05 (72.55)*
<i>Lb. brevis</i> KGPMF35	1.01 ± 0.04	0.97 ± 0.01 (96.04)	0.82 ± 0.02 (81.19)*	0.74 ± 0.04 (73.27)*
<i>Lb. plantarum</i> KGPMF62	1.08 ± 0.04	0.99 ± 0.02 (91.67)*	0.88 ± 0.00 (81.48)*	0.80 ± 0.03 (74.07)*
<i>Lb. plantarum</i> LP 299v	1.29 ± 0.08	1.16 ± 0.02 (89.92)*	1.15 ± 0.02 (89.15)*	1.13 ± 0.03 (87.6)*
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	1.04 ± 0.02	0.93 ± 0.00 (89.42)*	0.77 ± 0.01 (74.04)*	0.73 ± 0.03 (70.19)*
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF50	0.71 ± 0.01	0.65 ± 0.01 (91.55)*	0.21 ± 0.00 (29.58)*	0.16 ± 0.02 (22.54)*
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF54	1.02 ± 0.01	0.98 ± 0.02 (96.08)	0.86 ± 0.00 (84.31)*	0.78 ± 0.03 (76.47)*
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF55	0.84 ± 0.06	0.59 ± 0.02 (70.24)*	0.29 ± 0.03 (34.53)*	0.24 ± 0.00 (28.57)*
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF57	1.04 ± 0.05	1.00 ± 0.00 (96.15)	0.84 ± 0.01 (80.77)*	0.83 ± 0.00 (79.81)*
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF59	0.98 ± 0.05	0.85 ± 0.01 (86.73)*	0.74 ± 0.01 (75.51)*	0.69 ± 0.03 (70.41)*

Values are presented as mean ± SD measured at 600 nm; growth percentages are given in parentheses

*Statistical significance ($p < 0.05$) in growth of bacteria compared with growth control

Antibiotic sensitivity

The results of sensitivity of isolates to clinically relevant antibiotics was presented in Table 4. The results were checked according to LAB resistance criteria proposed for antibiotics of human and veterinary importance by the European Food Safety Authority (EFSA).

Lactobacillus spp. showed significant sensitivity to ampicillin and tetracycline, compared to the other tested antibiotics ($p < 0.05$). MIC values for lactobacilli obtained from 0.125 to 3 $\mu\text{g mL}^{-1}$ for tetracycline and from 0.195 to 64 $\mu\text{g mL}^{-1}$ for ampicillin. *Lactococcus* spp. showed significant sensitivity to ampicillin, tetracycline and vancomycin ($p < 0.05$). MIC values obtained from 1.56–3.125 $\mu\text{g mL}^{-1}$ for tetracycline, from 0.195–3.125 $\mu\text{g mL}^{-1}$ for ampicillin and from 1.56–3.125 $\mu\text{g mL}^{-1}$ for vancomycin.

Synthesis of biogenic amines, growth on media with phenol and haemolysis on blood agar

Tested LAB isolates showed no ability to synthesize histamine and tyramine (biogenic amines), which is desirable characteristic when selecting possible probiotics. In the medium with 0.1, 0.2 and 0.3% of phenol, the most of examined LABs grew well. The exceptions were KGPMF50 and KGPMF55 isolates, which showed no growth in medium with 0.3% of phenol. Lactobacilli and lactococci showed no α or β haemolysis.

The auto-aggregation and co-aggregation ability of LABs

The auto-aggregation ability of LAB was measured over a period of 5 h. Results indicated that the tested

Table 2 Tolerance to simulated gastric juice

Isolates	1 h	2 h	3 h	% of surviving after 3 h (CFU mL ⁻¹)
<i>Lb. fermentum</i> KGPMF28	0.63 ± 0.01 (90)*	0.62 ± 0.01 (88.57)*	0.55 ± 0.01 (78.57)	79.8 ± 0.11
<i>Lb. fermentum</i> KGPMF29	0.62 ± 0.02 (96.87)*	0.62 ± 0.02 (96.87)*	0.54 ± 0.02 (84.37)	81.6 ± 0.21
<i>Lb. brevis</i> KGPMF35	0.62 ± 0.01 (101.64)*	0.59 ± 0.03 (96.72)	0.55 ± 0.02 (90.13)	88.9 ± 0.12
<i>Lb. plantarum</i> KGPMF62	0.62 ± 0.01 (89.85)*	0.66 ± 0.02 (95.65)*	0.54 ± 0.01 (78.26)	79.5 ± 0.33
<i>Lb. plantarum</i> LP 299v	0.18 ± 0.02 (94.74)	0.17 ± 0.02 (89.47)	0.17 ± 0.00 (89.47)	89.6 ± 0.31
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	0.61 ± 0.00 (103.39)*	0.60 ± 0.04 (101.69)*	0.47 ± 0.02 (79.66)*	75.2 ± 0.20
<i>L. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i> KGPMF50	0.27 ± 0.00 (90)	0.25 ± 0.01 (83.33)*	0.25 ± 0.01 (83.33)*	86.6 ± 0.13
<i>L. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i> KGPMF54	0.58 ± 0.03 (84.06)*	0.56 ± 0.02 (81.16)	0.57 ± 0.04 (82.61)*	79.6 ± 0.26
<i>L. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i> KGPMF55	0.29 ± 0.00 (67.44)	0.31 ± 0.02 (72.09)	0.26 ± 0.01 (60.46)	58.2 ± 0.41
<i>L. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i> KGPMF57	0.60 ± 0.01 (92.31)*	0.57 ± 0.02 (87.69)*	0.54 ± 0.01 (83.08)	84.8 ± 0.33
<i>L. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i> KGPMF59	0.58 ± 0.03 (87.88)*	0.57 ± 0.02 (86.36)*	0.54 ± 0.02 (81.81)	80.4 ± 0.22

Values are presented as mean ± SD measured at 600 nm; percent of growth compared with growth control are given in parentheses

*Significant differences ($p < 0.05$) with 0 h

strains exhibited a strong auto-aggregating phenotype. The observed auto-aggregation was not lost after washing and suspending the bacterial cells in PBS, which is probably related to cell surface component. When compare the auto-aggregation between groups of bacteria, it could be concluded that it was slightly better in group of *Lactobacillus* spp. (Fig. 1), then in *Lactococcus* spp. (Fig. 2). The percentage of auto-aggregation of lactobacilli ranged from 41.89 to 53.74%. The best auto-aggregation ability was showed by *Lb. brevis* KGPMF35 isolate. The tested lactococci

showed auto-aggregation ability in a percentage ranged from 35.82 to 50.2% (Fig. 2).

Co-aggregation ability of isolated LAB with *E. coli* also was examined (Table 5). Results indicated that all tested *Lactobacillus* strains demonstrated co-aggregation with *E. coli*, while co-aggregation of *Lactococcus* spp. was a strain specific.

Table 3 Tolerance to stimulated small intestinal juice

Isolates	1 h	2 h	3 h	4 h	% of surviving after 4 h (CFU mL ⁻¹)
<i>Lb. fermentum</i> KGPMF28	0.46 ± 0.01 (59.74)	0.46 ± 0.02 (59.74)	0.40 ± 0.02 (51.95)	0.40 ± 0.01 (51.95)	41.9 ± 0.33
<i>Lb. fermentum</i> KGPMF29	0.44 ± 0.02 (61.97)	0.43 ± 0.03 (60.56)	0.36 ± 0.03 (50.70)	0.33 ± 0.01 (46.48)*	56.5 ± 0.52
<i>Lb. brevis</i> KGPMF35	0.56 ± 0.00 (90.32)	0.56 ± 0.03 (90.32)	0.50 ± 0.01 (80.65)	0.49 ± 0.00 (79.03)	63 ± 0.20
<i>Lb. plantarum</i> KGPMF62	0.51 ± 0.02 (69.86)	0.50 ± 0.01 (68.49)	0.41 ± 0.00 (56.16)*	0.41 ± 0.01 (56.16)*	26.2 ± 0.62
<i>Lb. plantarum</i> LP 299v	0.25 ± 0.01 (89.29)	0.21 ± 0.01 (75)	0.19 ± 0.02 (67.86)*	0.18 ± 0.00 (64.29)*	58.4 ± 0.20
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	0.46 ± 0.01 (71.87)*	0.49 ± 0.02 (76.56)*	0.48 ± 0.02 (75)	0.46 ± 0.01 (71.87)	52.8 ± 0.44
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF50	0.22 ± 0.00 (61.11)	0.24 ± 0.02 (66.67)*	0.22 ± 0.02 (61.11)	0.21 ± 0.01 (58.33)	52.3 ± 0.12
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF54	0.50 ± 0.01 (63.29)	0.49 ± 0.00 (62.02)	0.40 ± 0.02 (50.63)*	0.39 ± 0.02 (49.37)*	49.4 ± 0.08
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF55	0.25 ± 0.01 (56.82)	0.26 ± 0.02 (59.09)	0.23 ± 0.01 (52.27)*	0.22 ± 0.02 (50)	43.9 ± 0.23
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF57	0.49 ± 0.02 (79.03)*	0.47 ± 0.00 (75.81)	0.40 ± 0.00 (64.52)	0.38 ± 0.01 (61.29)	51.3 ± 0.21
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF59	0.45 ± 0.05 (62.5)	0.46 ± 0.03 (63.89)*	0.40 ± 0.02 (55.56)	0.39 ± 0.02 (54.17)	55.6 ± 0.64

Values are presented as mean ± SD measured at 600 nm; growth percentages are given in parentheses

*Significant differences ($p < 0.05$) with 0 h

The ability of adhesion to different solvents

The MATS method was used to evaluate the hydrophobic/hydrophilic of the cell surface properties of LABs. *Lb. plantarum* LP 299v was used for comparative purposes. In order to assess the Lewis acid–base characteristics of the bacterial cell surfaces, bacterial adhesion with chloroform and ethyl acetate was tested. The results indicated that tested bacteria had a stronger affinity with chloroform (10.79–21.57%), which is an acidic solvent and

electron acceptor, than with ethyl acetate (4.95–12.84%), which is a basic solvent and electron donor. Affinity for xylene was not observed (Table 6).

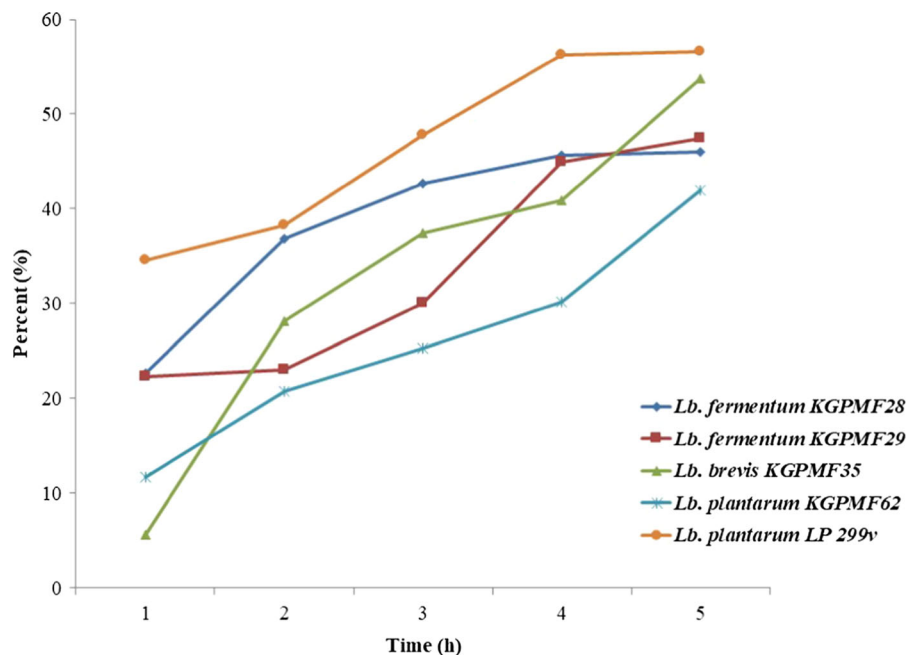
In vitro adhesion to pig intestinal epithelium

The ability of LABs isolated from cheese to adhere to pig epithelium, was investigated, using the fluorescence microscope. The adhesion was noticed for all selected bacteria (Fig. 3).

Table 4 Antibiotic sensitivity of isolated lactic acid bacteria

Isolates	Tetracycline MIC*	Gentamicin	Polymyxin B	Ampicillin	Vancomycin
<i>Lb. fermentum</i> KGPMF28	2	2.5	12.5	0.195	n.r
<i>Lb. fermentum</i> KGPMF29	2	2.5	12.5	0.195	n.r
<i>Lb. brevis</i> KGPMF35	3	2.5	12.5	0.195	n.r
<i>Lb. plantarum</i> KGPMF62	2	8	12.5	0.195	n.r
<i>Lb. plantarum</i> LP 299v	0.125	n.d	n.d	64	n.r
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	3.125	2	12.5	0.195	3.125
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetyllactis</i> KGPMF50	3.125	12.5	25	1.56	1.56
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetyllactis</i> KGPMF54	3.125	18.75	12.5	3.125	3.125
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetyllactis</i> KGPMF55	2.5	12.5	25	0.195	1.56
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetyllactis</i> KGPMF57	3.125	18.75	25	1.56	3.125
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetyllactis</i> KGPMF59	1.56	12.5	12.5	1.56	3.125

*MIC minimal inhibitory concentration; Values are given in $\mu\text{g mL}^{-1}$; *n.d.* not determined; *n.r.* not required according to EFSA

**Fig. 1** The auto-aggregation ability of *Lactobacillus* spp. isolated from cheese

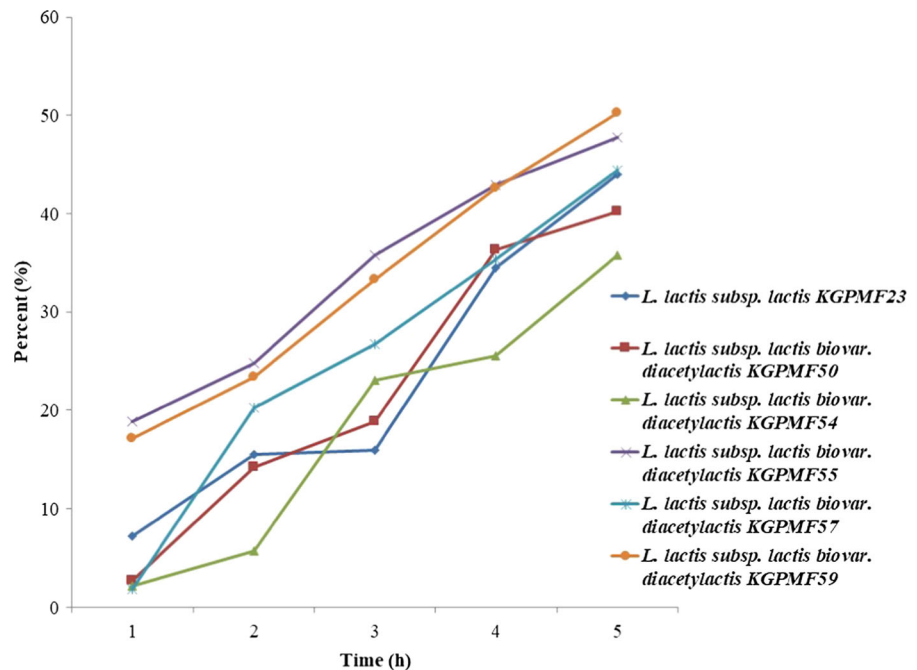
Discussion

In this paper, for the first time, it was investigated the probiotic potential, as well as the adhesion and aggregation ability of ten strains of LAB, isolated from traditionally made cheese from Southeastern Serbia. All isolates showed a tolerance to simulated

gastrointestinal conditions and potential for further investigation. Similar results have been previously reported by other studies, where authors analyzed LABs isolated from different fermented products (Ramos et al. 2013; Leite et al. 2015; Pavli et al. 2016).

Tetracycline, ampicillin, gentamicin, vancomycin and polymyxin B (a part of mixed therapy of bacterial

Fig. 2 The auto-aggregation ability of *Lactococcus* spp. isolated from cheese



vaginosis) are chosen in order to detect a wide range of determinants for resistance. According to EFSA (2012), “any bacterial strain carrying an acquired resistance to antimicrobial that is shown to be due to the acquisition of genetic determinant presents the greatest potential for horizontal spread and should not be used as a feed additive”. However, the use of probiotics is clinically relevant for patients using antimicrobial drugs, thus, being susceptible to antimicrobials, makes the strains unsuitable to this purpose. But, Pereira et al. (2015) indicated that there is a lack of agreement on the interpretive breakpoints for probiotic bacteria. Also, they indicated that data that confirms antimicrobial susceptibility of *Lactobacillus* strains are scarce. Uroić et al. (2014) investigated antibiotics sensitivity of LAB isolated from Serbian and Croatian cheeses and showed that all isolates were susceptible to the antibiotics, which was confirmed in our study. Leite et al. (2015) indicated that lactococci, isolated from Brazilian kefir, were susceptible to the tetracycline, erythromycin, clindamycin, ampicillin, and aminoglycosides. The susceptible to the vancomycin was the exception. In our study, lactococci were sensitive to vancomycin. The results observed in this paper conformed that tested LABs showed no resistance to tested antibiotics, according to EFSA (2012) breakpoints, so they are safe for clinical use.

But, non-parameterized MICs and breakpoints for probiotic bacteria are still a technical problem, because we do not have all needed parameters for antimicrobial susceptibility of LAB, given their clinical relevance as probiotics (Pereira et al. 2015).

There are many studies which indicated that the biofilm formation of LAB is associated with adhesion properties (Elhadidy and Zahran 2014; Živković et al. 2016; Popović et al. 2018). Previously studies of LAB, isolated from Sokobanja cheese, indicated that they showed the ability to produce moderate biofilm (Muruzović et al. 2018a, c). In the present study, their ability of adhesion with different solvents was demonstrated. Tested *Lactobacillus* isolates showed a significant correlation between auto-aggregation ability and affinity with chloroform ($p < 0.05$). In general, tested isolates showed a better affinity with chloroform (acidic solvent) than with ethyl acetate (basic solvent) ($p < 0.05$). Kaewnopparat et al. (2013) indicated that LAB possessed the ability to colonize the human gut and to increase the concentration of excreted antimicrobial substances in the process of co-aggregation. This could be one more mechanism for the control of development of pathogen strains. *Lactobacillus* spp., isolated from Serbian cheese, showed a high percent of co-aggregation with *E. coli* clinical isolate, after 2 h of incubation. Li et al. (2015)

Table 5 Co-aggregation ability of LAB in PBS

Isolates	Co-aggregation with <i>E. coli</i> (%)
<i>Lb. fermentum</i> KGPMF28	41.45 ± 1.30 ^a
<i>Lb. fermentum</i> KGPMF29	34.65 ± 2.68 ^b
<i>Lb. brevis</i> KGPMF35	39.06 ± 2.12 ^a
<i>Lb. plantarum</i> KGPMF62	31.82 ± 2.41 ^{b,c}
<i>Lb. plantarum</i> LP 299v	13.92 ± 3.22 ^d
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	2.84 ± 3.78 ^a
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF50	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF54	3.59 ± 2.46 ^a
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF55	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF57	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF59	0

Results are presented as mean ± standard deviation from three separate experiments; means of the isolates that's belong to different genera, with superscript with different letters, are significantly different ($p < 0.05$)

Table 6 Adhesion ability of LAB to xylene, chloroform and ethyl acetate

Isolates	Adhesion (%)		
	Xylene	Chloroform	Ethyl acetate
<i>Lb. fermentum</i> KGPMF28	0	16.83 ± 1.23a	10 ± 0.84 ^a
<i>Lb. fermentum</i> KGPMF29	0	18.31 ± 0.95a	12.84 ± 0.47 ^a
<i>Lb. brevis</i> KGPMF35	0	21.57 ± 1.02a	4.95 ± 1.07 ^b
<i>Lb. plantarum</i> KGPMF62	0	10.79 ± 1.85a	7.92 ± 0.26 ^a
<i>Lb. plantarum</i> LP 299v	0	21.54 ± 0.74a	4.43 ± 0.18 ^b
<i>L. lactis</i> subsp. <i>lactis</i> KGPMF23	0	8.15 ± 2.01	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF50	0	30.62 ± 0.65a	52.33 ± 0.03 ^b
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF54	0	93.46 ± 0.22	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF55	0	30.82 ± 0.25a	31.03 ± 0.28 ^a
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF57	0	91.08 ± 0.38	0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> KGPMF59	0	59.75 ± 0.75	0

Results are presented as mean ± standard deviation from three separate experiments; means in the two solvents columns or each particular isolate, with superscript with different letters, are significantly different ($p < 0.05$)

indicated that selecting LABs with higher adhering ability, according to the aggregation ability, is not a desirable method, because, so far, some authors showed that these characteristics were strain specific (Lee and Salminen 2009; Li et al. 2015), which was confirmed in our research, too. Janković et al. (2012) indicated that *Lb. plantarum*, isolated from home-made cow and sheep cheeses, showed a co-aggregation ability with some food-borne pathogens. Idoui (2014) demonstrated the ability of *Lb. fermentum* to co-aggregate with *Salmonella* spp., *Klebsiella* spp. and *E. coli*.

Some studies indicated that selection of probiotic LAB primarily was done based on their

hydrophobicity against xylene (Palomares et al. 2007), hexadecane (Pringsulaka et al. 2015) and toluene (Dowarah et al. 2018). The ability of adherence and colonization of intestinal epithelium cells of the host are important characteristic which have a role in the inhibition of the colonization by pathogens strains (Pringsulaka et al. 2015). Dowarah et al. (2018) reported the ability of in vitro adhesion by *Lb. acidophilus* PF01, *Lb. acidophilus* CF07 and *Pedio-coccus acidilactici* FT28 to duodenal epithelium cells of pig. The adhesion of LABs, isolated from Serbian cheese, was confirmed through the in vitro investigation of the adhesion ability to pig intestinal epithelium cells.

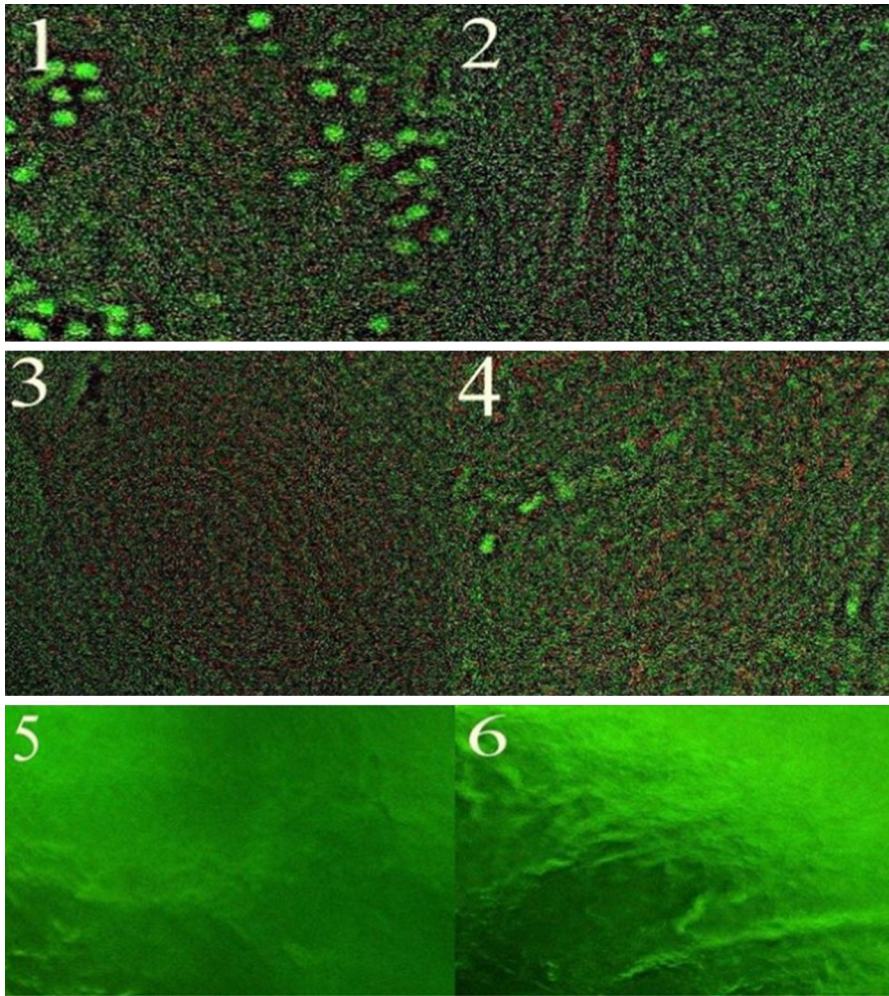


Fig. 3 Adhesion ability of selected LAB to pig intestinal epithelium (1-*Enterococcus faecalis* KGPMG47; 2-*Lb. brevis* KGPMF35; 3-*Lb. fermentum* KGPMF29; 4-*L. lactis* subsp. *lactis* biovar. *diacetylactis* KGPMF57; 5-Sterility control (MRS broth); 6-Sterility control (PBS buffer))

Conclusions

The tested LABs, isolated from Sokobanja's cheese, showed tolerance to extremes of gastrointestinal conditions, sensitivity to selected antibiotics, as well as the ability to survive in the presence of different concentrations of phenol. They showed no ability to synthesize histamine and tyramine, which is desirable characteristic when selecting possible probiotics or starter cultures. All isolates showed the ability of auto-aggregation and the co-aggregation with *E. coli* clinical isolate. The adhesion ability of bacteria is an important characteristic, because LAB, with process this ability, can be used as a mechanical barrier for

adhesion of other bacteria, like enterobacteria or potential pathogenic bacteria, to epithelium. Based on the results of tolerance to extremes of gastrointestinal conditions, adhesion and aggregation ability, it could be concluded that KGPMF28, KGPMF29, KGPMF35 and KGPMF57 isolates showed the potential for future investigation and usage.

Acknowledgements This investigation was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 41010).

Author contributions MŽG conceived and designed the experiments; DDN designed and photographed samples by florescent microscope and interpreted the results of adhesion;

MŽG and KGM processed the results; KGM and LRC take charge of the preparation of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest with the current work or its publication.

Research involving human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Bassyouni RH, Abdel-all WS, Fadl MG, Abdel-all S, Kamel Z (2012) Characterization of lactic acid bacteria isolated from dairy products in Egypt as a probiotic. *Life Sci J* 9:2924–2933
- Carasi P, Ambrosio NM, De Antoni GL, Bressollier P, Urdaci MC, de los Angeles Serradell M (2014) Adhesion properties of potentially probiotic *Lactobacillus kefir* to gastrointestinal mucus. *J Dairy Res* 81(1):16–23
- Collado MC, Meriluoto J, Salminen S (2008) Adhesion and aggregation properties of probiotic and pathogen strains. *Eur Food Res Technol* 226:1065–1073
- Dowarah R, Verma AK, Agarwal N, Singh P, Singh BR (2018) Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. *PLoS ONE* 13(3):e0192978. <https://doi.org/10.1371/journal.pone.0192978>
- Elhadidy M, Zahran E (2014) Biofilm mediates *Enterococcus faecalis* adhesion, invasion and survival into bovine mammary epithelial cells. *Lett Appl Microbiol* 58:248–254
- European Food Safety Authority-EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human or veterinary importance. *EFSA J* 10:1–10
- FAO/WHO (World Health Organization) (2006) Probiotics in food. Health and nutritional properties and guidelines for evaluation. *FAO Food Nutr Pap* 85:2.
- Garriga M, Rubio R, Aymerich T, Ruas-Madiedo P (2014) Potentially probiotic and bioprotective lactic acid bacteria starter cultures antagonise the *Listeria monocytogenes* adhesion to HT29 colonocyte-like cells. *Benef Microbes* 6(3):337–343
- Hernandez-Hernandez O, Muthaiyan A, Moreno FJ, Montilla A, Sanz ML, Rickett SC (2012) Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. *Food Microbiol* 30:355–361
- Huang Y, Adams MC (2004) *In vitro* assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. *Int J Food Microbiol* 91:253–260
- Idou T (2014) Probiotic properties of *Lactobacillus* strains isolated from gizzard of local poultry. *Iran J Microbiol* 6(2):120–126
- Janković T, Frece J, Abram M, Gobin I (2012) Aggregation ability of potential probiotic *Lactobacillus plantarum* strains. *Int J Sanit Eng Res* 6:19–24
- Jeong DW, Lee JH (2015) Antibiotic resistance, hemolysis and biogenic amine production assessments of *Leuconostoc* and *Weissella* isolates for kimchi starter development. *LWT Food Sci Technol* 64:1078–1084
- Kaewnopparat S, Dangmanee N, Kaewnopparat N, Srichana T, Chulasiri M, Settharaksa S (2013) *In vitro* probiotic properties of *Lactobacillus fermentum* SK5 isolated from vagina of a healthy woman. *Anaerobe* 22:6–13
- Kaktcham PM, Zambou NF, Tchouanguep FM, El-Soda M, Choudhary MI (2012) Antimicrobial and safety properties of lactobacilli isolated from two Cameroonian traditional fermented foods. *Sci Pharm* 80:189–203
- Kos B, Šušković J, Vuković S, Šimpraga M, Frece J, Matošić S (2003) Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *J Appl Microbiol* 94:981–987
- Kumar AM, Murugalatha N (2012) Isolation of *Lactobacillus plantarum* from cow milk and screening for the presence of sugar alcohol producing gene. *J Clin Microbiol Antimicrob* 4:16–22
- Lee YK, Salminen S (2009) Handbook of probiotics and prebiotics. Wiley, pp 386
- Leite AMO, Miguel MAL, Peixoto RS, Ruas-Madiedo P, Paschoalin VMF, Mayo B, Delgado S (2015) Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. *J Dairy Sci* 98:3622–3632
- Li Q, Liu X, Dong M, Zhou J, Wang Y (2015) Aggregation and adhesion abilities of 18 lactic acid bacteria strains isolated from traditional fermented food. *Int J Agric Policy Res* 3(2):84–92
- Magdoub MNI, Hassan ZMR, Effat BAM, Sadek ZIM, Tawfik NF, Mabrouk AMM (2015) Probiotic properties of some lactic acid bacteria isolated from Egyptian dairy products. *Int J Curr Microbiol Appl Sci* 4:758–766
- Muruzović MŽ, Mladenović KG, Stefanović OD, Vasić SM, Čomić LR (2016) Extracts of *Agrimonia eupatoria* L. as sources of biologically active compounds and evaluation of their antioxidant, antimicrobial, and antibiofilm activities. *J Food Drug Anal* 24:539–547
- Muruzović MŽ, Mladenović KG, Žugić Petrović TD, Čomić LR (2018a) Characterization of lactic acid bacteria isolated from traditionally made Serbian cheese and evaluation of their antagonistic potential against Enterobacteriaceae. *J Food Process Preserv* 42(4):e13577. <https://doi.org/10.1111/jfpp.13577>
- Muruzović MŽ, Mladenović KG, Đilas MD, Stefanović OD, Čomić LR (2018b) *In vitro* evaluation of antimicrobial potential and ability of biofilm formation of autochthonous *Lactobacillus* spp. and *Lactococcus* spp. isolated from traditionally made cheese from Southeastern Serbia. *J Food Process Preserv* 42(11):13776
- Muruzović MŽ, Mladenović KG, Čomić LR (2018) *In vitro* evaluation of resistance to environmental stress by planktonic and biofilm form of lactic acid bacteria isolated from traditionally made cheese from Serbia. *Food Biosci* 23:54–59
- Ocaña V, Nader-Macías ME (2002) Vaginal lactobacilli: self- and co-aggregation ability. *Br J Biomed Sci* 59:183–190
- Palomares IC, Morales PR, Felix AE (2007) Evaluation of probiotic properties in *Lactobacillus* isolated from small

- intestine of piglets. *Rev Latinoameric Microbiol* 49(3–4):46–54
- Pavli PG, Argyri AA, Papadopoulou OS, Nychas GE, Chorianopoulos NG, Tassou CC (2016) Probiotic potential of lactic acid bacteria from traditional fermented dairy and meat products: assessment by *in vitro* tests and molecular characterization. *J Probiotics Health* 4:157–165
- Pereira NG, Figueiredo FJB, Dias-Souza MV (2015) Antimicrobial susceptibility of commercial probiotic *Lactobacillus* strains. *J Appl Pharm Sci* 2(2):14–17
- Popović N, Dinić M, Tolinački M, Mihajlović S, Terzić-Vidojević A, Bojić S, Djokić J, Golić N, Veljović K (2018) New insight into biofilm formation ability, the presence of virulence genes and probiotic potential of *Enterococcus* sp. dairy isolates. *Front Microbiol* 9:78
- Pringsulaka O, Rueangyotchanthana K, Suwannasai N, Watanapokasin N, Amnueysit P, Sunthornthummas S, Sukkhum A, Sarawaneeayarak S, Rangsiruji A (2015) *In vitro* screening of lactic acid bacteria for multi-strain probiotics. *Livestock Sci* 174:66–73
- Ramos CL, Thorsen L, Schwan RF, Jespersen L (2013) Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiol* 36:22–29
- Rosenberg M, Gutnick D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett* 9:29–33
- Sarker SD, Nahar L, Kumarasamy Y (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* 42:321–324
- Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alithee NB, Jahromi MF, Ho YW (2014) Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. *BioMed Res Int* 2014:1–16
- Sitepu GR, Nursyirwani N, Efriyeldi E (2016) Adhesion of lactic acid bacteria (LAB) to intestinal epithelial cells of red snapper (*Lutjanus argentimaculatus*) in inhibiting *Vibrio alginolyticus*. *J Online Mahasiswa* 3(2):1–10
- Solieri L, Bianchi A, Mottolese G, Lemmetti F, Giudici P (2014) Tailoring the probiotic potential of non-starter *Lactobacillus* strains from ripened Parmigiano Reggiano cheese by *in vitro* screening and principal component analysis. *Food Microbiol* 38:240–249
- Šušková J, Kos B, Goreta J, Matosić S (2001) Role of lactic acid bacteria and bifidobacteria in symbiotic effect. *Food Technol Biotechnol* 39:227–235
- Tuo Y, Yu H, Ai L, Wu Z, Guo B, Chen W (2013) Aggregation and adhesion properties of 22 *Lactobacillus* strains. *J Dairy Sci* 96:4252–4257
- Uroić K, Nikolić M, Koslć B, Pavunc L, Beganović J, Lukić J, Jovčić B, Filipić B, Miljković M, Golić N, Topisirović L, Čadež N, Raspor P, Šušková J (2014) Probiotic properties of lactic acid bacteria isolated from Croatian fresh soft cheese and Serbian white pickled cheese. *Food Technol Biotechnol* 52:232–241
- Vesković Moračanin S, Djukić D, Zdolec N, Milijašević M, Mašković P (2017) Antimicrobial resistance of lactic acid bacteria in fermented food. *J Hyg Eng Des* 18:25–35
- Walsham ADS, MacKenzie DA, Cook V, Wemyss-Holden S, Hews CL, Juge N, Schüller S (2016) *Lactobacillus reuteri* inhibition of enteropathogenic *Escherichia coli* adherence to human intestinal epithelium. *Front Microbiol* 7:244
- Younes JA, van der Mei HC, van den Heuvel E, Busscher HJ, Reid G (2012) Adhesion forces and coaggregation between vaginal staphylococci and lactobacilli. *PLoS ONE* 7(5):1–8
- Živković M, Miljković MS, Ruas-Madiedo P, Markelić MB, Veljović K, Tolinački M, Sokocić S, Korać A, Golić N (2016) EPS-SJ exopolysaccharide produced by the strain *Lactobacillus paracasei* subsp. *paracasei* BGSJ2-8 is involved in adhesion to epithelial intestinal cells and decrease on *E. coli* association to Caco-2 cells. *Front Microbiol* 7:286
- Zoumpopoulou G, Foligne B, Christodoulou K, Grangette C, Pot B, Tsakalidou E (2008) *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential *in vitro* and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and *Salmonella* infection in murine models. *Int J Food Microbiol* 121:18–26

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.