

Original Article

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Screening of lactic acid bacteria isolated from Serbian kajmak for use in starter cultures

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Abstract:

Joković, N., Rajković, J., Veljović, K., Tolinački, M., Topisirović, Lj.: Screening of lactic acid bacteria isolated from Serbian kajmak for use in starter cultures. *Biologica Nyssana*, 5 (1), Septmeber 2014: 37-46.

One hundred and seventy eight isolates of lactic acid bacteria (LAB) were isolated by pour plate and enrichment techniques from a sample of milk used for kajmak production and three samples of kajmak from one month ripening period. The identification of isolates was performed by phenotypic characterization followed by molecular identification using (GTG)₅-PCR and sequence analysis of 16S rRNA gene. Isolates belonged to *Lactococcus lactis* and *Enterococcus faecium* were found in milk and kajmak samples while *Leuconostoc mesenteroides* and *Enterococcus durans* were the most frequently isolated species from kajmak samples. *Streptococcus thermophilus* were isolated from milk sample only with enrichment technique. Further characterization of LAB isolates was done for technological properties which are important for industrial application of LAB. Strains of *Lc. lactis* and *S. thermophilus* that showed very good acidification and proteolytic activities and *L. mesenteroides* strains that metabolized citrate can be used in development of starter cultures for eventual industrial production of kajmak. Additionally, producers of antimicrobial compounds belonged to *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* can be used for control of undesirable microflora in kajmak production.

Key words: Kajmak, Lactic acid bacteria, Technological characterization

Apstract:

Joković, N., Rajković, J., Veljović, K., Tolinački, M., Topisirović, Lj.: Ispitivanje bakterija mlečne kiseline izolovanih iz srpskog kajmaka za upotrebu u starter kulturama. *Biologica Nyssana*, 5 (1), Septmeber 2014: 37-46.

Sto i sedamdeset osam izolata bakterija mlečne kiseline (BMK) izolovano je metodom izlivanja ploča i tehnikom obogaćivanja iz uzorka mleka korišćenog za proizvodnju kajmaka i tri uzorka kajmaka starosti do jednog meseca. Preliminarna identifikacija izolata rađena je fenotipskim metodama dok su molekularne metode identifikacije, PCR sa (GTG)₅ prajmrom i sekvenciranje 16S rRNK gena, korišćene za identifikaciju izolata do nivoa vrste. Izolati identifikovani kao *Lactococcus lactis* i *Enterococcus faecium* izolovani su iz uzoraka mleka i kajmaka dok su vrste *Leuconostoc mesenteroides* i *Enterococcus durans* najčešće izolovane vrste iz uzoraka kajmaka. *Streptococcus thermophilus* izolati dobijeni su iz uzorka mleka samo tehnikom

obogaćivanja. Dalja karakterizacija BMK izolata rađena je na osnovu tehnoloških svojstava koja su značajna za industrijsku primenu BMK. Sojevi *Lc. lactis* i *S. thermophilus* koji su imali veoma dobru sposobnost acidifikacije i proteolitičku aktivnost kao i *L. mesenteroides* sojevi sa sposobnošću metabolizma citrata mogu se upotrebiti za razvoj starter kultura za industrijsku proizvodnju kajmaka. Osim toga, izolati identifikovani kao *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* koji sintetišu antimikrobna jedinjenja mogu se koristiti za kontrolu nepoželjne mikroflore u proizvodnji kajmaka.

Ključne reči: kajmak, bakterije mlečne kiseline, tehnološka karakterizacija izolata

Introduction

Kajmak is a delicious artisanal dairy product of Serbia and neighboring Balkan countries. It is made by fermentation of milk fat that rises on the top of cooked milk after slowly cooling. Thin layer of aggregated milk fats and proteins is then salted and placed layer by layer in special wooden vessels where ripening takes place. Kajmak can be consumed as a fresh up to 7 days of fermentation, or mature kajmak that can be aged for several months. In the most of the dairy products, lactic acid bacteria (LAB) are the dominant microbial population which metabolic products play crucial role in formation of the taste, smell, texture and quality of final product (Wouters et al., 2002). In a previous work, we have shown that the most ubiquitous LAB isolated from kajmak samples belong to genera *Leuconostoc*, *Enterococcus*, *Lactobacillus* and *Lactococcus* (Joković et al., 2008).

In Serbia, kajmak is made at farmhouse level in central and west part of the country and the sale is mainly limited to the open markets. Production of kajmak is always based on non-standardized traditional techniques without addition of starter cultures. Therefore, the fermentation process depends entirely on the natural microbial flora that originates from the milk and from the environment. Consequently, final products are sensitive to contamination and spoilage and have variable quality, poor hygiene and doubtful safety. On the other hand, some attempts for industrial production of kajmak did not succeed because sensory characteristics of final products were not the same as in original products from households (Pudja et al., 2008).

In modern dairy industry, the main approach to obtain traditional dairy products with improved and reproducible qualities is usage of defined commercial starter cultures made with strains isolated from original products (Chammas et al., 2006). These strains are best adapted on food substrate and their metabolic activities contribute to evolution of typical and unique organoleptic characteristics of traditional products. The searching for strains that can be used in construction of starter cultures includes screening of a large number of

isolates selected from naturally occurring processes in term of their technological capabilities that are relevant for obtaining more quality product (Asteri et al., 2009).

The aim of the present study was screening of LAB isolates that were predominant in the early phases of kajmak ripening. For that purpose, isolation and identification of LAB from the milk used for manufacturing of kajmak and samples of resulting kajmaks in the first period of ripening were performed. Additionally, the technological properties of obtained isolates were characterized. This information could make possible selection of strains that could be eventually used in development of starter cultures for the industrial production of kajmak.

Material and methods

Bacterial strains, media, and growth conditions

Bacterial strains used in this study are presented in **Table 1**. MRS broth (pH 5.7) (Merck, GmbH, Darmstadt, Germany) was used for growth of *Leuconostoc* and *Lactobacillus* strains whereas *Lactococcus*, *Streptococcus* and *Enterococcus* strains were cultured in M17 broth (pH 7.2) (Merck, GmbH, Darmstadt, Germany) supplemented with glucose (0.5%, w/v; GM17 broth). A solid medium was prepared by adding agar (2% w/v; Torlak, Belgrade, Serbia) to each medium. The inoculated media were incubated overnight at appropriate temperatures depending on the strain.

Manufacture and sampling of kajmak

One batch of kajmak was manufactured from cows' milk in a household in the western part of Serbia according to traditional procedures without addition of starter cultures. The household was selected as their kajmak had high quality properties.

Raw milk was cooked to boiling, with occasional stirring to prevent burnt. Then, hot milk was poured into the open wooden shallow vessels that were placed on the shelves in the special room for producing kajmak called "creamery". The next

Table 1. List of reference strains used

Bacterial strains
<i>Lactococcus lactis</i> NP45 ^a
<i>Lactococcus lactis</i> ssp. <i>cremoris</i> NS1 ^a
<i>Lactococcus lactis</i> subsp. <i>lactis</i> BGMN1-596 ^a
<i>Lactococcus lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> S50 ^a
<i>Enterococcus faecium</i> BGGJ8-3*
<i>Enterococcus durans</i> BGZLS20-35b*
<i>Streptococcus thermophilus</i> BGDK1-4a
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> BGBUK2-16/K4 ^a
<i>Lactobacillus plantarum</i> A112 ^a
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i> NRRL B-512
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i> NRRL B-3470

^a-Strains used in antimicrobial activity assay

*Strains identified by molecular methods in the Laboratorium voor Microbiologie, Universitet Gent, Gent, Belgium

NRRL – Agricultural Research Service Culture Collection, Peoria, IL, USA

12 h milk in vessels was cooling at room temperature with a gradual separation of cream on the surface of the milk. Afterwards, the formed layer of fats and proteins was carefully skimmed with perforated spoon, drained and placed in the wooden tub. Salt was added between the layers from different vessels. The tub was placed in the creamery where ripening took place for one month.

Sample of milk immediately after boiling, designated as BGNK1, and the samples of kajmak after 5, 15 and 30 days of ripening (designated as BGNK5, BGNK15 and BGNK30, respectively) were collected for microbiological analyses. The collected samples of milk and kajmak were kept in sterile bags at 4°C until the analyses which were performed within the following 12 h.

Isolation of lactic acid bacteria (LAB)

The isolation of LAB was performed by pour plate and enrichment techniques. In the pour plate technique, the samples (10 g or 10 mL) were homogenized in 90 mL of sterile 2% (w/v) sodium citrate solution (pH 7.5) preheated to 45°C. Decimal dilutions of the homogenates were prepared with 0.85% (w/v) sterile saline and were plated on suitable media for isolation of LAB: M17 agar (Merck, GmbH, Darmstadt, Germany) supplemented with glucose (GM17), MRS agar (Merck, GmbH, Darmstadt, Germany) and Mayeux, Sandine, Elliker (MSE) agar (Mayeux et al., 1962). The plates were incubated at 30°C and 45°C for 3 days. After the incubation period, five single

colonies were randomly picked from agar plates and streaked on new agar plates for purification.

In the enrichment technique, 0.1 mL of the first dilution was transferred into 10 mL of MRS or GM17 broth as well as into 10 mL of 10% skimmed milk. Tubes were incubated at 30 °C and 45 °C for 48 h. After the incubation, loopful of grown culture was streaked on MRS and GM17 agar plates and incubated for additional 48 h at appropriate temperatures and under anaerobic conditions (Anaerocult A, GasPakMerck, Germany). From each plate, few colonies showing different appearance were randomly picked and streaked on new plates in order to obtain pure colonies.

Phenotypic identification of isolates

Gram-positive, catalase-negative isolates were grouped and preliminary identified according to the following phenotypic tests: morphology under microscopic examination; the production of gas from glucose in MRS broth lacking beef extract and containing inverted Durham tubes; hydrolysis of arginine; hydrolysis of esculin in esculin broth (Torlak, Belgrade, Serbia); the growth in GM17 or MRS broth with 4% and 6.5% (w/v) NaCl for 5 days and the growth in 10% skimmed-milk medium. The growth at different temperatures was determined in MRS broth for bacilli and cocobacilli at 15°C and 45°C or in GM17 broth for cocci at 10°C and 45 for 5 days. Bile esculin agar (Himedia, Mubai, India) was used for the presumptive identification of enterococci.

Molecular identification of isolates

Molecular identification of selected isolates was done by rep-PCR method with (GTG)₅-primer and by sequencing of 16S rRNA gene.

The total DNA extraction from pure cultures, PCR amplification with (GTG)₅-primer, electrophoresis and statistical similarity among fingerprints of the isolates and the reference strains were performed as previously described (Joković et al., 2008).

Sequencing of the 16S rRNA gene was done by using total DNA as a template for PCR amplifications with U968 (5'-AACGCGAAGAACCTTAC-3') and L1401 (5'-GCGTGTGTACAAGACCC-3') primers (Zoetendal et al., 1998; Randazzo et al., 2002). The amplified fragments were sequenced by Macrogen (Sequencing Service Macrogen, Seoul, South Korea). The sequences were analysed in the NCBI database using BLAST, the standard nucleotide-nucleotide homology search (<http://www.ncbi.nlm.nih.gov/BLAST>).

Technological characterization of isolates

The acidifying activity of isolates was evaluated by measuring the pH of inoculating 10% (w/v) reconstructed skim milk (RSM). Overnight LAB cultures grown at appropriate temperatures were centrifuged at 5000 rpm for 10 min, washed with peptone water and inoculated (1% v/v) in 10 mL RSM. pH was measured after 6 and 24 h of incubation at appropriate temperature and values were expressed as pH decrease (Δ pH), in relation to the pH values of non-inoculated control milk.

The proteolytic activity of the isolates was detected after growing of isolates for 24 h in RSM as described for acidifying activity. After incubation, the proteins from 0.5 mL of the culture were precipitated with trichloroacetic acid. The proteolytic activity of isolates was determined by the o-PA (o-phthaldialdehyde) method of Church and associates (1983). The values were calculated from calibration curve with glycyl and expressed in mM_{Gly}L⁻¹.

Citrate utilization was detected on Kempler and McKay agar plates (Kempler & McKay, 1981). Citrate-positive colonies were blue after 48 h of incubation at appropriate temperature.

Production of diacetyl in milk was determined after a growing of isolates in RSM for 24 h as described above. Qualitative determination of diacetyl was done by addition of 0.5 mL of α -naphthol (1% w/v) and KOH (16% w/v) solution in

1 mL of milk culture. After incubation at 30°C for 10 min results were read. Appearance of red ring was considered as positive result (King, 1948).

Lipolytic activity of LAB was identified on tributyrin agar plates (Merck, GmbH, Darmstadt, Germany). The plates were incubated for 7 days at appropriate temperatures and observed daily for clear zones around the colonies.

Production of exopolysaccharides was detected on reconstructed MRS medium containing 100 g L⁻¹ of sucrose, glucose, lactose, maltose or fructose. The plates were incubated anaerobically for 48 h at appropriate temperature and isolates were tested for slime formation using the inoculated loop method.

The LAB isolates were screened for antimicrobial activity by the agar-well diffusion method (Tagg & Mc Given, 1971). Different indicator strains used in this study are listed in Table 1. The protein nature of antimicrobial compounds was confirmed by adding a crystal of pronase E (Sigma Chemie GmbH, Deisenhofen, Germany) near to the edge of the well containing the strains with a potential production of bacteriocins. The plates were incubated overnight at 30°C. The appearance of a clear zone of inhibition around the well, but not near of pronase E crystal, was considered as positive result for possible bacteriocin production

Results and Discussion

Identification of isolates

Sensory properties of dairy products manufactured without addition of starter cultures, as kajmak is, entirely depend on the microflora found in milk and the contaminants from the environment (Poznanski et al., 2004). In order to determine which part of the LAB populations derived directly from the milk, 178 Gram-positive and catalase-negative isolates were collected from milk used for kajmak production (BGNK1) and kajmak samples BGNK5, BGNK15 and BGNK30.

On the basis of physiological tests that were used for the preliminary identification of isolates to the genus level, all isolates were grouped into eight physiological groups (Tab. 2). Representatives of various physiological groups, a total of 76, were further selected for molecular identification by (GTG)₅-PCR fingerprinting. A dendrogram derived by comparing the similarity of digitally reproduced (GTG)₅ fingerprints of isolates and reference strains is presented in Fig. 1.

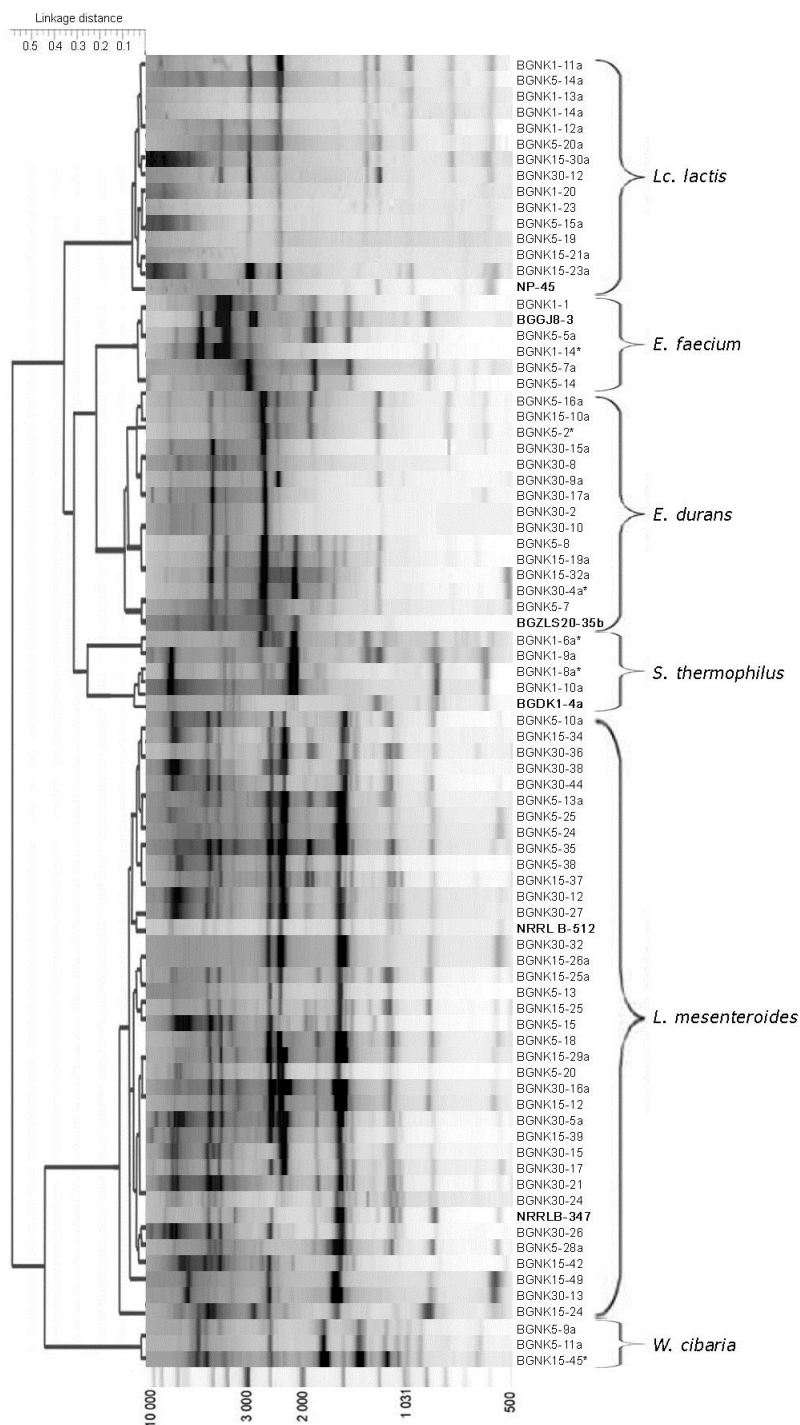


Figure 1. Dendrogram based on statistical analysis of the (GTG)₅-PCR fingerprints. *indicates the isolates that were subjected to sequencing of 16S rDNA

The results of physiological tests showed that three groups of coccal isolates can be distinguished (Tab. 2). Based on a dendrogram derived from (GTG)₅-PCR fingerprints these isolates were identified as *Lactococcus lactis*, *Streptococcus thermophilus*, *Enterococcus faecium* and *Enterococcus durans*. The (GTG)₅-PCR identification was confirmed by sequencing of 16S rDNA for two *S. thermophilus*, three *E. durans* and one *E. faecium* isolates.

Heterofermentative coccoid isolates showing arginine negative phenotype were supposed to belong to genus *Leuconostoc* (Tab. 2). These isolates were identified to species level as *Leuconostoc mesenteroides* (Fig. 1) as they had similar (GTG)₅-PCR fingerprints with *Leuconostoc mesenteroides* reference strains NRRL B-512 and NRRL B-347. Three isolates shaped as very short rods were also heterofermentative with the identical (GTG)₅-PCR fingerprints (Fig. 1). But these

Table 2. Differentiation of LAB isolated from milk and kajmaks samples based on morphological and physiological characteristics, and the identification of representative strains from each group by (GTG)₅-PCR and sequencing of 16S rDNA

Cell morphology and genus ¹	Production of CO ₂	Growth on			Growth in broth with NaCl		Milk clotting	Hydrolysis of		BSA ²	No isolates	Identified by	
		45°C	15°C	10°C	4%	6.5%		arginine	esculin			(GTG) ₅ -PCR as	sequencing of 16S rDNA as
Cocci													
<i>Lactococcus</i> sp. I	-	-	nd	+	+	-	+	+	+	-	14	<i>Lc. lactis</i> (14) ³	nd
<i>Enterococcus</i> sp.	-	+	nd	+	+	+	+	+	+	+	53	<i>E. faecium</i> (5) <i>E. durans</i> (14)	<i>E. faecium</i> (1) <i>E. durans</i> (3)
<i>Streptococcus</i> sp.	-	+	nd	-	+	-	+	-	-	-	7	<i>S. thermophilus</i> (4)	<i>S. thermophilus</i> (2)
Coccoid													
<i>Leuconostoc</i> sp. I	+	-	+	nd	+	+	+v	-	+	-	53	<i>L. mesenteroides</i> (15)	nd
<i>Leuconostoc</i> sp. II	+	-	+	nd	+	-	+v	-	+	-	28	<i>L. mesenteroides</i> (13)	nd
<i>Leuconostoc</i> sp. III	+	-	+	nd	+	+	+v	-	-	-	13	<i>L. mesenteroides</i> (5)	nd
<i>Leuconostoc</i> sp. IV	+	-	+	nd	+	-	+	-	-	-	7	<i>L. mesenteroides</i> (3)	nd
Rods (very short)													
<i>Weissella</i> sp.	+	-	+	nd	+	-	-	+	+	-	3	nd (3)	<i>W. cibaria</i> (1)

+ positive; - negative; +v reaction positive for some isolates; nd – not determined

¹-classification based on morphological and physiological characteristics

²-Black colonies on BSA (bile esculin agar)

³-numbers in parenthesis represent the number of isolates that underwent genetic identification

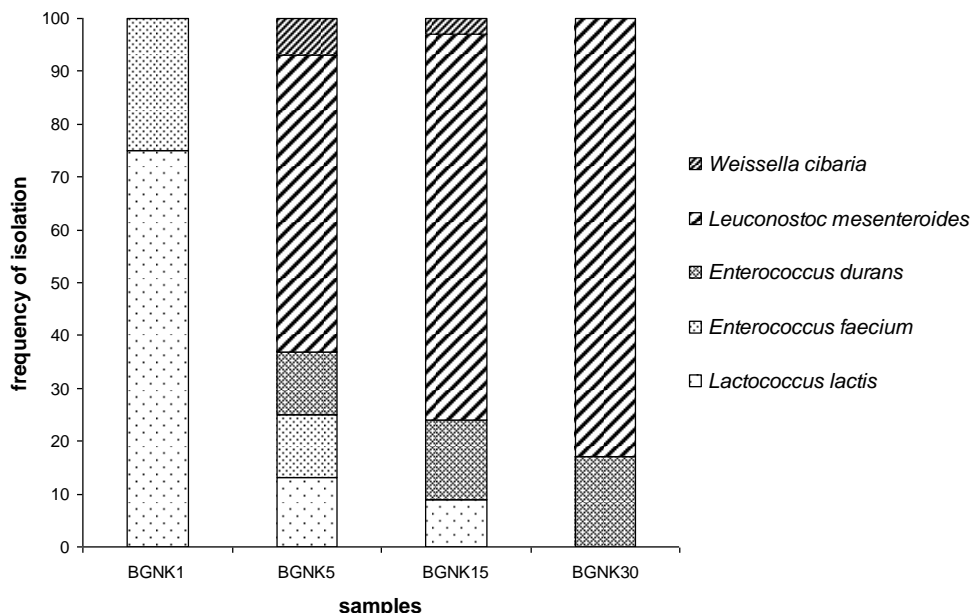


Figure 2. Distribution of LAB species in milk and kajmak samples based on a pour-plate technique

isolates were not identified with rep-PCR method because (GTG)₅-PCR fingerprints of these isolates were not related to any band pattern of reference strains from our collection. The 16S rDNA sequencing result of one representative isolate from this group showed that it belonged to the species *Weissella cibaria* (Table 2).

The identification of isolates allowed us to describe the distribution of LAB in milk (BGNK1) and kajmak samples (BGNK5, BGNK15 and BGNK30). LAB species isolated from milk and kajmak samples were different (Fig. 2).

E. faecium and *Lc. lactis* were only species isolated from milk sample by a pour plate technique,

while *S. thermophilus* was isolated from the milk by enrichment technique. *Lc. lactis*, *E. faecium*, and *S. thermophilus* are commonly isolated species from milk, while the lactobacilli and leuconostocs are always isolated in lower percentage (Franciosi et al., 2009; Giannino et al., 2009). On the other hand, *L. mesenteroides* was the most dominant species in all analyzed kajmak samples. This species was found in high percentage (56%) in 5-day-old sample of kajmak (BGNK5) and its frequency of isolation increased throughout of the ripening (Fig. 2). Leuconostocs are ubiquitous in nature due to their ability to survive longer on the surface of different materials or during pasteurization (Martley & Crow, 1993; Ogier et al., 2008). Since leuconostocs were not isolated from the milk, they probably got into the kajmak from the environment or they were present in the milk in such small numbers that they could not be detected by used isolation techniques. *Lc. lactis* isolates were isolated by pour plate technique from 5-day-old (BGNK5) and 15-day-old samples of kajmaks (BGNK15) although its frequency of isolation was very low and decreased during fermentation (13% and 9%, respectively) (Fig. 2). In the 30-day-old kajmak sample (BGNK30) lactococci were isolated only with enrichment technique. The reduction in the number of lactococci is probably the result of a slight salt content and dry matter increase and pH values decrease during the ripening of kajmak (Joković et al., 2008). These conditions inhibited the growth of lactococci, which are much more sensitive to adverse conditions prevailing during the ripening of dairy products compared to leuconostocs and enterococci (Lopez-Diaz et al., 2000). Two species of enterococcal isolates were detected in kajmak samples. *E. faecium* isolates detected in milk, were also isolated from 5-day-old kajmak sample (BGNK5) while *E. durans* isolates were detected in all samples of kajmak and their number increased slightly during ripening (Fig. 2). *E. durans* isolates were not previously detected in the analyzed samples of kajmak (Joković et al., 2008) but they were found commonly in milk and different types of cheeses (Ogier & Serror, 2008). Enterococci have a high tolerance to adverse conditions such as salt and acidity during the ripening of dairy products which explains their dominance in many dairy products (Marino et al., 2003). Isolates identified as *W. cibaria* were found in 5-day-old (BGNK5) and 15-day-old samples of kajmak (BGNK15) in low percentage (7% and 3%, respectively). *W. cibaria* isolates have been detected in foods of various origins, as well as among some clinical isolates of human and animal origin

(Bjorkroth et al., 2002; Ouadghiri et al., 2009).

Technological characterization of isolates

All isolates were tested for technological characteristics important for use of LAB in dairy industry. Assays included acidifying, proteolytic and lipolytic activity, ability to metabolize citrate and produce diacetyl and production of EPS and inhibitory substances.

Acidifying and proteolytic activity

In all dairy products rapid production of lactic acid by LAB at the beginning of fermentation is a crucial step for getting products with good sensory and hygienic properties. Fast production of lactic acid by LAB strains depends on their proteolytic system, ability to metabolize lactose and bacterial resistance to acid stress (Galia et al., 2009). Therefore, acidifying and proteolytic activities of the isolates from dairy products are very important for their selection in starter cultures.

In our study, streptococci showed the highest acidifying activity in milk (Fig. 3) and all strains tested clotted skimmed milk after 24 h of incubation. Four of them decreased pH of milk for 6 h of incubation to the value around 5.30. Lactococcal strains had lower initial values of ΔpH than streptococci but in latter stage of growing in milk some of them enhanced their acidifying activity reaching pH around 4.40 after 24 h incubation period. *Lc. lactis* and *S. thermophilus* strains isolated from different dairy products, most often, have better activity in milk than other LAB isolates (Ayad et al., 2004; Badis et al., 2004). Enterococcal strains were weak acid producers and no of the isolate could decrease pH of milk under 5.00 after 24 h of growth in the milk. All strains of *L. mesenteroides* showed low acidifying ability and 30% could not reduce pH of milk at all while strains of *W. cibaria* practically could not grow in milk (Fig. 3). A limited number of LAB strains with good activity in milk was detected by analyzing LAB isolates from other artisanal dairy products (Astari et al., 2009; Mohammed et al., 2009).

The assay for proteolytic activity using the o-phthaldialdehyde method showed that lactococci released the highest amount of amino groups in milk with average value of $1.97 \text{ mM}_{\text{Gly}} \text{ L}^{-1}$ (Fig. 3). *S. thermophilus* isolates had lower proteolytic activity followed by *E. durans* and *E. faecium* isolates.

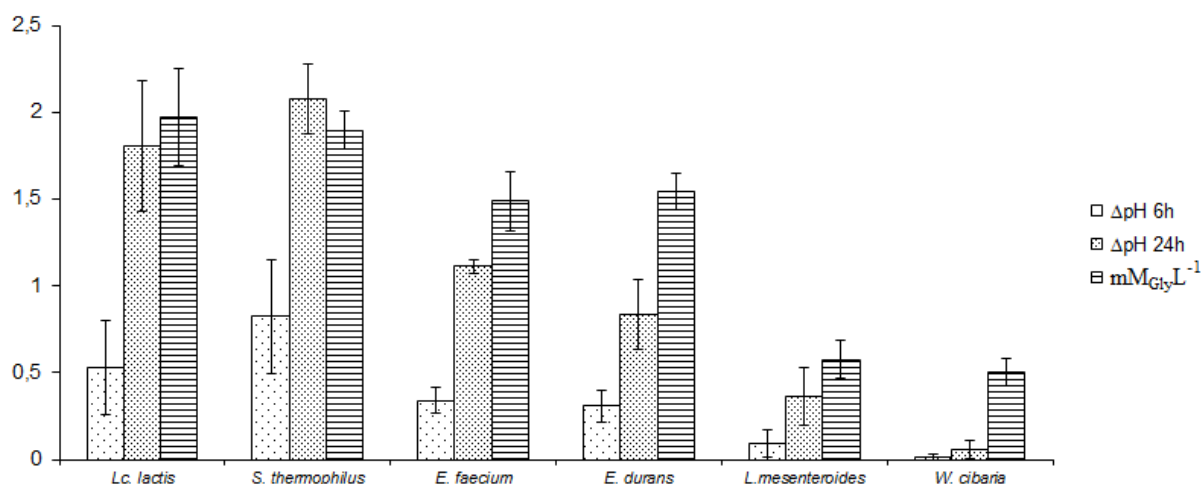


Figure 3. Acidifying and proteolytic activities of isolates
The error bars represent the means of standard deviation

Isolates identified as leuconostocs and weisella showed the lowest level of proteolytic activity (Fig. 3). These results were similar to that described by other authors who showed that lactococci had the best proteolytic activity among LAB (Gonzalez et al., 2010).

Five of the fourteen strains of *Lc. lactis* with the highest level of proteolytic activity (2.08-2.28 mM_{Gly}L⁻¹) were the most rapidly acidifying lactococcal strains. These strains are good candidates for construction of starter cultures for kajmak production.

Utilization of citrate and production of diacetyl

Possibility of diacetyl synthesis from citrate is characteristic of certain LAB strains included in the composition of starter cultures. Diacetyl is one of the most important compounds that affect the aroma of dairy products giving them flavor similar to that of butter (Madera et al., 2003).

In this study, six strains of *Lc. lactis* had blue appearance on Kempler and McKey agar plates and produced diacetyl in milk after 24 h of incubation. Therefore, these isolates were identified as *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*. The most of enterococcal isolates had ability of diacetyl production (five *E. faecium* and twenty-five *E. durans* isolates) but none of them could use citrate on Kempler and McKey agar. These results indicate that enterococci might use other substrates for the synthesis of diacetyl, as have been shown for some lactococci (Bars & Yvon, 2008). On the other hand, thirty four leuconostoc isolates were able to metabolize citrate on Kempler and McKey agar plates but diacetyl synthesis in

milk was not detected. Since leuconostocs produce diacetyl only in medium with lower pH values (Garabal et al., 2008) that had not been detected in this study due to their poor growth in milk, more detailed investigations on this matter are required.

Lipolytic activity

Five *E. durans* and one *E. faecium* strains showed lipolytic activity when assayed on tributirin agar. These strains were weakly lipolytic as halos around colonies were very small. In general, LAB have poor lipolytic activity, but for certain strains it has been shown to hydrolyze mainly triglycerides containing fatty acids with medium length chains (Katz et al., 2002). Lipolytic strains of the genus *Enterococcus* have increased lipase activity compared to the strains of the genera *Lactococcus* and *Leuconostoc* (Astari et al., 2009).

Production of EPS

In the dairy industry, LAB strains that synthesize EPS have effects on the organoleptic properties of the product and use of these strains in functional starter cultures may be significant (Leroy & De Vuyst, 2004). Among the tested strains, only sixty-seven *L. mesenteroides* and three *W. cibaria* isolates produced EPS on the MRS medium with the addition of sucrose.

Antimicrobial activity

The most important antimicrobial substances that are produced by LAB are bacteriocins due to their possible application in food biopreservation (Simova et al., 2009). Results showed that four *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* strains

produced bacteriocins with inhibitory effect on the growth of lactococcal indicator strains *Lc. lactis* ssp. *cremoris* NS1 and *Lc. lactis* subsp. *lactis* BGMN1-596 used in the experiment. LAB strains that produce bacteriocins are a part of starter cultures for improving the microbiological quality and safety of dairy products, while the adjunct starters composed of sensitive strains and bacteriocin producer strains lead to increasing in the degree of autolysis and accelerate the ripening (Ayad et al., 2004).

Conclusion

Kajmak is a delicatessen dairy product of Balkan countries that is not present in the world market because of not standardized production in households that leads to the variability and poor safety of final products. These problems can be overcome by the industrial production of kajmak with the addition of starter cultures composed of the appropriate strains isolated from kajmak samples and well characterized. The results of the present research indicate that among isolated LAB a few interesting strains could be used in construction of starter cultures for industrial production of kajmak. Four lactococcal strains that showed fast acidification rate and good proteolytic activity as well as rapid acidifying *S. thermophilus* strains could be used in starter cultures for rapid production of lactic acid at the beginning of kajmak fermentation. Strains of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* that produce diacetyl and protein antimicrobial substances may have double role, in getting the specific flavor and control of undesirable microflora. Among *L. mesenteroides* isolates, strains that metabolized citrate, also may have important role in the formation of sensory properties of kajmak.

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