ANTISTAPHYLOCOCCAL POTENTIAL AND APPLICATION OF AUTOCHTHONOUS ENTEROCOCCI AND LACTOBACILLI IN PILOT CHEESE PRODUCTION

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This study was undertaken to prepare tailor-made starter culture (TMSC) for Karst ewe's cheese production. Therefore, basic technological characteristics (growth ability in milk, acid production, and proteolytic activity) and antistaphylococcal potential were assessed for autochthonous enterococci and lactobacilli. Beside good growth in milk with numbers as high as 8 log CFU ml^{-1}, certain enterococci and lactobacilli also reduced pH below 5.0 and showed proteolytic activity. In antistaphylococcal testing, only pure strains of enterococci and lactobacilli were moderately antagonistic, but not in coagulated milks and coagulated milk extracts. Enterococci and lactobacilli with most relevant technological/antistaphylococcal properties were combined and tested as TMSC.

In control (C) cheese-making, milk was inoculated with TMSC, while staphylococci (SC) cheese-making included contamination with staphylococci. In C trials, high logarithmic counts per g of cheese for enterococci (8.07–8.80) and lactobacilli (7.49–9.98) throughout the ripening period were found, and their authenticity was monitored by RAPD method. Furthermore, cheese extracts failed to inhibit pure cultures of staphylococci, while cheese pieces inhibited *Staphylococcus* sp. ST17. In SC trials, population dynamics of enterococci (7.81–9.04) and lactobacilli (7.98–9.63) corroborated the results in milk and in C trials, with staphylococci still present at the end of the ripening period but at lower counts than in fresh cheese.

Keywords: tailor-made starter culture, lactobacilli, enterococci, staphylococci, cheese, RAPD

Traditional cheeses are synonymous with natural, autochthonous, and artisanal dairy products that have been made for centuries from raw milk of different dairy animals following traditional protocols. Their unique organoleptic characteristics reflect properties of milk combined with the fermentation and ripening processes caused by strains of lactic acid bacteria (LAB), which are determined by the cheese-making environment and induced by the cheese-production technology.

Awareness of the importance to preserve unique traditional dairy products together with the increasing distrust of consumers towards additives, preservatives, and new food-transmitted diseases force producers and scientists to find new, safe, and natural ways to fulfil consumers' requests for authentic and safe traditional raw milk dairy products. In south European countries (Garabal et al., 2008; Litopoulou-Tzanetaki & Tzanetakis, 2011; Radulović et al., 2011; Terzić-Vidojević et al., 2015; Carafa et al., 2016; Domingos-Lopes et al., 2017) as well as in Slovenia (Čanžek Majhenič et al., 2005; Čanžek Majhenič et al., 2007; Mohar Lorbeg et al., 2009), the last two decades were very prolific in preserving production and consumption of traditional dairy products, in Slovenia particularly cheeses such as Karst ewe's cheese, Bovec cheese, Mohant, and Tolminc (Čanžek Majhenič & Mohar Lorbeg, 2017). Being produced from raw milk, traditional cheeses could represent a

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potential microbiological threat in spite of good hygiene rules. Raw cow milk pathogens that adversely influence cheese processing as well as sensory quality most frequently belong to *Bacillus cereus, Listeria monocytogenes, Pseudomonas* spp., and *Staphylococcus aureus* (Touch & Deeth, 2009; Cokal et al., 2012), to *Staphylococcus aureus*, *Bacillus* spp., *Escherichia coli*, *Salmonella* spp., and *Clostridium perfringens* in raw sheep's milk (Fotou et al., 2011; De Garnica et al., 2013), and to *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serotype *paratyphi* B, and *Listeria monocytogenes* in raw goat's milk (Silanikove et al., 2010). Regardless the type of milk, *Staphylococcus aureus* seems to be one of the prevalent pathogens and a potential risk in raw milk cheeses, also in Slovenian hard type cheeses. The microbiological safety of traditional cheeses is of great concern, and the antimicrobial activity of indigenous milk/cheese microbiota can play an important role, as examined by Trmčić and co-workers (2010). LAB consortia, extracted from Tolminc and Karst ewe's cheeses, respectively, were applied in challenge tests against *Staphylococcus aureus* in milk and cheese, where conditions for the "actual" Tolminc cheese production were simulated. All cheese consortia successfully suppressed the growth of *Staphylococcus aureus*. Their research addressed the safety issue using cheese consortia of LAB in pathogen inhibition, where the next step is introduction of selected LAB, active against pathogenic or spoilage bacteria, in real cheeses, but not affecting the species involved in ripening (Čanžek Majhenič et al., 2015). Unfortunately, data regarding the use of such TMSC in real cheese production models are limited.

Therefore, the present study focused on the construction of TMSC and its application for traditional cheese production.

1. Materials and methods

1.1. Microorganisms and media

Enterococci (EC; n=11) (Mohar et al., 2005) and lactobacilli (LB; n=10) (Čanžek Majhenič et al., 2007) were autochthonous isolates from Karst ewe's cheese. Coagulase positive staphylococci (ST; n=33) were selected from Karst ewe's cheese, Bovec cheese, Mohant, and Tolminc according to the ISO 6888-2 (1999). Cultivations were performed with MRS broth/agar and Rogosa agar (Merck, Darmstadt, Germany) for LB, M17 broth/agar and Citrate-Azide-Tween-Carbonate agar (CATC) (Merck) for EC, and Brain Heart Infusion broth/soft agar (BHI) (Merck) and Baird Parker agar (BP; Biolife, Milan, Italy) supplemented with Rabbit Plasma Fibrinogen (RPF; Biolife) for ST. Incubation temperature of 37 °C was applied, while incubations for LB, EC, and ST lasted for 48–72 h, 24 h, and 24 h, respectively. Viable counts of pure overnight LB and EC cultures were between 8.59–9.13 log CFU ml^{-1} and 9.08–9.24 log CFU ml^{-1}, respectively.

1.2. Basic technological properties of pure cultures

Sterilized (110 °C, 15 min) reconstituted skim milk (RSM; 100 g l^{-1}; Merck) was individually inoculated with 1% (v/v) of overnight LB and EC, and incubated. Growth ability in RSM was monitored every 3 h using colony count technique. The acidifying power of LB and EC in RSM was determined as pH decrease at 6, 12, and 24 h of incubation. Clotting time was recorded as well. Proteolytic activity of LB and EC was evaluated by applying 5 µl of pure cultures on MRS and M17 agar, respectively, supplemented with 20% (v/v) of RSM. Strains

that after incubation decreased the turbid zone were considered positive for proteolytic activity.

1.3. Antistaphylococcal activity of pure cultures, coagulated milks, and coagulated milk extracts

Antistaphylococcal activity of pure LB and EC cultures was assayed by the agar spot method (ČANŽEK MAJHENIČ et al., 2007). After incubation, the size of halos and the shape of their edges (sharp/blurred) were recorded.

To determine antistaphylococcal effect of coagulated milks and of coagulated milk extracts, RSM was first coagulated (37 °C for 24 h) with each LAB, and further tested with slightly modified agar spot method. Plates were overlaid with 4 ml of BHI soft agar inoculated with staphylococci. Soft agar hardening was followed by direct application of coagulated milks (15 µl) and of coagulated milk extracts, separately. To prepare the latter, coagulated milks were centrifuged (6000 g, 10 min) and supernatants were carefully applied onto soft agar in three consecutive steps of 5 µl (total volume 15 µl). After incubation, zones of inhibition were recorded. Sampling and testing of coagulated milks and of coagulated milk extracts were performed at 9 h, 12 h, and 24 h of coagulation.

1.4. Experimental design: cheese manufacture and microbial dynamics during ripening

Cheeses were made from 80 l of thermized (65 °C, 30 min) milk. After cooling to 32 °C, milk was inoculated with 1% (v/v) TMSC, and renneted with CHY-MAX™ Powder Extra (Chr. Hansen, Denmark). About 30 min later, the curd was cut and scalded (39 °C, 20 min), and transferred into molds. After whey draining, cheeses were pressed at room temperature for about 20 h (pH of cheese dropped to approx. 5.4). Cheeses were salted in 20% brine for 7 h and ripened at 15 °C for 8 weeks.

Cheese-making process was repeated in three consecutive weeks. Each week, control cheeses (C1, C2, and C3) inoculated with TMSC, and staphylococci cheeses (SC1, SC2, and SC3) additionally contaminated with the mix of selected staphylococci (1%, v/v) were made. Three cheese wheels of approx. 2.5 kg in weight were obtained in each cheese-making process.

pH in cheeses was measured with pH meter MP 120 (Mettler Toledo) equipped with puncture electrode, while microbial dynamics was monitored by colony count technique. pH measurement and aseptic sampling were performed immediately after salting (t=0), and then bi-weekly (t=2, t=4, t=6, t=8). Sample preparation and decimal dilutions were made according to the ISO 6887-5 (2010). Total LB were determined on Rogosa agar (Merck), total EC on CATC agar (Merck), and total ST on BP agar (Biolife) supplemented with RPF (Biolife).

1.5. RAPD authenticity of LB and EC from C cheeses

To verify their authenticity, LB and EC from C cheese-making trials were screened with M13 primer and their RAPD patterns were compared to those of the original *L. paracasei* K2/3, *L. paracasei* K29/3, *L. brevis* K31/5, *E. faecalis* K9/2, *Enterococcus* sp. K29/7, and *E. faecalis* K50/6 strains. At two cheese sampling times (t=2, t=8), 21 LB and 21 EC colonies were randomly selected from Rogosa and CATC agar, respectively. Prior RAPD, LB and EC colonies were subcultured in MRS or M17 broth. Genomic DNA was extracted from overnight cultures using Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). RAPD mix and protocol used were as previously described (TORRIANI et al., 1999).

1.6. Antistaphylococcal activity of cheese pieces and cheese extracts

Antistaphylococcal activity was assayed with C1, C2, and C3 cheese pieces and extracts, separately, obtained by three different extraction protocols (Fig. 1). Following extraction solutions were used: 2% (w/v) dipotassium hydrogen phosphate solution (K_2HPO_4; Merck), citrate buffer (CB; pH 2.5; 46.5 ml 0.1 mol l^{-1} citric acid, 3.5 ml 0.1 mol l^{-1} sodium citrate dihydrate), and dH_2O. Blending was accomplished in BagMixer® 400 (Interscience, St Nom, France). Immediately when obtained, all supernatants were filter-sterilized.
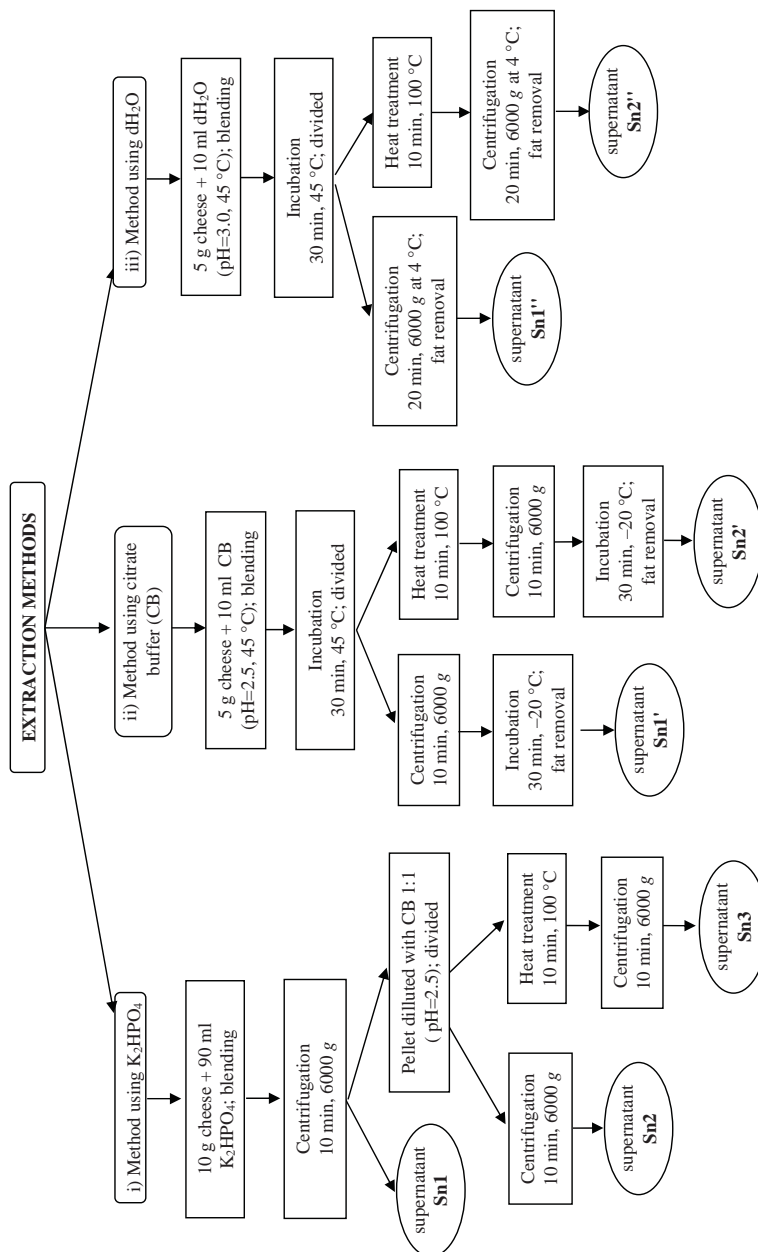

Fig. 1. Schematic presentation of three protocols applied in preparing of cheese extracts using i) K_2HPO_4, ii) citrate buffer (CB) and iii) dH_2O

Using cheese drill, uniform cheese pieces from C1, C2, and C3 cheeses-making trials were collected and tested for antimicrobial potential.

Supernatants (each in total volume of 15 μl) and cheese pieces were placed onto pour plates of BHI soft agar inoculated with the indicator ST. After incubation, plates were checked for a clear halo around the applied supernatants or cheese pieces.

2. Results and discussion

2.1. Technological properties of EC and LB

Good growth in milk was observed for all isolates tested, where the viable counts of LB and EC increased from the initial 6–7 log CFU ml^{-1} of milk up to 8–9 log CFU ml^{-1}.

Acidifying activity, clotting time, and proteolytic character of LB and EC are summarized in Table 1. Ability of isolates to reduce pH is essential to suppress the development of adventitious bacterial populations (CARAFA et al., 2016), while proteolytic activity is aiding the ripening, texture and aroma development of cheeses (DOMINGOS-LOPES et al., 2017). Acidifying activity of LB and EC was slow to moderate initially but accelerated later. After 24 h of incubation, 15 tested strains were able to coagulate milk, and 17 lowered pH into the range of 3.5–5.11. Similar observations for autochthonous LB and EC isolates from various dairy products were reported (LITOPOULOU-TZANETAKI & TZANETAKIS, 2011; RADULOVIĆ et al., 2011; TERZIĆ-VIDOJEVIĆ et al., 2015; DOMINGOS-LOPES et al., 2017). In proteolytic activity evaluation, EC were more efficient in milk protein degradation (5/11) compared to LB (1/10).

Table 1. Acidifying activity, clotting time, and proteolytic character of lactobacilli and enterococci

Strain	pH			Clotting time	Proteolytic character
	6 h	12 h	24 h	(h)	(mm)
L. paracasei **K29/3**	5.92	5.68	3.50	9	+ (9)
L. paracasei **K2/3**	5.78	5.06	4.30	24	–
L. brevis **K31/5**	6.17	6.08	5.11	24	–
L. paracasei M11/1	6.19	5.51	4.46	24	–
L. paracasei M13/3	6.00	5.73	5.48	/	–
L. paracasei K20/2	6.24	5.90	5.64	/	–
L. paracasei M13/5	6.17	5.80	5.90	/	–
L. brevis K27/1	6.28	6.05	6.10	/	–
L. paracasei M2/3	6.10	5.45	4.80	24	–
L. paracasei K30/2	6.14	5.82	4.98	24	–
E. faecalis **K9/2**	5.98	5.76	4.55	12	+ (11)
E. faecalis **K50/6**	6.01	5.66	5.09	/	–
Enterococcus sp. **K29/7**	6.22	5.90	4.94	24	+ (5)
E. faecalis K32/1	5.77	5.02	4.48	24	–
Enterococcus sp. K26/3	6.05	5.62	4.58	24	+ (9)
Enterococcus sp. K30/10	6.08	5.77	4.56	24	+ (7)
E. faecalis M10/3	5.97	5.52	4.87	24	–
E. faecalis M13/2	5.97	5.58	4.94	24	–
E. faecalis M1/6	5.88	5.57	4.97	24	–
E. faecalis K2/2	6.14	5.87	4.98	/	–
Enterococcus sp. K29/6	6.22	5.80	4.99	24	+ (4)

pH of the uninoculated RSM was 6.7; /: clotting not observed; –: no proteolytic activity detected; bold: strains used as TMSC

2.2. Antistaphylococcal activity of pure cultures, coagulated milks, and coagulated milk extracts

Antistaphylococcal activity of pure LB and EC varied, ranging from sharp to blurred or no inhibition halos (data not shown). LB inhibited more ST, but they mostly formed weak and blurred halos, while EC inhibited fewer ST in number, but their antagonistic activity was recognized as more efficient, because they formed clean and sharp halos. *L. paracasei* K29/3 and *L. paracasei* K30/2 revealed completely identical antistaphylococcal potential (data not shown), but they differed in final pH value, clotting time, and proteolytic ability (Table 1). On the contrary, acidifying activity, clotting time, and proteolytic character seemed to be alike for *Enterococcus* sp. K29/6 and *Enterococcus* sp. K29/7 (Table 1), but their antistaphylococcal activities were different. Antistaphylococcal activity of LAB is rather strain-dependent and can range from bacteriostatic to no inhibitory effect (CHARLIER et al., 2008).

When coagulated milks and coagulated milk extracts, were tested against ST, coagulated milk extracts were completely ineffective, while direct application of coagulated milk samples exerted negligible antistaphylococcal activity, with formation of hardly visible and hazy halos (data not shown). Results were always the same, irrespective of the time of sampling of coagulated milks.

2.3. Cheese pH and viable counts of LB, EC, and ST in cheeses during ripening

Possessing most relevant characteristics, *L. paracasei* K2/3, *L. paracasei* K29/3, and *L. brevis* K31/5, and enterococci *E. faecalis* K9/2, *Enterococcus* sp. K29/7, and *E. faecalis* K50/6 were selected, mixed, and applied as TMSC culture in C cheese production. Additionally, a combination of selected ST (n=5) was used as a contaminating mix in SC cheese production. pH and growth dynamics of total LB, EC, and ST populations in C and SC cheeses throughout ripening are presented in Figs 2 and 3. pH profile was virtually identical for all vats in both C and SC cheese-making trials throughout ripening. From milk inoculation to the beginning of ripening time (t=0), a pH decrease from 6.6 in cheese milk to 5.6 in fresh cheese and a rise in the counts of TMSC were observed in all cheeses (data not shown). The trend of pH decrease and microbial count increase for LB and EC continued for week 2 in C and SC cheeses, which coincided with an evident decline of ST of 1.66 log units in SC cheeses, indicating a certain inhibitory effect probably coming from both the cheese environment and TMSC (TRMČIĆ et al., 2010). Peaks of mean log bacterial counts of LB and EC together with pH values, noted at t=2 (Figs 2 and 3), suggested that LB and EC probably reached their maximal growth and metabolic activity, which led to pH lowering that obviously reduced ST growth. After, viable counts of LB and EC remained almost unchanged in both cheeses, indicating good stability and adaptation of the TMSC. The first 2 weeks were important for a suppression of viable staphylococci counts, but from then on, their counts of about 5 log CFU g^{-1} were retained to the end of ripening (Fig. 3). These counts are the maximum limit of coagulase-positive staphylococci in cheeses made from raw milk (EC, 2005), but already levels of 3 log CFU g^{-1} can be enough for enterotoxin production (CHARLIER et al., 2008). Nevertheless, LAB antagonism against staphylococci in food matrix such as cheese is probably a complex activity of different factors, such as acidification, antimicrobials production, nutritional competition, influence of LAB/staphylococci ratio, and inter- and intra-species variability of the inhibition capacity, that all form a complex and intricate network that aggravates evaluation of the prevalence of one inhibition factor over another.
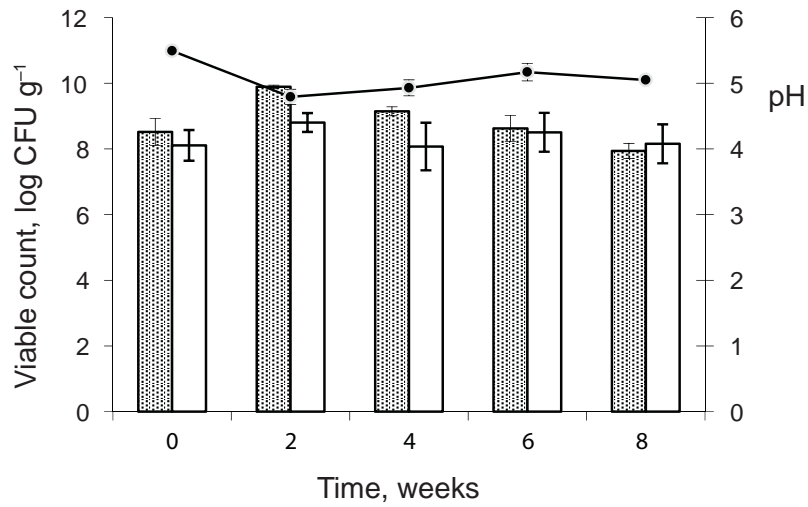
Fig. 2. Counts (mean log CFU g^{-1}±SD) of lactobacilli (LB) and enterococci (EC) and pH (mean value ±SD) examined in cheeses during ripening. Data are the mean values of C1, C2, and C3 cheese-making trials
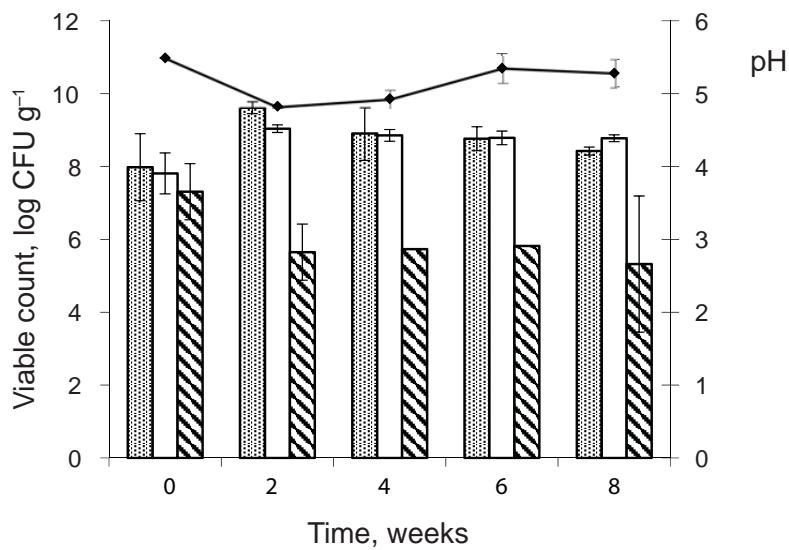
▓▓▓: LB; ▭: EC; ●—: pH

Fig. 3. Counts (mean log CFU g^{-1}±SD) of lactobacilli (LB), enterococci (EC) and staphylococci (ST) and pH (mean value ±SD) values examined in cheeses during ripening. Data are the mean values of SC1, SC2, and SC3 cheese-making trials

▓▓▓: LB; ▭: EC; ▨: ST; ●—: pH

2.4. RAPD authenticity of LB and EC from C cheeses

When 21 EC colonies from cheese C2 (t=2) were analysed, 19 RAPD patterns were authentic to *E. faecalis* K50/6, 1 to *E. faecalis* K9/2, and 1 was unidentified (Fig. 4). Similarly, 19 out of 21 EC from C2 cheese at t=8 were authentic to *E. faecalis* K50/6, 1 to *E. faecalis* K9/2, and 1 to *Enterococcus* sp. K29/7 (Fig. 5). Although all three EC strains (K50/6, K9/2, and K29/7) were inoculated at approximately the same ratio, *E. faecalis* K50/6 evidently outgrew the other two. Results support findings of MOHAR and co-workers (2005), who reported *E. faecalis* K50/6 as the most abundant EC strain inhabiting Karst ewe's cheese. Similar results were obtained for LB, where *L. paracasei* K2/3 RAPD pattern predominated over *L. paracasei* K29/3 and *L. brevis* K31/5 (data not shown), and was the second most abundant strain in Karst ewe's cheese (ČANŽEK MAJHENIČ et al., 2007). Results of RAPD analysis highly correlated with the natural appearance of selected strains in their original habitat.
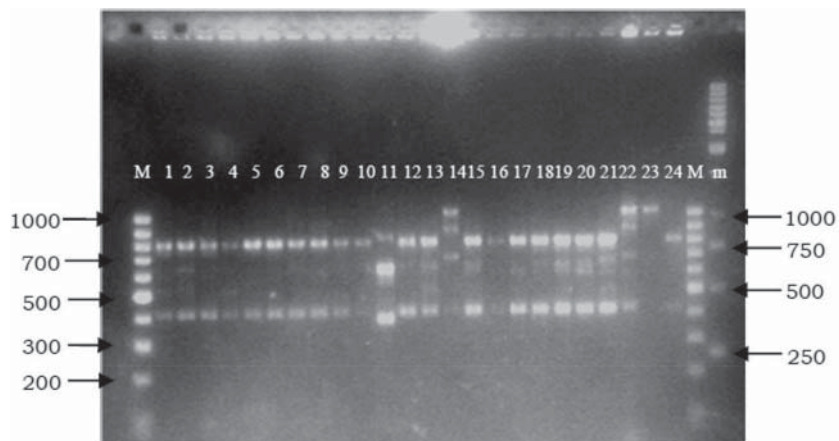

Fig. 4. RAPD fingerprints of C cheese isolates. Enterococci from C2 (t=2). 1–21: EC colonies from CATC agar, 22–24: controls *E. faecalis* K9/2, *Enterococcus* sp. K29/7, and *E. faecalis* K50/6; M: 100 bp DNA ladder; m: 1 kb DNA ladder
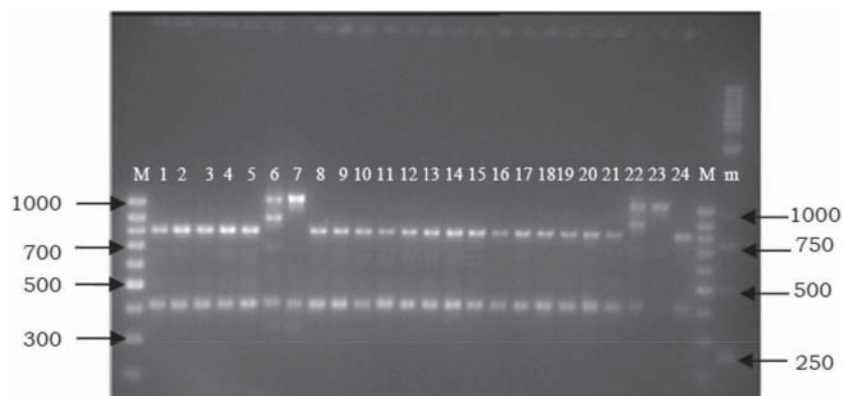

Fig. 5. RAPD fingerprints of C cheese isolates. Enterococci from C2 (t=8). 1–21: EC colonies from CATC agar; 22–24: controls *E. faecalis* K9/2, *Enterococcus* sp. K29/7, and *E. faecalis* K50/6; M: 100 bp DNA ladder; m: 1 kb DNA ladder

2.5. Antistaphylococcal activity of cheese extracts and cheese pieces

When cheese extracts were applied on BHI soft agar lawn inoculated with indicator ST, no inhibition was observed, irrespective of the extraction protocol, sampling time, or cheese samples (C1, C2, or C3) extracts analysed. Although three different extraction methods, reported as applicable in antimicrobial/bacteriocinogenic activity detection in cheese extracts (FOULQUIE MORENO et al., 2003; ANASTASIOU et al., 2007), were used, we failed to determine any inhibitory action. On the contrary, when intact pieces of C cheese samples were placed on soft agar plates containing indicator staphylococci, evident and clear inhibition zones against *Staphylococcus* sp. ST17 were detected (Fig. 6) throughout the 8-weeks ripening.
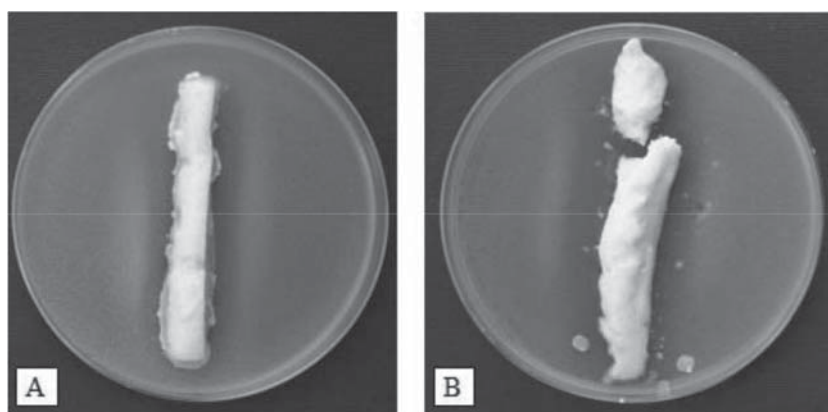

Fig. 6. Antistaphylococcal activity of C cheese pieces. C2 cheese exerting antistaphylococcal activity against *Staphylococcus* sp. ST17 at t=2 (A) and t=8 (B) weeks of ripening

Successful detection of bacteriocinogenic activity in cheese throughout ripening by placing intact cheese samples on agar plates containing indicator strain was reported by FOULQUIE MORENO and co-workers (2003) and ANASTASIOU and co-workers (2007). Although in both cases, using either intact cheese pieces or cheese extracts, authors detected inhibitory activity, this activity was more pronounced, when intact cheese samples compared to cheese extracts were used. Authors assumed that this might be due to the distribution of inhibitory substance in different phases of extraction solutions. We agree that although no specific antimicrobial compound(s) was determined in our pure LAB strains or in cheese extracts, the complex cheese matrix obviously offers the optimal environment for LAB growth and antimicrobial production, which, on some occasions, were better expressed when tested as a complete unit (cheese piece) rather than individual components (bacteria, coagulated milk, or cheese extracts).

3. Conclusions

After analyzing basic technological parameters and antistaphylococcal activity, 3 lactobacilli strains and 3 enterococci strains were selected and used as a potential TMSC for traditional cheese production. Two types of cheeses were made, one inoculated only with TMSC, other additionally contaminated with staphylococci. Growth dynamics, persistence, and authenticity

of TMSC throughout ripening by plate counting and RAPD analysis in cheeses were tested. TMSC exerted satisfactory antistaphylococcal activity in contaminated cheese. Evidently, the use of such TMSC can improve hygienic status and preserve the uniqueness of distinctive artisanal cheeses.

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References

ANASTASIOU, R., GEORGALAKI, M., MANOLOPOULOU, E., KANDARAKISA, I., DE VUYST, L. & TSAKALIDOU, E. (2007): The performance of *Streptococcus macedonicus* ACA-DC 198 as starter culture in Kasseri cheese production. *Int. Dairy J.*, *17*, 208–217.

CARAFA, I., CLEMENTI, F., TUOHY, K. & FRANCIOSI, E. (2016): Microbial evolution of traditional mountain cheese and characterization of early fermentation cocci for selection of autochthonous dairy starter strains. *Food Microbiol.*, *53*, 94–103.

CHARLIER, C., EVEN, S., GAUTIER, M. & LE LOIR, Y. (2008): Acidification is not involved in the early inhibition of *Staphylococcus aureus* growth by *Lactococcus lactis* in milk. *Int. Dairy J.*, *18*, 197–203.

COKAL, Y., DAGDELEN, A., CENET, O. & GUNSEN, U. (2012): Presence of *L. monocytogenes* and some bacterial pathogens in two Turkish traditional foods, Mihalic cheese and Hosmerim dessert. *Food Control*, *26*, 337–340.

EC (2005): Commission Regulation No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. (2005): *OJ of the EU L 338*, 1–26.

ČANŽEK MAJHENIČ, A., PERKO, B. & ROGELJ, I. (2005): Enterococci from Tolminc cheese: population structure, antibiotic susceptibility and incidence of virulence determinants. *Int. J. Food Microbiol.*, *102*, 239–244.

ČANŽEK MAJHENIČ, A., MOHAR, P. & ROGELJ, I. (2007): Characterisation of the *Lactobacillus* community in traditional Karst ewe's cheese. *Int. J. Dairy Technol.*, *60*, 182–190.

ČANŽEK MAJHENIČ, A., BOGOVIČ MATIJAŠIĆ, B., TRMČIĆ, A. & ROGELJ, I. (2015): Tailor-made starter cultures for preserving the uniqueness of traditional cheeses. -in: RAI, V.R. & BAI, J.A. (Eds) *Beneficial microbes in fermented and functional foods*. CRC Press, Boca Raton, pp. 15–34.

ČANŽEK MAJHENIČ, A. & MOHAR LORBEG, P. (2017): Slovenian dairy products. -in: CRUZ, R.M.S. & VIEIRA, M.C. (Eds) *Mediterranean foods: Ccomposition and processing*. CRC Press, Boca Raton, pp. 121–140.

DE GARNICA, M.L., LINAGE, B., CARRIEDO, J.A., SANTOS, J.A. & GONZALO, C. (2013): *Staphylococcus aureus* and *Escherichia coli* prevalence in ovine bulk tank milk. *Small Ruminant Res.*, *115*, 108–112.

DOMINGOS-LOPES, M.F.P., STANTON, C., ROSS, P.R., DAPKEVICIUS, M.L.E. & SILVA, C.C.G. (2017): Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese. *Food Microbiol.*, *63*, 178–190.

FOTOU, K., TZORA, A., VOIDAROU, CH., ALEXOPOULOS, A., PLESSAS, S., AVGERIS, I., BEZIRTZOGLOU, E., AKRIDA-DEMERTZI, K. & DEMERTZIS, P.G. (2011): Isolation of microbial pathogens of subclinical mastitis from raw sheep's milk of Epirus (Greece) and their role in its hygiene. *Anaerobe, 17*, 315–319.

FOULQUIE MORENO, M.R., REA, M.C., COGAN, T.M. & DE VUYST, L. (2003): Applicability of a bacteriocin-producing *Enterococcus faecium* as a co-culture in Cheddar cheese manufacture. *Int. J. Food Microbiol.*, *81*, 73–84.

GARABAL, J.I., RODRIGUEZ-ALONSO, P. & CENTENO, J.A. (2008): Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). *LWT – Food Sci. Technol.*, *41*, 1452–1458.

GIRAFFA, G. (2003): Functionality of enterococci in dairy products. *Int. J. Food Microbiol.*, *88*, 215–222.

ISO 6887-5 (2010): *Microbiology of food and animal feeding stuffs*. Part 5.

ISO 6888-2 (1999): *Microbiology of food and animal feeding stuffs*. Part 2.

LITOPOULOU-TZANETAKI, E. & TZANETAKIS, N. (2011): Microbiological characteristics of Greek traditional cheeses. *Small Ruminant Res.*, *101*, 1–32.

MOHAR, P., ČANŽEK MAJHENIČ, A. & ROGELJ, I. (2005): Phenotypic characterisation, antibiotic susceptibility and antimicrobial activity of enterococcal population from Karst ewe's cheese. *2nd International ASM-FEMS Conference on Enterococci, Helsingor, Denmark,* p. 39.

MOHAR LORBEG, P., ČANŽEK MAJHENIČ, A. & ROGELJ, I. (2009): Evaluation of different primers for PCR-DGGE analysis of cheese-associated enterococci. *J. Dairy Res.*, *76*, 1–7.

RADULOVIĆ, Z., MIOČINOVIĆ, J., PUĐA, P., BARAĆ, M., MILORADOVIĆ, Z., PAUNOVIĆ, D. & OBRADOVIĆ, D. (2011): The application of autochthonous lactic acid bacteria in white brined cheese production. *Mljekarstvo, 61*, 15–25.

SILANIKOVE, N., LEITNER, G., MERIN, U. & PROSSER, C.G. (2010): Recent advances in exploiting goat's milk: Quality, safety and production aspects. *Small Ruminant Res.*, *89*, 110–124.

TERZIĆ-VIDOJEVIĆ, A., TONKOVIĆ, K., LEBOŠ PAVUNC, A., BEGANOVIĆ, J., STRAHINIĆ, I., KOJIĆ, M., VELJOVIĆ, K., GOLIĆ, N., KOS, B., ČADEŽ, N., GREGUREK, L., ŠUŠKOVIĆ, J., RASPOR, P. & TOPISIROVIĆ, L. (2015): Evaluation of autochthonous lactic acid bacteria as starter cultures for production of white pickled and fresh soft cheeses. *LWT – Food Sci. Technol.*, *63*, 298–306.

TORRIANI, S., ZAPPAROLI, G. & DELLAGLIO, F. (1999): Use of PCR-based methods for rapid differentiation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis. Appl. Environ. Microb.*, *65*, 4351–4356.

TOUCH, V. & DEETH, H.C.P. (2009): Microbiology of raw milk and market milks. -in: TAMIME, A.Y. (Ed.), *Milk processing and quality management*. Oxford, Blackwell Publishing, pp. 48–71.

TRMČIĆ, A., OBERMAJER, T., ČANŽEK MAJHENIČ, A., ROGELJ, I. & BOGOVIČ MATIJAŠIĆ, B. (2010): *In-situ* inhibition of *Staphylococcus aureus* by lactic acid bacteria consortia from two traditional Slovenian raw milk cheeses. *Mljekarstvo*, *60*, 183–190.