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CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM ARTISANAL ZLATAR CHEESES PRODUCED AT TWO DIFFERENT GEOGRAPHICAL LOCATION

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Eighty-one strains of lactic acid bacteria (LAB) were isolated from white semi-hard homemade cheese, designated Zlatar BGNV, which was taken from household settled on Northern side of mountain Zlatar. The Zlatar BGNV cheese was manufactured from raw cow's milk without addition of the starter culture. All isolates of LAB were characterized by

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phenotypic and genotypic tests. Identification of strains was done by the repetitive extragenic palindromic-polymerase chain reaction (rep-PCR) with $(GTG)_5$ primer and 16S rDNA sequence analysis. The most present species in Zlatar BGNV cheese were *Lactobacillus casei/paracasei* (65.43%) and *Enterococcus faecalis* (29.63%). Two facultative heterofermentative rods were identified as *Lactobacillus plantarum* (2.47%), and two obligate hetrofermentative LAB isolates as *Lactobacillus parabuchneri* (2.47%). Among all 81 tested isolates, only eight enterococci were producers of antimicrobial compounds. Fourteen of 16 tested lactobacilli isolates showed medium to very good proteolytic activity. All 57 lactobacilli from the Zlatar BGNV cheese curdled milk very slowly or did not curdle milk at all. However, three isolates of enterococci, BGNV1-63, BGNV1-76 and BGNV1-80, showed very good activity in milk and curdled milk within 5 h. They showed very high proteolytic activity hydrolyzing completely α_{s1}- and κ-casein after 3 h, and β-casein after 30 min of incubation. In addition, those three enterococcal isolates degraded gelatin. Comparing obtained results with those previously achieved in examination of LAB microflora in another Zlatar BGZLS cheese made also from raw cow's milk, it can be concluded that LAB microflora in the Zlatar BGNV cheese is less diverse.

Key words: Zlatar cheese, lactic acid bacteria, proteolytic activity, rep-PCR, 16S rDNA

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INTRODUCTION

Its morphology, climate (combination of Mediterranean and mountain climate) and authentic flora and fauna consider the mountain Zlatar the nature reserve. In the country households, the domestic colorful ox is present and that is the reason why many households practice cheese manufacturing. It is so called "Zlatar cheese" which is prepared from raw cow's milk without adding the starter culture. Specific attributes of raw milk, the nutrition of dairy cows, the basic traditional cheese making process and the natural micro biota responsible for the fermentation process and ripening, have very significant role in formation of the particular flavor and typical organoleptic properties of raw milk cheeses (CORROLER *et al*., 1998; GRAPPIN and BEUVIER, 1998; BERESFORD *et al*., 2001). It has been determined that the cheeses made from raw milk have more intensive and more typical flavor than cheeses made from pasteurized milk (DEMARIGNY *et al*., 1997; BEUVIER *et al*., 1997; ALBENZIO *et al*., 2001).

A thorough study of the microbial community involved in the fermentation process has to be done in order to understand better the role played by the nonstarter lactic acid bacteria (NSLAB) in the production of traditional cheeses. The identification of species and strains involved in the fermentation of raw milk cheese and the study of cheese micro biota were done thanks to the

advanced molecular approaches (WOUTERS *et al.*, 2002). In artisanal cheeses, the micro biota is quite heterogeneous (MOREA *et al.*, 1999). Its composition changes during the cheese ripening. The strains dominating the first stages are not necessarily predominating in the later periods (MANNU and PABA, 2002).

In the present study, we evaluated the dominant NSLAB species of 90 days old Zlatar BGNV cheese, which was taken from one household settled on Northern side of mountain Zlatar, combining conventional microbiological and molecular techniques, rep-PCR and 16S rDNA sequence analysis of bacterial isolates. The results are compared with previously obtained results dealing with NSLAB population isolated from the Zlatar BGZLS cheeses sampled in country household settled on South-Eastern side of mountain Zlatar. The aim was to obtain a more comprehensive view of lactic acid bacteria (LAB) microflora isolated from Zlatar cheeses that are produced from raw cow's milk in households located at different geographical locations

MATERIAL AND METHODS

Cheese-making manufacture and sampling. – Cheese, designated as Zlatar BGNV, was taken from the household settled in village Amzići at 700 m of altitude on Northern side of mountain Zlatar, while samples of the Zlatar BGZLS cheese described previously (TERZIC-VIDOJEVIC *et al.*, 2007) were collected from household settled in village Bukovik over 1200 m of altitude on South-Eastern side of mountain Zlatar. The Zlatar BGNV-cheese is made from raw cow's whole milk by mixing the milk from the morning and evening milking without adding the starter cultures. It belongs to the group of white semi-hard cheeses.

The commercial rennet called "Maja" (Čačak, Serbia) with strength of 1:5000 was used for the production of Zlatar BGNV cheese. The rennet was added in tepid milk (temperature about 25-30ºC). One tablespoon of rennet was added on every 10 l of milk. The forming of a curd lasted 80 min. The end of the curdling process was when the curd detached from the walls of the container and when the 2 cm layer go light-green whey was created above the curd. After the curdling, the curd was transferred into the cotton cloth strainer. The draining of the curd was done spontaneously, without pressing, during about 30 min. Afterwards the curd is drained in the strainer, under pressure (the curd was pressed with the 3 kg stone) during 3-4 h. The cheese had a shape of the 30 cm x 1.5 cm bannock. It was salted with a dry salt (1 kg of salt on 10 kg of cheese). The salt was put first on the bottom of container, and than on every cheese slice. When the container with cheese slices was full, the cheese was covered with the brine so that no slices stuck out. The cheese container was left in the cool room at temperature of 15-18ºC during 30-40 days on summer and longer on winter. After that period, the cheese was used for eating. It needs six liters of milk to produce of 1 kg of cheese.

The sample of Zlatar BGNV cheese was taken under sterile conditions and transported to the laboratory in portable refrigerator for microbiological analysis.

Chemical analysis. - The sample of Zlatar BGNV cheese was subjected to chemical analysis comprising dry matter, protein, fat, fat in dry matter, NaCl content and pH value. Chemical analysis was done according to the same methods described previously (TERZIC-VIDOJEVIC *et al*., 2007). All analyses were performed in triplicate.

Bacterial strains, media, and growth conditions. - The list of reference strains used in this study is shown in Table 1. Lactobacilli was cultivated in MRS broth (pH 5.7) (Merck GmbH, Darmstadt, Germany) while lactococci and enterococci were grown in M17 broth (pH 7.2) (Merck GmbH) supplemented with 0.5% (w/v) glucose (GM17 broth). The incubation was carried out at appropriate growth temperature for each bacterium. Solid medium was obtained by adding 2% (w/v) agar (Torlak, Belgrade, Serbia) to broth. The plates were incubated 24-48 h in corresponding conditions of incubation depending on the strain. Anaerobic conditions were obtained using the Anaerocult A (Merck GmbH) in anaerobic jars. The pure culture was stored at –80ºC in appropriate media containing 15% glycerol (w/v). When active cultures were needed, the frozen cultures were inoculated in corresponding broth at 30ºC (37ºC) during 24 h., Purity was checked by streaking on GM17 or MRS agar plates depending on the strain.

Table 1. The list of strains used in this study.

Bacterial strains	Source of reference
Lactobacillus plantarum A112[a,b]	VUJCIC AND TOPISISROVIC, 1993
Lactobacillus casei ATCC334[b]	ATCC
Lactobacillus paracasei subsp. *paracasei* LMG10774[t]	BCCM/LMG
Lactobacillus paracasei subsp. *paracasei* LMG4560[b]	BCCM/LMG
Lactobacillus paracasei subsp. *paracasei* BGBUK2-16[a,b] and BGBUK2-16/K4[a]	LOZO *ET AL.*, 2004
Lactobacillus paracasei subsp. *paracasei* BGSJ2-8[b]	LOZO *ET AL.*, 2007
Lactobacillus brevis ATCC14869[b]	ATCC
Lactococcus lactis subsp. *lactis* NP45[a]	Laboratory collection
Lactococcus lactis subsp. *lactis* BGMN1-596[a]	GAJIC *ET AL.*, 1999
Lactococcus lactis subsp. *cremoris* NS1[a]	Laboratory collection
Lactococcus lactis subsp. *lactis* biovar. *diacetylactis* S	KOJIC *ET AL.*, 1991B
Enterococcus faecalis BGZLS60-26a[b]	Laboratory collection[c]

[a] Used for BLIS-activity detection; [b] Used for rep-PCR.[c] This strain was identified by molecular methods, AFLP, SDS-PAGE and rep-PCR with $(GTG)_5$ primer in the Laboratorium voor Microbiologie, Universitet Gent, Gent, Belgium.ATCC - American Type Culture Collection, Rockville, Md, USA..BCCM/LMG - Bacteria Collection, Laboratorium voor Microbiologie - Universiteit Gent, Gent, Belgium.

Microbiological analyses. - Isolation of lactic acid bacteria from Zlatar BGNV cheese was done according the procedure described previously (TERZIC-VIDOJEVIC *et al*., 2007). The enumeration of total mesophilic and thermophilic bacteria was performed by using MRS and GM17 agar plates, and the incubation was done at 30ºC, 37ºC and 45ºC for 72 h. The results were expressed as colony forming units (CFU) per gram of cheese.

Eighty-one isolate were characterized using the same tests as for the characterization of LAB isolates from Zlatar BGZLS cheese (TERZIC-VIDOJEVIC *et al*., 2007). In addition, the following tests were performed, too: growth at 15^{o}C and growth in broth with 2% and 8% of NaCl (those three tests were repeated three times). Production of acetoin from glucose or Voges-Proskauer (V.P.) test (ZOURARI *et al*., 1991); growth and production of slime from sucrose on MSE agar plates (MAYEUX *et al*., 1962), as well as and formation of black zone on bile-esculin agar (BEA) (Himedia, Mumbai, India) only for cocci-like LAB were examined too.

Identification of LAB isolates based on their phenotypic characteristics was performed according to the methods and criteria of SHARPE (1979), GARVIE *et al*. (1986), KANDLER and WEISS (1986), MUNDT (1986a, b) and SNEATH *et al*. (1986).

Detection of antimicrobial activity. - The ability of bacteriocin production by isolated LAB was preliminary screened by agar-well diffusion method (TAGG and MCGIVEN, 1971) using the indicator stains presented in Table 1. To confirm the production of antimicrobial compounds of proteinaceous nature, a crystal protease TYPE XIV (Sigma Chemie GmbH, Deisenhofen, Germany) was placed close to the edge of the well containing antimicrobial compound. The plates were incubated overnight at 30ºC. A clear zone of inhibition around the well, but not near the protease crystal, was taken as an indicator possible antimicrobial compounds production.

Proteolytic activity. –Caseinolytic activity by chosen of LAB isolates were assayed previously described (KOJIC *et al*., 1999a) with minor modification. LAB strains were grown on milk-citrate agar MCA plates for 48 h at 30°C. MCA plates containing 4.4% reconstituted skimmed milk (RSM), 0.8% sodium citrate, 0.1% yeast extract, 0.5% glucose and 1.5% agar Fresh cells were collected (10 mg approximate density 10^{10} cells per ml) and resuspended in 100 mM sodium-phosphate buffer, pH 6.8. The cell suspension was mixed with 5 mg of α_{s1}-, β- or κ-casein (Sigma, St. Louis, MO, USA) per ml dissolved in the same buffer at a 1:1 volume ratio. The mixtures were incubated for 3 h at 30ºC. Centrifugation of the mixtures was done 5 min at 12,000 rpm to remove the cells. The clear supernatants were mixed with solubilisation buffer (125 mM TrisHCl, pH 6.8, 10 mM disodium

EDTA, 4% sodium dodecyl sulphate (SDS), 25% glycerol., 5% 2-mercaptoethanol and 0.07% bromophenolblue) at a 1:1 volume ratio. Samples were heated at 100°C for 2 min. Electrophoresis was carried out on 12.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for 20 h at 10 mA constant current. Gels were stained with Coomassie brilliant blue R250 (Serva, Heidelberg, Germany) and distained in a mix of methanol (20%) and acetic acid (7%).

Gelatinase activity assay. - The phenotypic assay of gelatinase activity was performed as described by SU *et al*. (1991). A drop of culture was deposited on plate containing Gelatin Agar, with the following formulation per litter: peptone (Torlak) 5 g, yeast extracts (Torlak) 3 g, gelatin (Himedia, Mumbai, India) 30 g, agar (Torlak) 17 g, pH 7.0. After incubation at 37ºC for 48 h, the plates were flooded with a saturated solution of ammonium sulphate (BRL, Gaithersburg, MD, USA). Gelatin precipitates and transparent halo around cells appear around gelatinase producers.

rep-PCR analysis and 16S rDNA sequencing of LAB isolates. - The method for isolation and purification of total DNA from LAB isolates given by HOPWOOD *et al*. (1985) was used in this study.For rep-PCR analysis total DNA from different isolates of LAB were used as templates for PCR amplifications with $(GTG)_5$ (5´-GTGGTGGTGGTGGTG-3´) oligonucleotide primer, with its optimal PCR program (VERSALOVIC *et al.*, 1994), using Taq DNA polymerase (Fermentas UAB, Vilnius, Lithuania).

The PCR reactions were performed in a 50 µl reaction mixture containing 1 µg DNA template, 20 mmol/l Tris-HCl (pH 8.4), 50 mmol/l KCl, 3 mmol/l $MgCl_2$, 50 mmol/l each of the four deoxynucleotide triphosphates (dNTP), 1 U of *Taq* polymerase and 5 pmol/l of primer (Fermentas UAB, Vilnius, Lithuania).

The samples were amplified in GeneAmp PCR System 2700 (Applied Biosystems, Foster City, Calif.) using the following programme: initial denaturation of DNA for 7 min at 95ºC, 32 cycles of 1 min at 94ºC, 1 min at 40ºC, and 8 min at 65ºC; and extension of incomplete products for 16 min at 65ºC. PCR products were quantified by electrophoresis on a 1% agarose gel containing ethidium bromide and visualized by CCD camera Biometra BDR2/5/6 (Bio Doc Analyze GmbH, Göttingen, Germany).

For the sequencing of 16S rDNA, total DNA from chosen LAB isolate was utilized 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), using the same PCR reaction mixture (50 µl) as described previously (TERZIC-VIDOJEVIC *et al.*, 2007). Obtained PCR amplicons were purified by QIAquick PCR Purification Kit/250 (Qiagen GmbH, Hilden, Germany), and sequenced by sequencing service Macrogen, Soeul, Korea. The BLAST algorithm was used to determine the most related sequence relatives in the NCBI nucleotide sequence database (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS

Chemical analysis. - The chemical composition of the 90 days old Zlatar BGNV cheese is shown in detail in Table 2. The values of dry matter (45.90%) and fat in dry matter (64.03%) in this cheese showed that it belongs to extra fat white semi-hard cheese. The protein content in cheese was 13.50 %, and the salt content was 2.28%.

Table 2. Chemical composition of Zlatar BGNV cheese.

Chemical characteristics	
Dry matter (%)	45.90
Proteins (%)	13.50
Fat (%)	19.00
Fat in dry matter (%)	64.03
NaCl content (%)	2.28
pH	4.95

Mean values of three independent measurements

Total count of bacteria. - Total count of viable bacteria grown at different incubation temperature was higher on GM17 agar plates than on MRS agar plates (Table 3). The average count of total bacteria on GM17 medium ranged between 1.84 x 10^6 CFU/g and 7.93 x 10^7 CFU/g of cheese when grew at 45ºC and 30ºC, respectively. On the other hand, when grown on MRS agar plates total count of bacteria was 1.08 x 10^8 CFU/g at 30ºC and 5.66 x 10^7 CFU/g of cheese at 37ºC, whilst the growth of bacteria on MRS agar plates at 45ºC was not recorded.

Table 3. Total number of viable bacteria in Zlatar BGNV cheese on MRS and GM17 agar plates.

	Agar plates and temperature of incubation					
	MRS at 30ºC	MRS at 37ºC	MRS at 45ºC	GM17 at 30ºC	GM17 at 37ºC	GM17 at 45ºC
CFU/g of cheese[a]	1.08 x 10^8	5.66 x 10^7	No growth	4.72 x 10^7	7.93 x 10^7	1.84 x 10^6

[a] Average values of three independent experiments

Physiological characteristics of LAB isolated from Zlatar BGNV cheese. – In total, 81 Gram-positive and catalase-negative isolates (57 rods and 24 cocci) were obtained from Zlatar BGNV cheese. Randomly chosen isolates were designated as BGNV1-1 to BGNV1-81. The results obtained by testing the physiological abilities showed that 50 isolates belong to the *Lactobacillus*

plantarum species, 4 isolates to *Lactobacillus paracasei*, 3 isolates to *Lactobacillus brevis* and 24 isolates belonged to the genus *Enterococcus* (Table 4).

Table 4. Physiological characteristics of isolated LAB from Zlatar BGNV cheese.

Tests	Lactic acid bacteria			
	Lb. plantarum	*Lb. paracasei*	*Lb. brevis*	*Enterococcus* spp.
Morphology	Rods	Rods	Rods	Cocci
Growth at 15°C	+[a]	+	+	+
Growth at 30°C	+	+	+	+
Growth at 45°C	-	-	-	+
Growth in 2% NaCl	+	+	+	+
Growth in 4% NaCl	+	+[b]	-[b]	+
Growth in 6.5% NaCl	+	-	-	+
Growth in 8% NaCl	+[a]	-	-	+
Hydrolysis of arginine	-	-	+	+
Utilization of citrate	+[a]	+	+	-
Hydrolysis of esculin	+[a]	+	-	+
Production of CO_2	-	-	+	-
Production of diacetyl	-	-	-	-
V.P. test	+[b]	+[b]	-[b]	+
Black zone on bile esculin agar	NT	NT	NT	+
Slime on MSE agar	-	-	-	-
Activity in milk (h)	24[a]	ACR[b]	No activity	24[b]
Litmus milk	ACR	48[b]	P	ACR
Number of strains	50	4	3	24

Lb. – *Lactobacillus.*, [a] More than 90% of the strains; [b] Between 10% and 90% of the strains.NT - not tested. A - acid production; C - curd formation; R - litmus reduction; P - purple (unchanged reaction).

All enterococci isolates grown in broth at 45ºC. More than 90% of the most numerous group of the rod shaped isolates, as well as enterococci, showed the growth in broth containing 8% of NaCl.. None of isolate showed the ability of diacetyl production. However, more than 90% of all isolates could use citrate and produced acetoin. Generally, activity in milk of all isolates was very low. The exception was three isolates (12.5%) of enterococci (BGNV1-63, BGNV1-76 and BGNV1-80), which were able to curdle milk within 5 h. Other isolates curdled milk after 24 h or 48 h of incubation or did not curdle milk at all.

Antimicrobial activity of LAB. - Eight of total 81 tested LAB isolates showed the ability to produce of antimicrobial compounds. Experiments with protease revealed a proteinaceous nature of antimicrobial compounds, indicating the possibility they could be bacteriocin-like substances (BLIS). All BLIS producers belonged to species *Enterococcus faecalis* as identified by rep-PCR and 16S rDNA sequencing. These isolates gave clear or turbid inhibition zones on six indicator strains used in this study (Table 5).

Table 5. Antimicrobial activity of LAB from ZlatarBGNV cheese.

Isolates	Indicator strains[a]					
	BGMN1-596	S50	NS1	NP45	A112	BUK2-16/K4
BGNV1-55	4 mm (c)	3 mm (c)	4 mm (c)	1 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-59	5 mm (c)	3 mm (c)	5 mm (c)	2 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-61	4 mm (c)	2 mm (c)	4 mm (c)	1 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-68	4 mm (c)	2 mm (c)	4 mm (c)	2 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-70	5 mm (c)	3 mm (c)	5 mm (c)	2 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-71	5 mm (c)	3 mm (c)	5 mm (c)	2 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-75	5 mm (c)	3 mm (c)	5 mm (c)	1 mm (c)	0.5 mm (t)	0.5 mm (t)
BGNV1-76	4 mm (c)	2 mm (c)	3 mm (c)	1 mm (c)	0.5 mm (t)	0.5 mm (t)

[a] The names of indicator strains are indicated in Table 1.,c - clear zone of inhibition; t - turbid zone of inhibition.

Proteolytic activity. - According to the phenotypic characterization and rep-PCR results, 16 lactobacilli and 9 enterococci were chosen for the analysis of proteolytic activity (Figure 1). Among isolates identified as *Lactobacillus paracasei*, five (BGNV1-23, BGNV1-30, BGNV1-36, BGNV1-38 and BGNV1-45) showed very good whereas seven showed medium ability to degrade β-casein. No, activity towards β-casein hydrolysis was detected in two (BGNV1-3 and BGNV1-27) isolates. In addition, two isolates identified as *Lactobacillus plantarum* species (BGNV1-73 and BGNV1-74) also showed very good ability to degrade β-casein. Within the group of nine isolated *Enteroccoccus faecalis,* three (BGNV1-63, BGNV1-76 and BGNV1-80) showed a significant ability to degrade β-casein while six isolates (BGNV1-57, BGNV1-67, BGNV1-69, BGNV1-70, BGNV1-71 and BGNV1-72) did not show proteolytic activity. Since enterococci generally degrade β-casein poorly, three isolate of enteroccoci, which showed good ability to hydrolyze β-casein, were chosen for further examination. Obtained results revealed that all three isolates of enterococci (BGNV1-63, BGNV1-76 and

BGNV1-80) completely degrade α_{s1}- and κ-casein after 3 h of incubation and β-casein after 30 min of incubation (Figure 2).

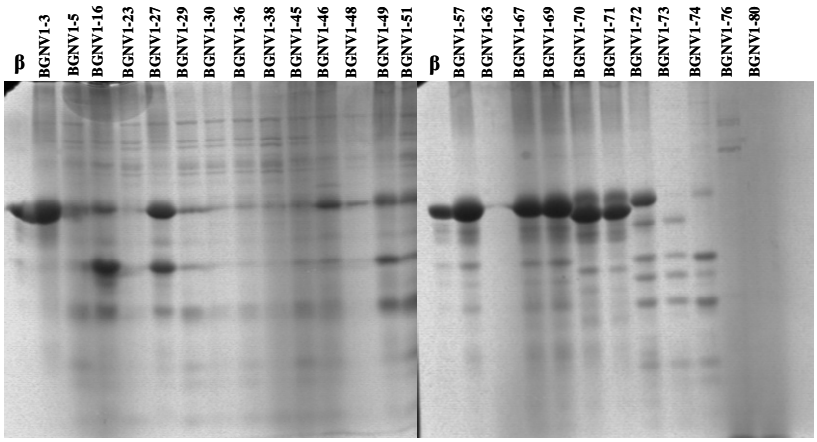

Figure 1. Proteolytic activity of chosen LAB isolated from artisanal Zlatar BGNVcheese. Reaction mixtures were incubated at 30ºC for 3 h. β: substrate β-casein.

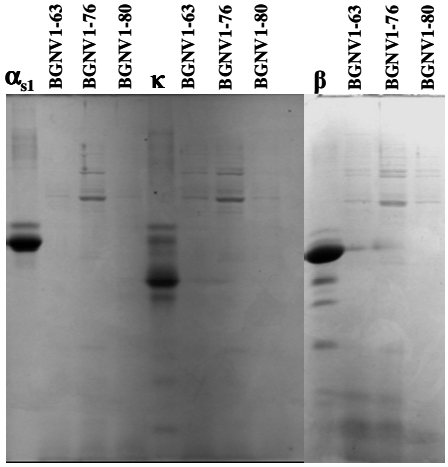

Figure 2. Hydrolysis of casein by three enterococcal isolates.Degradation of α_{s1}- and κ-casein after 3 h of incubation at 30ºC and degradation of β-casein after 30 min of incubation at 30ºC. α_{s1}, β and κ: substrates α_{s1}-casein, β-casein and κ-casein, respectively.

The assay of gelatinase activity with mentioned isolated of enterococci was also performed. All three isolates formed a clear zone on the medium described in Materials and Methods suggesting that they are producers of gelatinase (data not shown).

rep-PCR and 16S rDNA sequence analysis. - All 81 isolate were analyzed according to rep-PCR with $(GTG)_5$ primer. After those 11 groups of isolates with different band patterns was made. Per one isolate from each group was chosen for presentation of rep-PCR analysis (Figure 3). It was confirmed that in the Zlatar BGNV cheese two species were dominant: One group of rod-shaped LAB (53 isolates) was identified as *Lactobacillus casei/paracasei* because showed significant similarity with *Lactobacillus casei* and *Lactobacillus paracasei* reference strains. Second group of coccal-shaped LAB (24 isolates) was identified as *Enterococcus faecalis.* Two lactobacilli, which showed same band patterns belong to the species *Lactobacillus plantarum* (BGNV1-73 and BGNV1-74). However, two lactobacilli shearing same band patterns were unidentified (BGNV1-22 and BGNV1-24).

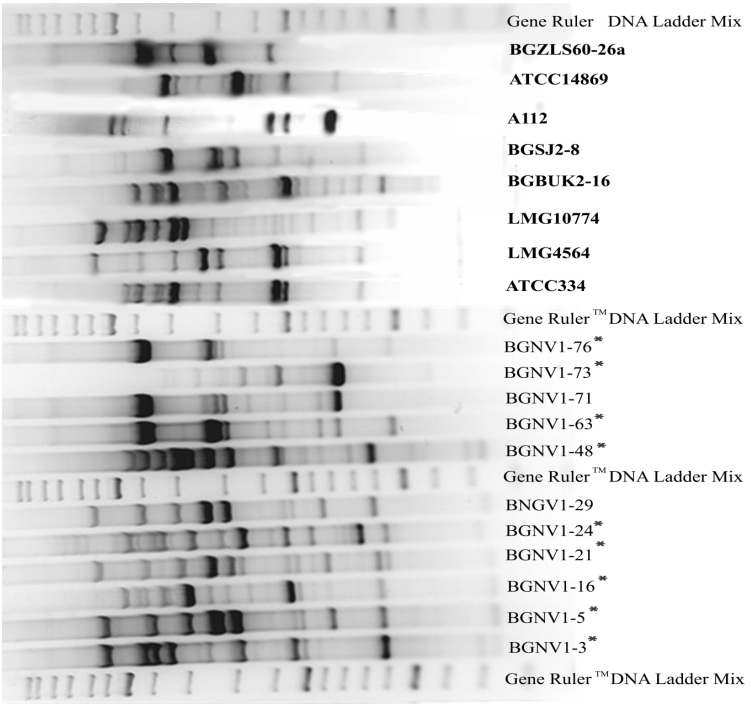

Figure 3. The rep-PCR analysis of LAB isolated from artisanal Zlatar NV cheese. Reference strains used in the test are given in bold letters. *Isolates which are also identified by 16S rDNA sequencing.

Since discrepancies in band patterns between analyzed isolates by rep-PCR and references strains were obtained, nine isolates were subjected to 16S rDNA gene sequencing. The results of sequencing showed that two of seven chosen lactobacilli were identified as *Lactobacillus casei* (BGNV1-3 and BGNV1-5), three as *Lactobacillus casei/paracasei* (BGNV!-16, BGNV1-21 and BGNV1-48), one as *Lactobacillus pentosus/plantarum* (BGNV1-73), and one lactobacilli was identified as heterofermentative species *Lactobacillus parabuchneri* (BGNV1-24). Based on the same method, two isolate of enterococci (BGNV1-63 and BGNV1-76) are identified as *Enterococcus faecalis* (Table 6).

Table 6. The identification of isolated LAB by rep-PCR with $(GTG)_5$ primer and 16S rDNA sequencing.

Isolates	Identification by rep-PCR	Identification by 16S rDNA sequencing	(% identity)[a]
BGNV1-3	*Lb. casei/paracasei*	*Lb. casei*	(100%)
BGNV1-5	*Lb. casei/paracasei*	*Lb. casei*	(100%)
BGNV1-16	*Lb. casei/paracasei*	*Lb. casei/paracasei*	(95%)
BGNV1-21	*Lb. casei/paracasei*	*Lb. casei/paracasei*	(98%)
BGNV1-24	Unidentified	*Lb. parabuchneri*	(98%)
BGNV1-29	*Lb. plantarum*	ND	(94%)
BGNV1-48	*Lb. paracasei*	*Lb. casei/paracasei*	(98%)
BGNV1-63	*Ec. faecalis*	*Ec. faecalis*	(99%)
BGNV1-71	*Ec. faecalis*	ND	(99%)
BGNV1-73	*Lb. plantarum*	*Lb. pentosus/ plantarum*	(99%)
BGNV1-76	*Ec. faecalis*	*Ec. faecalis*	(89%)

Lb. – Lactobacillus; *Ec. – Enterococcus*.

ND - Not determined.

[a] Demonstrate identity with 16S rDNA sequences of relevant species deposited in GenBank database (NCBI).

DISCUSSION

The chemical analysis of Zlatar BGNV and BGZLS cheeses showed that significant variation exists between them. Based on the fat content in dry matter (64.03%) Zlatar BGNV cheese could be classified as extra fat cheese. On the other hand, Zlatar BGZLS cheese belongs to the group of high fat cheeses since the fat

content in dry matter ranged between 50.0% in 1-day-old cheese and 54.9% in 60 days old cheese as described previously (TERZIC-VIDOJEVIC *et al*., 2007). Since the manufacturing of artisanal Zlatar BGNV and BGZLS cheeses is not standardized, there are lot of variations in chemical parameters, especially concerning content of salt, proteins and fat as well as pH values. The technological procedures, which are specific for each household, significantly affect these values. Many authors reported on values of chemical parameters of artisanal cheeses. For example, ESTEPAR *et al*. (1999) mentioned that the pH value in artisanal 30 days old Peñamellera cheese is 6.32, NaCl content 1.80%, and fat content is 33.67%. Fat content in dry matter in 60 days old Ibores cheese was 52.64%, NaCl content 2.50%, and pH values 5.18 (MAS *et al*., 2002). DERVISOGLU *et al*. (2001) determined that the NaCl content in 90 days old Kulek cheese was 5.63% and pH value 5.33. The data shown by PISANO *et al*. (2006), got by analyzing the traditional Fiore Sardo cheese show that the NaCl content in 90 days old cheese was 4.56 %, fat content in dry matter 45.35%, and pH value was 5.13.

The counts of viable microorganisms in Zlatar BGNV cheese ranged between 10^6-10^8 CFU/g of cheese depending on growth media. The data on microorganism numbers in Zlatar BGZLS cheese, (VELJOVIC *et al*., 2007), show that the total number of microorganisms ranged between 10^4 and 10^9 CFU/g of cheese. Similar data on count of total viable microorganisms in other artisanal cheeses are described by DE ANGELIS *et al*. (2001), FITZSIMONS *et al*. (2001), as well as and KAGKLI *et al*. (2007).

The presence of lactobacilli is 2.5 times higher than cocci in the Zlatar BGNV cheese. In contrast, the number of isolated lactobacilli and cocci was approximately the same in the 60 days old Zlatar BGZLS cheese. Based on the physiological characteristics, lactobacilli in the Zlatar BGNV cheese were preliminary determined as *Lactobacillus plantarum* species. However, using rep-PCR and 16S rDNA sequencing of these isolates revealed that they are *Lactobacillus casei* and *Lactobacillus paracasei* subsp. *paracasei* species. The species *Lactobacillus paracasei* subsp. *paracasei* is often present in the raw milk cheeses which other authors also point to (ALBENZIO *et al*., 2001; POZNANSKI *et al*., 2004; NIKOLIC *et al*., 2007; ABRIOUEL *et al*., 2008). This species was also the most present in the Zlatar BGZLS cheese regardless of ripening period (TERZIC-VIDOJEVIC *et al*., 2007; VELJOVIC *et al*., 2007). As reported earlier, the species *Lactobacillus casei/paracasei* and *Lactobacillus plantarum* were the only microorganisms, which continued to multiply during cheese ripening (MANNU *et al*., 2000). One of the reason for this fact is their capability to use citrate as a potential source of carbon (PALLES *et al*., 1998), which significantly contributes the formation of the typical flavor of artisanal cheeses given by mesophilic lactobacilli which are generally the main part of NSLAB (ALBENZIO *et al*., 2001).

The rep-PCR and 16S rDNA sequence analyses also showed that only two of 57 lacobacilli isolated from the Zlatar BGNV cheese were identified as *Lactobacillus plantarum*. In the Zlatar BGZLS cheese, the species *Lactobacillus*

plantarum was also less present then *Lactobacillus paracasei* subsp. *paracasei* species (TERZIC-VIDOJEVIC *et al*., 2007; VELJOVIC *et al*., 2007).

Two heterofermentative lactobacilli isolated from the Zlatar BGNV cheese were characterized as *Lactobacillus brevis* according to physiological tests. However, further characterization by 16S rDNA sequencing revealed that both isolates are *Lactobacillus parabuchneri. Lactobacillus parabuchneri* is an obligate heterofermentative LAB isolated occasionally from cheeses. GOBBETTI *et al*. (2002) identified a significant number of *Lactobacillus parabuchneri* strains from 42 and 60 days old Caciocavallo Pugliese cheese. On the other hand, in the Zlatar BGZLS cheese *Lactobacillus brevis* was the only detected heterofermentative species (TERZIC-VIDOJEVIC *et al*., 2007; VELJOVIC *et al*., 2007).

The second present LAB group in the Zlatar cheeses is enterococci. Enterococci are widely distributed in nature. *Enterococcus faecalis* is the most frequently found species in dairy products (ANDRIGHETTO *et al.*, 2001; PRODROMOU *et al*., 2001; AYAD *et al*., 2004; PSONI *et al*., 2006). Analysis showed that enterococci are the only cocci-shaped LAB isolated from the Zlatar BGNV cheese. However, both lactococci and enterococci were found among cocci-shaped LAB in the Zlatar BGZLS cheese (TERZIC-VIDOJEVIC *et al*., 2007; VELJOVIC *et al*., 2007).

The ability of bacteriocinogenic enterococci to inhibit the growth of certain pathogens and spoilage microorganisms shows their great potential to be used in food preservation (ANDRIGHETTO *et al.*, 2001; GIRAFFA, 2002; FOULQUIÉ-MORENO *et al.*, 2006). Testing of all 81 isolates from Zlatar BGNV cheese for antimicrobial activity revealed that only eight isolates of eneterococci produced bacteriocin-like substance. Conversely, the bacteriocin-like substance producers found in the Zlatar BGZLS cheese belong to *Lactococcus* and *Lactobacillus* strains, besides enterococci (VELJOVIC *et al*., 2007; TOPISIROVIC *et al*., 2007)

All 50 of *Lactobacillus casei/paracesi*, as well as two *Lactobacillus plantarum* strains showed a low activity in milk since milk curdling occurred after 24 h. Similarly, *Lactobacillus casei/paracesi* and *Lactobacillus plantarum* strains isolated from Armada cheese, made from raw goat's milk show milk curdling after 24 h (HERREROS *et al*., 2003) showed. Strains of *Lactobacillus casei/paracasei* showed variable proteolytic activity. Most of them exhibited a very low proteolytic activity, while approximately 35% of the tested strains was highly proteolytic, which correspond with the results on proteolytic activity of the *Lactobacillus casei/paracasei* strains described formerly (MADRAU *et al.,* 2006; MANGIA *et al*., 2008). Both examined *Lactobacillus plantarum* strains isolated from the Zlatar BGNV cheese showed good proteolytic activity. *Lactobacillus plantarum* strains isolated from Fiore Sardo cheese during the ripening also showed a good proteolytic activity (MANGIA *et al*., 2008). One group of tested *Lactbacillus paracasei* subsp. *paracasei* isolates from the Zlatar BGZLS cheese exhibited strong proteolytic activity. Second group of *Lactobacillus paracasei* subsp. *paracasei* isolates, as well as *Lactobacillus plantarum* strains from same cheese

degraded β-casein poorly or not at all (VELJOVIC *et al*., 2007; TOPISIROVIC *et al*., 2007)

Three *Enterococs faecalis* isolates from Zlatar BGNV cheese were able to coagulate milk and showed very high proteolytic activity. In addition, they showed ability to hydrolyze gelatin indicating that that produce extracellular zinc metalloproteinase gelatinase. The examination of gelatinase activity in dairy enterococci revealed that besides *Enterococcus faecium* and *Enterococcus faecalis*, this enzyme could be procuced by *Enterococcus durans* and *Enterococcus hirae* (LOPES *et al*., 2006). *Enterococcus* species isolated from the Zlatar BGZLS cheese exhibited weak proteolytic activity (TOPISIROVIC *et al*., 2007). ANDRIGHETTO *et al*. (2001) reported that 21% of strains isolated from Italian cheeses and identified as *Enterococcus faecalis* curdled milk after 6 h, and all except one, showed proteolytic activity. The high proteolytic activity shown by some strains belonging to the species *Enterococus faecalis* could contribute the sensorial and textural properties of cheese (CENTENO *et al*., 1999).

In this study, comparison of LAB microflora from the Zlatar BGNV and BGZLS cheeses produced at two different geographical locations was performed. Four species of LAB were isolated from artisanal Zlatar BGNV cheese. Among them *Lactobacillus casei/paracasei* is dominant group comprising 65.4% of microflora. The next numerous LAB species (29.6%) was *Enterococcus faecalis.* Only two *Lactobacillus plantarum* were found in Zlatar BGNV cheese (2.5%) and two isolates of heterofermentative species *Lactobacillus parabuchneri* (2.5%). Antimicrobial compounds producers were found solely among enterococci. Lactobacilli showed a variable proteolytic activity whereas only three enterococci isolates showed good acidification and proteolytic activity as well as an ability to degrade gelatin. In contrast, previous examination of LAB microflora in the Zlatar BGZLS cheese showed that microflora of the Zlatar BGZLS cheese is richer in LAB species and it is composed of lactococci, lactobacilli and enterococci. In addition, there are a significant number of bacteriocin producers including nisin producers among lactococci. However, taking into consideration all available data it is hard to conclude that this variation of microflora is the consequence of different geographical origin of cheeses. It is rather linked to the specificities of household cheese manufacturing.

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REFERENCES

ABRIOUEL, H., A. MARTIN-PLATEREO, M. MAQUEDA, E. VALDIVIA, and M. MARTINEZ-BUENO (2008): Biodiversity of the microbial community in a Spanish farmhouse cheese as revealed by culture-dependent and culture-independent methods. Int. J. Food Microbiol., *127*, 200-208.

ALBENZIO, M., M.R. CORBO, S.U. REHMAN, P.F. FOX, M. DE ANGELIS, A. CORSETTI, A. SEVI, and M. GOBBETTI (2001): Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk, or by heating the curd in hot way. Int. J. Food Microbiol., *67*, 35-48.

ANDRIGHETTO, C., E. KNIJFF, A. LOMBARDI, S. TORRIANI, M. VANCANNEYT, K. KERSTERS, J. SWINGS, and F. DELLAGLIO (2001): Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. J. Dairy Res., *68*, 303-316.

AYAD, E.H.E., S. NASHAT, N. EL-SADEK, H. METWALY, and M. EL-SODA (2004): Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol., *21*, 715-725.

BERESFORD, T.P., N.A. FITZSIMONS, N.L. BRENNAN, and T.M. COGAN (2001): Recent advances in cheese microbiology. Int. Dairy J., *11*, 259-274.

BEUVIER, E., K. BERTHAUD, S. CEGARRA, A. DASEN, S. POCHET, S. BUCHIN, and G. DUBOZ (1997): Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. Int. Dairy J., *7*, 311-323.

CENTENO, J.A., S. MENENDEZ, M.A. HERMIDA, and J.L. RODRIGUEZ-OTERO (1999): Effects of the addition of *Enterococcus faecalis* in Cebreiro cheese manufacture. Int. J. Food Microbiol., *48*, 97-111.

CORROLER, D., I. MANGIN, N. DESMASURES, and M. GUEGUEN (1998): An ecological study of lactococci isolated from raw milk in the Camembert cheese registered designation of origin area. Appl. Environ. Microbiol., 64, 4729-4735.

DE ANGELIS, M., A. CORSETTI, N. TOSTI, J. ROSSI, R. CORBO, and M. GOBBETTI (2001): Characterization of non-starter lactic acid bacteria from Italian ewe cheeses based on phenotypic, genotypic, and cell wall protein analyses. Appl. Environ. Microbiol., 67, 2011-2020.

DERVISOGLU, M., and F. YAZICI (2001): Ripening changes of Kulek cheese in wooden and plastic containers. J. Food Engin., 48, 243-249.

DEMARIGNY, Y., E. BEUVIER, S. BUCHIN, S. POCHET, and R. GRAPPIN (1997): Influence of raw milk microflora on the characteristics of Swiss-type cheeses: II. Biochemical and sensory characteristics. Lait. 77, 151-167.

ESTEPAR, J., M. DEL MAR SÁNCHEZ, L. ALONSO, and B. MAYO (1999): Biochemical and microbiological characterization of artisanal 'Peñamellera' cheese: analysis of its indigenous lactic acid bacteria. Int. Dairy J., 9, 737-746.

FITZSIMONS, A.N., T.M. COGAN, S. CONDON, and T. BERESFORD (2001): Spatial and temporal distribution of non-starter lactic acid bacteria in cheddar cheese. J. Appl. Microbiol., 90, 600-608.

FOULQUIÉ-MORENO, M.R., P. SARANTINOPOULOS, E. TSAKALIDOU, and L. DE VUYST (2006): The role of application of enterococci in food and health. Int. J. Food Microbiol., 106, 1-24.

GAJIC, O., M. KOJIC, A. BANINA, and L. TOPISIROVIC (1999): Characterization of natural isolate Lactococcus lactis subsp. lactis BGMN1-5, a strain producing two bacteriocins, cell wall-associated proteinase and showing clumping phenotype. Arch. Biol. Sci., 51, 69-78.

GARVIE, E. (1986): Genus Leuconostoc. In: Bergey's Manual of Systematic Bacteriology, 6th ed. Vol. 2. P.H. Sneath (Ed.), Williams and Wilkins Co, Baltimore, 1071-1075.

GIRAFFA, G. (2002): Enterococci from foods. FEMS Microbiol. Rev., 26, 163-171.

GOBBETTI, M., M. MOREA, F. BARUZZI, M.R. CORBO, A. MATARANTE, T. CONSIDINE, R. DI CAGNO, T. GUINEE, and P.F. FOX (2002): Microbiological, compositional, biochemical and textural characterisation of Caciocavallo Pugliese cheese during ripening. Int. Dairy J., 12, 511-523.

GRAPPIN, R., and E. BEUVIER (1998): Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese: a review. Bull. Int. Dairy Fed., 327, 16-19.

HERREROS, M.A., J.M. FRESNO, M.J. GONZALEZ PRIETO, and M.E. TORNADIJO. (2003): Technological characterization of lactic acid bacteria isolated from Armado cheese (a Spanish goats' milk cheese). Int. Dairy J., 13, 469-479.

HOPWOOD, D.A., J.M. BIBB, K.F. CHATER, T. KIESER, C.J. BRUTON, H.M. KIESER, K.M. LYDIATE, C.P. SMITH, J.M. WARD, and H. SCHREMPF (1985): Genetic manipulation of Streptomyces, a laboratory manual. The John Innes Foundation, Norwich, UK.

KAGKLI, D.M., M. VANCANNEYT, C. HILL, P. VANDAMME, and T.M. COGAN (2007): Enterococcus and Lactobacillus contamination of raw milk in a farm dairy environment. Int. J. Food Microbiol., 114, 243-251.

KANDLER, O., and N. WEISS (1986): Genus Lactobacillus Beijerinck 1901. In: Bergey's Manual of Systematic Bacteriology, Vol 2. P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.), Williams and Wilkins Co, Baltimore, 1209-1243.

KOJIC, M., D. FIRA, A. BANINA, and L. TOPISIROVIC (1991a): Characterisation of the cell-bound proteinase of Lactobacillus casei HN14. Appl. Environ. Microbiol., 57, 1753-1757.

KOJIC, M., J. SVIRCEVIC, A. BANINA, and L. TOPISIROVIC (1991b): Bacteriocin producing strains of Lactococcus lactis subsp. diacetylactis S50. Appl. Environ. Microbiol., 57, 1835-1837.

LOZO, J., M. VUKASINOVIC, I. STRAHINIC, and L. TOPISIROVIC (2004): Characterization and antimicrobial activity of bacteriocin 217 produced by natural isolate Lactobacillus paracasei subsp. paracasei BGBUK2-16. J. Food Prot., 67, 2727-2734.

LOPES, M.F.S., A.P. SIMÕES, R. TENREIRO, J.J.F. MARQUES, and M.T.B. CRESPO (2006): Activity and expression of a virulence factor, gelatinase, in dairy enterococci. Int. J. Food Microbiol., 112, 208-214.

LOZO, J., B. JOVCIC, M. KOJIC, M. DALGALARRONDO, J. CHOBERT, T. HAERTLÉ, and L. TOPISIROVIC (2007): Molecular characterization of a novel bacteriocin and an unusually large aggregation factor of Lactobacillus paracasei subsp. paracasei BGSJ2-8, a natural isolate from home-made cheese. Curr. Microbiol., 55, 266-271.

MADRAU, M.A., N.P. MANGIA, M.A. MURGIA, M.G. SANNA, G. GARAU, L. LECCIS, M. CAREDDA, and P. DEIANA (2006): Employment of autochthonous microflora in Pecorino Sardo cheese manufacturing and evolution of physicochemical parameters during ripening. Int. Dairy J., 16, 876-885.

MANGIA, N.P., M.A. MURGIA, G. GARAU, M.G. SANNA, and P. DEIANA (2008): Influence of selected lab cultures on the evolution of free amino acids, free fetty acids and Fiore Sardo cheese microflora during the ripening. Food Microbiol., 25, 366-377.

MANNU, L., R. COMUNIAN, and M.F. SCINTU (2000): Mesophilic lactobacilli in Fiore Sardo cheese: PCR-identification and evolution during cheese ripening. Int. Dairy J., 10, 383-389.

MANNU, L., and A. PABA (2002): Genetic diversity of lactococci and enterococci isolated from home-made Pecorino Sardo ewes' milk cheese. J. Appl. Microbiol., 92, 55-62.

MAS, M., R. TABLA, J. MORICHE, I. ROA, J. GONZALEZ, E.J. REBOLLO, and P. CÁCERES (2002): Ibores goat's milk cheese: Microbiological and physiological changes throughout ripening. Lait. 82, 579-587.

MAYEUX, J.V., W.W.E. SANDINE, and P.R. ELLIKER (1962): A selective medium for detecting Leuconostoc organisms in mixed isolate starter cultures. J. Dairy Sci., 45, 655-656.

MOREA, M., F. BARUZZI, and P.S. COCCONCELLI (1999): Molecular and physiological characterization of dominant populations in traditional Mozzarella cheese processing. J. Appl. Microbiol., 87, 574-582.

MUNDT, J.O. (1986a): Lactic acid streptococci. In: Bergey's Manual of Systematic Bacteriology, Vol 2. P.H.A. Sneath, N.S. Mair, M. E. Sharpe, and J.G. Holt (Eds.), Williams and Wilkins Co, Baltimore, 1064-1071.

MUNDT, J.O. (1986b): Enterococci. In: Bergey's Manual of Systematic Bacteriology. J. P. Butler (Ed.), Williams and Wilkins Co, Baltimore, 1063-1065.

NIKOLIC, M., A. TERZIC-VIDOJEVIC, B. JOVCIC, J. BEGOVIC, N. GOLIC, and L. TOPISIROVIC (2008): Characterization of lactic acid bacteria isolated from Bukuljac, a homemade goat's milk cheese. Int. J. Food Microbiol., 122, 162-170.

PALLES, T., T. BERESFORD, S. CONDON, and T.M. COGAN (1998): Citrate metabolism in Lactobacillus casei and Lactobacillus plantarum. J. Appl. Microbiol., 85, 147-154.

PISANO, M.B., M.E. FADDA, M. DEPLANO, A. CORDA, and S. COSENTINO (2006): Microbiological and chemical characterization of Fiore Sardo, a traditional Sardinian cheese made from ewe's milk. Int. J. Dairy Technol., 59, 171-179.

POZNANSKI, E., A. CAVAZZA, F. CAPPA, and P.S. COCCONCELLI (2004): Indigenous milk microbiota influences the bacterial development in traditional cheese from an alpine natural park. Int. J. Food Microbiol., 92, 141-51.

PRODROMOU, K., P. THASITOU, E. HARITONIDOU, N. TZANETAKIS, and E. LITOPOULOU-TZANETAKI (2001): Microbiology of "Orynotyri", a ewe's milk cheese from the Greek mountains. Food Microbiol., 18, 319-328.

PSONI, L., C. KOTZAMANIDES, C. ANDRIGHETO, A. LOMBARDI, N. TZANETAKIS, and E. LITOPOULOU-TZANETAKI (2006): Genotypic and phenotypic heterogeneity in Enterococcus isolates from Batzos, a raw goat milk cheese. Int. J. Food Microbiol., 109, 109-120.

RANDAZZO, C.L., S. TORRIANI, A.D.L. AKKERMANS, W.M. DE VOS, and E.E. VAUGHAN (2002): Diversity, dynamics, and activity of bacterial communities during production of an artisanal Sicilian cheese as evaluated by 16S rRNA analysis. Appl. Environ. Microbiol., 68, 1882-1892.

SHARPE, M.E. (1979): Identification of lactic acid bacteria. In: Identification Methods for Microbiologists. F.A. Skinner, and D.W. Lovelock (Eds.), Academic Press, London. 233-259.

SNEATH, P.H.A., N.S. MAIR, M.E. SHARPE, and J.G. HOLT (1986): Bergey's Manual of Systematic Bacteriology, Vol 2. Williams and Wilkins Co, Baltimore.

SU, Y.A., M.C. SULAVIK, P. HE, K.K. MAKINEN, P. MAKINEN, S. FIEDLER, R. WIRTH, and D.B. CLEWELL (1991): Nucleotide sequence of the gelatinase gene from Enterococcus faecalis subsp. liquefaciens. Infect. Immun., 59, 415-420.

TAGG, J.R., and A.R. MC GIVEN (1971) Assay system for bacteriocins. Appl. Microbiol., 21, 943.

TERZIC-VIDOJEVIC, A., M. VUKASINOVIC, K. VELJOVIC, M. OSTOJIC, and L. TOPISIROVIC (2007): Characterization of microflora in homemade semi-hard white Zlatar cheese. Int. J. Food Microbiol., 114, 36-42.

TOPISIROVIC, L., K. VELJOVIC, A. TERZIC-VIDOJEVIC, I. STRAHINIC, and M. KOJIC (2007): Comparative analysis of antimicrobial and proteolytic activity of lactic acid bacteria isolated from Zlatar cheese. Genetika. 39, 125-138.

VELJOVIC, K., A. TERZIC-VIDOJEVIC, M. VUKASINOVIC, I. STRAHINIC, J. BEGOVIC, J. LOZO, M. OSTOJIC, and L. TOPISIROVIC (2007): Preliminary characterization of lactic acid bacteria isolated from Zlatar cheese. J. Appl. Microbiol., 103, 2142-2152.

VERSALOVIC, J., M. SCHNEIDER, F.J. DE BRUIJN, and J.R. LUPSKI (1994): Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol. Cell. Biol., 5, 25-40.

VUJCIC, M., and L. TOPISIROVIC (1993): Molecular analysis of the rolling-circle replicating plasmid pA1 of Lactobacillus plantarum A112. Appl. Environ. Microbiol., 59, 274-280.

WOUTERS, J.T.M., E.H.E. AYAD, J. HUGENHOLTY, and G. SMIT (2002): Microbes from raw milk for fermented dairy products. Int. Dairy J., 12, 91-109.

ZOETENDAL, E.G., A.D.L. AKKERMANS, and W.M. DE VOS (1998) Temperature gradient gel electrophoresis analysis of 16s rRNA for human fecal samples reveals stable and host-specific communities of active bacteria. Appl. Environ. Microbiol., 64, 3854-3859.

ZOURARI, A., S. ROGER, C. CHABANET, and M. DESMAZEAUD (1991): Caractérisation de bacteries lactiques thermophiles isolées de yaourts artisanaux grecs. Souches de Streptococcus salivarius subsp. thermophilus. Lait. 71, 445-461.

KARAKTERIZACIJA BAKTERIJA MLEČNE KISELINE IZOLOVANIH IZ ZLATARSKIH SIREVA PROIZVEDENIH U DOMAĆINSTU NA DVE GEOGRAFSKI RAZLIČITE LOKACIJE

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I z v o d

Iz belog polutvrdog zlatarskog sira, označenog kao BGNV, izolovan je osamdeset jedan soj bakterija mlečne kiseline (BMK). Sir je uzet iz jednog seoskog domaćinstva smeštenog na severnoj strani planine Zlatar. Zlatarski BGNV sir je napravljen od svežeg kravljeg mleka bez dodatka starter kulture. Svi izolati BMK su okarakterisani fenotipskim i genotipskim testovima. Identifikacija sojeva je rađena rep-PCR analizom sa $(GTG)_5$ prajmerom i 16S rDNK sekvenciranjem. Najzastupljenije vrste u zlatarskom BGNV siru su bile Lactobacillus casei/paracasei (65.43%) i Enterococcus faecalis (29.63%. Dva fakultetivno anaerobna štapića su identifikovana kao Lactobacillus plantarum (2.47%), a dva obligatna heterofermentativna izolata BMK su identifikovani kao Lactobacillus parabuchneri (2.47%). Od svih 81 testiranih izolata, samo osam eneterokoka su bili proizvođači antimikrobnih komponenti. Četrnaest od 16 testiranih izolata laktobacila je pokazivalo srednju do vrlo dobru proteolitičku aktivnost. Svih 57 laktobacila iz zlatarskog BGNV sira veoma sporo grušaju mleko, ili ga uopšte ne grušaju. Međutim, tri izolata enterokoka, BGNV1-63, BGNV1-76 i BGNV1-80, su pokazivala vrlo dobru aktivnost u mleku i grušala su ga za 5 h. Ove enterokoke su pokazivale vrlo visoku proteolitičku aktivnost potpuno hidrolizirajući α_{s1}- i κ-kazein nakon 3 h, a β-kazein nakon 30 min inkubacije. Pored toga, ova tri izolata enterokoka degradovala su želatin. Upoređujući dobijene rezultate sa onima prethodno dobijenim ispitivanjem BMK u drugom zlatarskom BGZLS siru, napravljenom takođe, od svežeg kravljeg mleka, može se zaključiti da je mikroflora BMK zlatraskog BGNV sira manje raznovrsna.

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