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Probiotic properties and safety assessment of lactic acid bacteria isolated from kajmak

Jelena Rajković, Nataša Joković[a]*

University of Niš, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Višegradska 33, 18000 Niš, Serbia

** E-mail: jole35@mts.rs*

***Abstract*:**

Rajković, J., Joković, N.: ***Probiotic properties and safety assessment of lactic acid bacteria isolated from kajmak. Biologica Nyssana, 6 (2), December 2015: 81-89.***

One hundred forty-one strains of lactic acid bacteria (LAB) were tested for probiotic features such as antimicrobial activities, autoaggregation ability, hydrophobicity of bacterial cell walls and ability to grow on and hydrolyze bile salts. Additionally, resistance to antibiotics and production of biogenic amines were performed as safety assessment of LAB strains. Five *Lc. lactis* ssp. *lactis* and six *Lc. raffinolactis* strains showed antibacterial activities against used indicator strains. Protein nature of antibacterial compounds was proven by the addition of pronase. *Enterococcus durans* strains had the highest degree of autoaggregation and hydrophobicity while the rest of the strains showed low degree for both characteristics. *Lb. paracasei, Lb. plantarum*, *Ln. mesenteroides*, *En. faecium*, *En. faecalis* and *En. durans* strains could growth on bile salts and some of them were able to hydrolyse them. Safety assessment of LAB indicates that many strains of enterococci were resistant to chloramphenicol, tetracycline, cephalosporin and to the lowest used concentration of erythromycin. The strains from other LAB genera were resistant to antibiotics used in lower concentration and in smaller numbers when compared to enterococci.

Key words: Lactic acid bacteria, Probiotic properties, Safety assessment

***Apstrakt*:**

Rajković, J., Joković, N.: ***Probiotska svojstva i bezbednost upotrebe mlečno kiselinskih bakterija izolovanih i kajmaka. Biologica Nyssana, 6 (2), December 2015: 81-89.***

Probiotska svojstva mlečno kiselinskih bakterija kao što su antimikrobna aktivnost, autoagregacija, hidrofobnost i sposobnost da rastu na žučnim solima i vrše njihovu hidrolizu ispitivana su na sto četrdeset jednom bakterijskom izolatu. Pored toga, ispitivana je rezistentnost ovih bakterijih izolata na antibiotike i sposobnost produkcije biogenih amina u okviru procene bezbednosti izolata za upotrebu u starter kulturama. Pet *Lc. lactis* ssp. *lactis* i šest *Lc. raffinolactis* izolata pokazala su antimikrobno delovanje prema korišćenim indikatorskim sojevima. Proteinska priroda antimikrobnih jedinjenja je potvrdjena dodavanjem pronaze. Najveći stepen hidrofobnosti i autoagregacije imali su *En. durans* izolati, dok su kod ostalih izolata ova probiotska svojstava bila izražena u manjem stepenu. *Lb. paracasei, Lb. plantarum, Ln. mesenteroides, En. faecium, En. faecalis* i *En. durans* izolati su pokazali rast na podlogama sa žučnim solima, dok su neki od njih imali sposobnost da vrše njihovu hidrolizu. Procenom sigurnosti upotrebe izolata utvrđeno je da su mnogi izolati enterokoka bili rezistentni na hloramfenikol, tetraciklin, cefalosporin i na najniže korišćene koncentracije eritromicina. Izolati iz ostalih rodova mlečno kiselinskih bakterija su bili rezistentni na niske koncentracije antibiotika, ali u manjem broju u poređenju sa enterokokama.

Key words: mlečno kiselinske bakterije, probiotska svojstva, procena bezbednosti

Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of bacteria, including Gram-positive cocci and bacilli, with common feature to produce lactic acid as the end-product of metabolism. They are mainly isolated from the various fermented foods produced from milk, meat or vegetables but also present a normal microflora of the nasopharyngeal, intestinal and vaginal mucosa of humans and animals (Stiles & Holzapfel, 1997). Various strains of LAB are widely used in the industry as a part of starter cultures for improving the quality and shelf life of fermented foods in different food processes.

In recent years, LAB has been intensively studied as probiotic bacteria. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit to the host" (FAO/WHO, 2002). In dairy products bifidobacteria and lactobacilli are commonly used as probiotic bacteria, but other LAB, such as enterococci, lactococci or streptococci, also may possess probiotic properties (Pringsulaka et al., 2015). Intake of probiotic bacteria could lead to the establishment of intestinal microbial balance, prevention of allergies and alleviation of certain intolerances. Antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis, and immunomodulatory effects on the host have been also reported for some probiotic bacteria (Chiang & Pan, 2012).

Considering the fact that beneficial effects of LAB are strain-dependent, screening of LAB isolated from different sources for probiotic properties is a common way for searching new probiotic bacteria. Synthesis of antimicrobial compounds, adhesion potential to the intestinal epithelium and ability to survive conditions in the gastrointestinal tract are the characteristics that have been studied for preliminary selection of potential probiotics (Bautista-Gallego et al., 2013; Pringsulaka et al., 2015; Angmo et al., 2016). The strains of LAB which have capacity to produce different antimicrobial compounds could inhibit the pathogenic bacteria in gastrointestinal tract, thus reducing the risk of gastrointestinal diseases. In order to achieve the effect in intestine, probiotic bacteria must have ability to pass through upper parts of gastrointestinal tract and adhere to intestinal epithelial cells. Conditions in the upper gastrointestinal tract, such as pH values and bile salts, may adversely affect the probiotic bacteria. Therefore, it is necessary to examine the possibility of survival of the probiotic bacteria in the presence of bile salts. Aggregation ability and hydrophobicity of bacterial cell walls are correlated to adherence properties of probiotic bacteria (Duary et al., 2011; Janković et al., 2012). Required property of potential probiotic bacteria is the safety for human consumption. Resistance to different antibiotics, the presence of virulence factors and synthesis of biogenic amines are commonly tested undesirable characteristics of probiotic bacteria (Chamba & Jamet, 2008).

Traditional fermented products are the source of the new probiotic bacteria that can be used in the production of various types of functional foods. The aim of current study was screening of LAB isolated from Serbian traditional dairy product, kajmak, for some probiotics features such as antimicrobial activities, autoaggregation ability, hydrophobicity of bacterial cell walls and the ability to grow on and to hydrolyze bile salts. Additionally, LAB strains were tested for resistance to antibiotics and production of biogenic amines as safety assessment of strains.

Material and methods

Bacterial strains, media, and growth conditions

One hundred forty-one strains of LAB isolated from different samples of kajmak were used in this study. All strains were previously identified by molecular methods to the species level as *Lactococcus lactis* ssp. *lactis* (19), *Lactococcus raffinolactis* (6), *Streptococcus thermophilus* (4), *Enterococcus faecalis* (9), *Enterococcus faecium* (19), *Enterococcus durans* (10), *Leuconostoc mesenteroides* (50), *Lactobacillus plantarum* (15), *Lactobacillus paracasei* (6), *Lactobacillus satsumensis* (1), *Lactobacillus kefiri* (1) and *Lactobacillus kefiranofaciens* (1) (Jokovic et al., 2008; Joković et al., 2014). The strains were stored at -20 °C and activated by triple streaking on appropriate media.

MRS agar (Merck, GmbH, Darmstadt, Germany) was used for growth of *Leuconostoc* and *Lactobacillus* strains whereas *Lactococcus*, *Streptococcus* and *Enterococcus* strains were cultured on M17 agar (Merck, GmbH, Darmstadt, Germany) supplemented with glucose (0.5%, w/v; GM17 broth). The inoculated media were incubated overnight at 30 °C for mesophilic and 37 °C for thermophilic strains.

Tryptic soy agar (Himedia, India) was used for the growth of non-LAB strains (*Escherichia coli*, *Bacillus subtilis* and *Listeria innocua*) which were incubated at 37 °C for 24 h. Yeast (*Candida albicans*) was cultured on Sabouraud maltose agar (Torlak, Serbia) at 30 °C for 48 h.

Antimicrobial activity of strains

The LAB strains were screened for antimicrobial activity by the agar-well diffusion method (Tagg & McGiven, 1971) using various indicator strains that are listed in Table 1. Asssay was performed by overlaying solid MRS or GM17 medium in Petri dishes with 5 ml of soft (0.7% agar) MRS, GM17, Tryptic soy and Sabouraud maltose media inoculated with 10^5-10^6 cells of indicator strain/ml medium. The wells, 5 mm in diameter, were made in soft agar and filled with 100 μl of overnight strain culture with a potential of bacteriocin production. The presence of antimicrobial substances was detected by the appearance of clear zones around wells as a result of growth inhibition of sensitive bacterial strains.

The protein nature of bacteriocins was confirmed by adding a cristal of pronase E near to the edge of the well. Absence of a zone of inhibition in the place where pronase E crystals were added indicates the protein nature of bacteriocins.

Adhesive properties of strains

Bacterial adhesion to hydrocarbons was determined by the method described by Fortina et al. (2008). Cells from overnight culture were struck down by centrifugation at 5000 rpm for 15 min, washed twice in PBS buffer and resuspended in 0.1 M KNO_3 (pH 6.2). The initial absorbance of the cell suspension was adjusted to 0.5-0.6 at 600 nm (A_0). Then, 3 ml of cell suspension was mixed with 1 ml of *n*-hexadecane. The mixture was incubated at room temperature for 10 min and mixed well on vortex for 2 min. After 20 min of incubation at room temperature, the aqueous phase was carefully separated and the optical density was measured at 600 nm (A_t). The percentage of bacterial adhesion to *n*-hexadecane was expressed as: % hydrophobicity = 1- (A_t/A_0) x100, where A_0 and A_t represent absorbance values of aqueous phase before and after contact with *n*-hexadecane.

Aggregation of strains was monitored in phosphate buffered saline (PBS) according to Fortina et al. (2008). Bacterial cells from overnight cultures were centrifuged at 5000 rpm for 15 min, washed twice in PBS and resuspended in 4.0 mL of PBS to give an OD_{600nm} of 1.0. The cell suspensions were further well mixed on a vortex for 10 s and autoaggregation was determined during 5 h of incubation at room temperature. Every hour, 100 μl from the surface of the suspension was transferred to new microtube containing 900 μl PBS buffer and OD_{600nm} was measured. The percentage of aggregated cells was calculated as 1-(A_t/A_0) x100 where A_0 represents absorbance values at time t=0, while A_t represents absorbance values at time t= 5 h.

The growth of strains on bile salts

The growth of strains in the presence of bile salts was monitored on LAPTg medium consisted of: 15 g/l peptone, 10 g/l tryptone, 10 g/l yeast extract, 10 g/l glucose, 15 g/l agar, 0.01 ml/l of tween 80 and 0.3 g/l bovine bile (Fortina et al., 2008).

The ability of strains to hydrolyse bile salts was detected by precipitation of deconjugated bile acids around the colony that grew on the LAPTg medium without bovine bile and with the addition of 0.05 g/l bile salts No. 3 (Torlak, Belgrade, Serbia) and 0.2 g/l $CaCl_2$. A positive reaction for the presence of hydrolase is the appearance of a precipitate around the colonies.

Safety assessment

The resistance of strains to antibiotics was tested by spot agar test on antibiotic-containing GM17 and MRS agar media optimal for LAB growth. Tests were done for the following antibiotics and concentration: chloramphenicol (10 μg/ml, 20 μg/ml), tetracycline (10 μg/ml, 20 μg/ml, 30 μg/ml) and erythromycin (5 μg/ml, 10 μg/ml).

Table 1. List of strains used in antimicrobial activity assay

Bacterial strains	Source or reference
Lactococcus lactis ssp. *lactis* biovar. Diacetylactis, S50	(Kojic et al., 2005)
Lactococcus lactis ssp.*cremoris,* NS1	(Kojic et al., 1991)
Lactococcus lactis ssp. *Lactis,* BGMN1-596	(Gajic et al., 1999)
Lactococcus lactis ssp. *Lactis,* NP45	Lab. kolekcija
Lactobacillus paracasei ssp. *Paracasei,* BGBUK2-16/K4	(Lozo et al., 2004)
Lactobacillus plantarum, A112	(Vujcic & Topisirovic, 1993)
Escherichia coli, ATCC 25923	ATCC
Bacilus subtilis, ATCC 6633	ATCC
Listeria innocua, ATCC 33090	ATCC
Candida albicans, ATCC 10231	ATCC

The ability of LAB isolates to produce biogenic amines was determined according to the method described by Bover-Cid and Holzapfel (1999). Histidine, tyrosine, ornithine and lysine were used as precursors for the biogenic amines synthesis. The color change of colonies to purple or occurrence of precipitates around the colonies in case of tyramine synthesis indicated the decarboxylation activities of isolates.

Results and discussion

Antimicrobial activity

LAB produce different substances that have antimicrobial activity, such as an organic acid (lactic, formic, acetic acid etc.), CO_2, H_2O_2, diacetyl, ethanol, and bacteriocins. Among these products, bacteriocins are the most important because of their possible application in the food industry as biopreservatives (De Vuyst & Leroy, 2007). Since bacteriocins have the highest inhibitory effect against related Gram positive bacteria, bacteria from genus *Lactococcus*, *Lactobacillus*, *Bacillus* and *Listeria* were used as indicator strains (**Tab. 1**). Additionally, Gram negative bacteria *E. coli* and yeast *C. albicans* were also used in antimicrobial assays.

Results of antimicrobial assays showed that a small number of strains could synthesize bacteriocins (**Tab. 2**). Five *Lc. lactis* ssp. *lactis*, and six *Lc. raffinolactis* strains produced bacteriocins with inhibitory effect on the growth of lactococcal indicator strains *Lc. lactis* ssp. *cremoris* NS1, *Lc. lactis* ssp. *lactis* BGMN1-596 and *Lc. lactis* ssp. *lactis* NP45 (**Tab. 2**). Lactococcal strains formed

Table 2. Antimicrobial effect of tested strains shown through the zones of inhibition

Species	***No. of isolates***	***Indicator strains***					
		Lactococcus lactis ssp. *lactis* BGMN1-596	*Lactococcusl actis* ssp. *cremoris* NS1	*Lactococcus lactis* ssp. *lactis* NP45	*Lactococcus lactis* ssp. *lactis* biovar. diacetylactis S50	*Escherichia coli* ATCC2592	*Listeria innocua* ATCC 33090
Lc. lactis ssp. *lactis*	3	1 mm S	1 mm S	1 mm S	-	-	-
Lc. lactis ssp. *lactis* biovar. diacetylactis	4	7 mm S	7 mm S	3 mm S	-	-	-
Lc. raffinolactis	6	5 mm S	5 mm S	3 mm S	2 mm S	1 mm S	2 mm S
En. faecium	1	-	-	-	2 mm S		5 mm S
En. faecalis	1	4 mm S	2 mm S	-	-	-	0.5 mm S

mm – zone width; S – clear zone.

Table 3. Autoaggregation, hydrophobicity, growth on bile salts and hydrolysis of bile salts of tested LAB strains

	Lc. lactis ssp. *lactis* (19)[1]	*Lc. raffinolactis (6)*	*St. thermophilus* (4)	*En. faecalis (9)*	*En. faecium (19)*	*En. durans (10)*	*Ln. mesenteroides (50)*	*Lb. parcasei (6)*	*Lb. plantarum (15)*	*Lb. satsumensis (1)*	*Lb. kefiri (1)*	*Lb. kefiranofaciens (1)*
Hydrophobicity (%)[2]	19	2	10	14	28	45	17	6	6	27	2	3
Autoaggregation (%)[2]	39	31	36	31	44	71	36	50	27	36	8	37
Growth in the presence of bile salts[3]	-	-	-	4	10	10	3	3	9	-	-	-
Hydrolysis of bile salts [3]	-	-	-	1	4	5	3	3	8	-	-	-

1 -numbers in parenthesis represent the number of isolates
2 -results are presented as mean value
3 -results are shown as the number of isolates that has a certain property

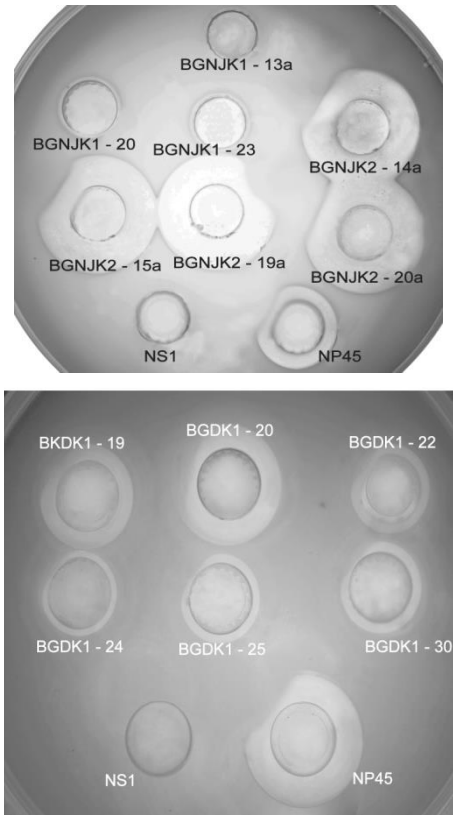

Fig. 1. Inhibitory effects of *Lc. lactis* ssp. *lactis* and *Lc. raffinolactis* strains on *Lc. lactis* ssp. *cremoris* NS1 as indicator strain

clear zones of inhibition, which lacked on the points where pronase E was added (**Fig. 1**). *Lc. raffinolactis* strains also inhibited the growth of *Lc. lactis* ssp. *lactis* biovar. diacetylactis S50, *E. coli* ATCC 2592 and *L. inoccua* ATCC 33090 but zones of inhibition were smaller in these cases (**Tab. 2**).

One strain of *En. faecium* formed clear zones of inhibition against *Lc. lactis* ssp. *lactis* biovar. diacetylactis S50, and *L. innocua* ATCC 33090 while *En. faecalis* strain had inhibitory effects on *Lc. lactis* ssp. *cremoris* NS1, *Lc. lactis* ssp. *lactis* BGMN1-596 and *L. inoccua* ATCC 33090 (**Tab. 2**). Zones of inhibition formed by enterococci were not sensitive to pronase E, so it can be concluded that the antimicrobial substance doesn't have protein nature. Strains which have been found to have antimicrobial activity against any of the above mention indicator strains, did not act inhibitory against *Lb. plantarum* A112, *Lb. paracasei* ssp. *paracasei* BGBUK2-16 / K4, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231 indicator strains.

Synthesis of bacteriocins is significant characteristics of probiotic bacteria because bacteriocin producing strains could inhibit pathogenic bacteria in the gastrointestinal tract. Except probiotic effects that strains with antimicrobial activities could have, bacteriocin producing LAB strains are a part of starter cultures for improving the microbiological quality and safety of dairy products, or for accelerating the ripening of fermented foods (Ayad et al., 2004; Mohammed et al., 2009).

Adhesive properties of strains

Autoaggregation and hydrophobicity, as surface characteristics of the LAB strains, indicate potential adhesion ability of probiotic LAB strains to epithelial cells of human intestine (Fortina et al., 2008). The high degree of autoaggregation indicates better adhesive capability of isolates, while physicochemical properties of the cell wall of LAB may probably affect autoaggregation and adhesion (Fortina et al., 2008). According to Maldonado et al. (2012), three different groups of LAB strains can be distinguished on the basis of autoaggregation and hydrophobicity degrees: high (60–100%), medium (30–60%) and low (0–30%). The average values of these characteristics of tested LAB species are summarized in Table 3.

Enterococcus durans strains showed the highest autoaggregation and hydrophobicity degrees with the average values of 71% and 45%, respectively. Among six *En. durans* strains, three of them aggregated quickly after 15 min, forming a precipitate and clear solution. These strains also expressed hydrophobicity values higher than 60%. *Lc. lactis, Lc. raffinolactis, St. thermophilus, En. faecalis, En. faecium, Ln. mesenteroides, Lb. paracasei, Lb. satsumensis and Lb. kefiranofaciens* strains showed a medium degree of autoaggregation. *En. faecium* and *Lb. paracasei* strains had the highest degree of autoaggregation in this group (44% and 50%, respectively). All strains in this group had very low level of binding to *n*-hexadecane and only *En. faecium* strains had average degree of hydrophobicity higher than 20%. *Lb. plantarum* strains had low degree of autoaggregation and hydrophobicity (27% and 6%, respectively) while one *Lb. kefiranofaciens* strain had the lowest degree of both properties among tested strains.

High intraspecies variability in autoaggregation and hydrophobicity degrees was observed for LAB strains evidencing that these properties seems to be strain specific. Similar findings have been reported by other authors (Todorov et al., 2008; Giaouris et al., 2009; Iyer et al., 2010).

Growth in the presence of bile salts

The growth of the LAB strains in the presence of bile salts is a significant characteristic of probiotic LAB strains, which indicates the possibility of their survival in gastrointestinal tract (Dunne et al., 2001).

Table 4. Resistance of LAB strains to different antibiotics

Antibiotic	*Chloramphenicol, μg/ml*		*Tetracycline, μg/ml*			*Erythromycin, μg/ml*		*Cephalosporin, μg/ml*		
LAB species	10	20	10	20	30	5	10	10	20	30
Lc. lactis ssp. *lactis* (19)[1,2]	5	-	19	18	-	8	-	19	18	18
Lc. raffinolactis (6)	-	-	-	-	-	-	-	-	-	-
St. thermophilus (4)	-	-	4	2	1	-	-	4	2	2
En. faecalis (9)	9	6	9	9	3	8	-	9	9	9
En. faecium (19)	19	13	19	19	5	19	-	19	19	19
En. durans (10)	8	-	10	10	7	-	-	10	10	10
Ln. mesenteroides(50)	-	-	50	45	4	40	-	50	40	9
Lb. parcasei (6)	-	-	6	6	-	4	-	6	6	4
Lb. plantarum (15)	-	-	15	14	-	14	4	15	14	12
Lb. satsumensis (1)	-	-	1	1	-	1	-	1	1	1
Lb. kefiri (1)	-	-	1	1	-	-	-	1	1	1
Lb. kefiranofaciens (1)	-	-	-	-	-	-	-	-	-	-

1 -numbers in parenthesis represent the number of isolates of the certain strain
2 -the results are presented as number of isolates which are resistant to a particular antibiotic

The ability of bile salts hydrolysis is desirable probiotic characteristics of LAB strains, because the bile salts deconjugation contributes to the intestinal microflora balance (Mathara et al., 2008). Selection of probiotic strains based on their deconjugation activities is also important, because of the correlation between this activity and the ability of LAB strains to decrease cholesterol level (Pereira et al., 2003).

In our study, both characteristics were detected only in strains of genus *Enterococcus*, *Leuconostoc* and *Lactobacillus* (**Tab. 3**). The most of the strains that could grow in the presence of bile salts belonged to species *En. faecium*, *En. durans* and *Lb. plantarum* while half of the *Lb. plantarum* strains could hydrolyse bile salts. The growth of *Lb. plantarum*, *Lb. paracasei* and *En. faecium* strains on the media with the addition of bile salts was also found in earlier studies (Xanthopoulos et al., 2000; Saavedra et al., 2003; Todorov et al., 2008). Certain *Lb. plantarum*, *Lb. paracasei* and *En. faecium* strains were found to be able to deconjugate bile salts (Saavedra et al., 2003; Maragkoudakis et al., 2006; Nguyen et al., 2007).

Safety assessment

Although LAB has GRAS (generally recognized as safe) status, the strains isolated from fermented foods that can be used as starter culture should be tested for their potentially adverse effects on human health. Antibiotic resistance and the synthesis of biogenic amines are mostly analyzed characteristics of LAB strains important for their safe use in starter cultures (Franciosi et al., 2009).

Antibiotic resistance of LAB strains is a major problem in the food industry after the discovery that certain strains of lactococci, lactobacilli and enterococci have genes for antibiotics resistance located on plasmids and transposons. Thus, antibiotic resistance can be transferred to other bacterial species (Kastner et al., 2006). In our work, resistance to antibiotics was determined by growing the isolates on the appropriate medium with various antibiotics (chloramphenicol, tetracycline, erythromycin, and cephalosporin) added in certain concentrations (**Tab. 4**). Cephalosporin belongs to the group of antibiotics which inhibit the synthesis of the cell wall, while tetracycline, erythromycin, chloramphenicol affect the protein synthesis in the bacterial cell.

Results from the present study showed that the most of the tested LAB strains were resistant to cephalosporin (**Tab. 4**). All enterococci, *Lb. satsumensis* strain, *Lb. kefiri* strain and the most of *Lc. lactis* ssp. *lactis*, *St. thermophilus*, *Lb. paracasei* and *Lb. plantarum* strains grew well on the media where cephalosporin was added in the concentration of 30 μg/ml. Strains belonging to species *Ln. mesenteroides* showed the highest sensitivity to cephalosporin with only 9 strains resistant to the highest concentration of cephalosporin used (**Tab. 4**).

The most of the LAB strains were resistant to tetracycline when it was added in media in low concentration (10 μg/ml and 20 μg/ml). Increasing of tetracycline concentration in media reduced the number of resistant strains for all LAB species but some strains of enterococci were resistant to this high concentration of tetracycline. When chloramphenicol was added in the concentration of 10 μg/ml, all enterococcal strains and a small number of lactococcal strains were resistant. Increasing concentrations of chloramphenicol at 30 μg/ml did not affect growth of the most of *En. faecium* and *En. faecalis* strains.

Addition of erythromycin in a concentration of 5 μg/ml in media did not inhibit the growth of *En. faecium*, *En. faecalis*, *Ln. mesenteroides*, *Lb. paracasei*, *Lb. plantarum* and *Lb. satsumensis* strains, but some of *Lc. lactis*, all *St. thermophilus* and *En. durans* strains and one *Lb. kefiri* strain were sensitive to addition of this antibiotic in low concentration. Only four *Lb. plantarum* strains were resistant to the higher concentration of erythromycin (10 μg/ml). The most sensitive to the tested antibiotics were *Lc. raffinolactis* and *Lb. kefiranofaciens* strains that showed no growth on any of the medium with the addition of antibiotics.

Antibiotic resistant strains from dairy environment were also found by other researchers (Flórez et al., 2005; Devirgiliis et al., 2010). Data on proposed minimum inhibitory concentrations of antibiotics used for LAB isolates from dairy products are different in the literature due to the differences between selected methods (Ammor et al., 2007). Overall, LAB isolates from dairy products are the most sensitive to erythromycin, while their resistance is most pronounced to cephalosporins (Katla et al., 2001; Temmerman et al., 2003). Resistance to chloramphenicol and tetracycline is distinguished among LAB isolates from different genus and it is usual to obtain strains with a much higher resistance compared to the proposed minimal inhibitory concentration for a particular species (Danielsen & Wind, 2003; Flórez et al., 2005; Maragkoudakis et al., 2006).

In fermented food, biogenic amines formed by bacterial decarboxylation of amino acids histidine, tyrosine, lysine or ornithine, lead to the undesirable flavor of final product and may have negative impact on human health (Landete et al., 2007). Among analyzed LAB strains, all *En. faecalis* strains, 12 strains of *En. faecium* and 5 strains of *En. durans* synthesized tyramine from tyrosine. Production of tyramine is commonly detected characteristics of enterococci from dairy products (Martín-Platero et al., 2009).

Conclusion

Traditional fermented products, such kajmak is, are new sources of LAB strains with interesting characteristics. In recent years, LAB strains that have probiotic properties are particularly popular because they can be used for the production of new types of functional foods. The results of the present research indicate that among analyzed LAB strains isolated from kajmak, a few strains had interesting probiotic characteristics. Five *Lc. lactis* ssp. *lactis* and six *Lc. raffinolactis* strains synthesized bacteriocins, so they could be used for a control of pathogenic bacteria. *Enterococcus durans* strains with high degree of autoaggregation and hydrophobicity should be further tested for adhesion to human cell lines. Strains of *Lb. paracasei*, *Lb. plantarum* and *Ln. mesenteroides* that hydrolyze bile salts could have cholesterol lowering effects. All the above-mentioned strains were low resistance to used antibiotics, and they didn't have the ability to synthesize biogenic amines. On the other hand, all enterococcal strains that hydrolyze bile salts showed high resistance to used antibiotics and could decarboxylate amino acids.

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