Journal of Food Safety and Food Quality 70, Heft 1 (2019), Seiten 1–30

Arch Lebensmittelhyg 70,
4–9 (2019)
DOI 10.2376/0003-925X-70-4

ISSN 0003-925X

Korrespondenzadresse:
btaban@ankara.edu.tr

[1]) Hacettepe University, Faculty of Engineering, Food Engineering Department, Beytepe, Ankara, Turkey; [2]) Ankara University, Faculty of Agriculture, Dairy Technology Department, Diskapi, Ankara, Turkey; [3]) Ankara University, Faculty of Science, Biology Department, Tandogan, Ankara, Turkey

The effects of autochthonous probiotic strains of *Lactobacillus brevis* and *Pediococcus pentosaceus* on *Staphylococcus aureus* during production and ripening of white-pickled cheeses

Auswirkungen von autochthonen probiotischen Stämmen von Lactobacillus brevis und Pediococcus pentosaceus auf Staphylococcus aureus bei der Herstellung und Reifung weisser Käse in Salzlake

Münevver Yücebay[1]), G. Seval Karslı[1]), Birce Mercanoglu Taban[2]), S. Aykut Aytaç[1]), H. Barbaros Özer[2]), Ayşe Gürsoy[2]), Mustafa Akçelik[3])

Summary

The effects of two autochthonous probiotic strains of *Lactobacillus brevis* BG18 and *Pediococcus pentosaceus* BH105 were investigated on the growth of *Staphylococcus aureus*. *S. aureus* counts were monitored separately during production and ripening of white-pickled cheeses by BAM's 5-tube MPN method. Pasteurized milk used for cheese production with either 1 % *Lb. brevis* or *P. pentosaceus,* was artificially contaminated with *S. aureus* to the mean levels of 6.252 log MPN/mL and 5.952 log MPN/mL, respectively. The study was also carried out with control group cheeses produced without adjunct culture. Each positive tube of MPN method was confirmed by PCR amplification of a 400 bp region of the nuc gene. As a result, *Lb. brevis* BG18 could reduce *S. aureus* count by 0.4 and 0.7 log units separately during the production (22nd hours) and ripening (92nd day), respectively, when compared to control group cheeses. Whereas *P. pentosaceus* BH105 could sharply reduce *S. aureus* counts by 1.8 log units during ripening (92nd day), when compared to control group cheeses. As a result, the present study demonstrates the potential use of these autochthonous strains as adjunct cultures in white-pickled cheese production to prevent *S. aureus* growth which is a great point of importance in respect of food technology as well as food safety.

Keywords: *Lactobacillus brevis, Pediococcus pentosaceus, Staphylococcus aureus,* white-pickled cheese, bacteriocin

Zusammenfassung

Die Auswirkungen von zwei autochthonen probiotischen Stämmen von *Lactobacillus brevis* BG18 und *Pediococcus pentosaceus* BH105 auf das Wachstum von *Staphylococcus aureus* wurden untersucht. Die Zählung von *S. aureus* wurde während der Produktion und Reifung von weißem Käse in Salzlake durch das BAM's 5-Röhren-MPN-Verfahren separat überwacht. Pasteurisierte Milch unter Zusatz von 1 % *Lb. brevis* bzw. *P. pentosaceus* wurde mit *S. aureus* bis zu mittleren Konzentrationen von 6,252 log MPN/ml und 5,952 log MPN/ml künstlich kontaminiert. Die Studie wurde auch mit Kontrollgruppen durchgeführt, die ohne Zusatzkultur hergestellt wurden. Jedes positive Röhrchen der MPN-Methode wurde durch PCR-Amplifikation einer 400 bp-Region des Nuc-Gens bestätigt. Die Ergebnisse zeigten, *Lb. brevis* BG18 konnte die Anzahl der *S. aureus* um 0,4 und 0,7 log Einheiten während der Produktion (22. Stunde) und der Reifung (92. Tag) im Vergleich zu Kontrollgruppen deutlich reduzieren. Während *P. pentosaceus* BH105 *S. aureus*-Zählungen während der Reifung (92. Tag) um 1,8 log-Einheiten stark reduzieren konnte, verglichen mit Kontrollgruppen-Käse. Die vorliegende Studie zeigt die potentielle Verwendung die potentielle Verwendung dieser autochthonen Stämme als Zusatzkulturen in der weißer Käse in Salzlake Produktion, um das Wachstum von *S. aureus* zu verhindern, was ein wichtiger Punkt in Bezug auf die Lebensmitteltechnologie und die Lebensmittelsicherheit ist.

Schlüsselwörter: *Lactobacillus brevis, Pediococcus pentosaceus, Staphylococcus aureus,* weißer Käse in Salzlake, bacteriocin

Introduction

White-pickled cheese (also known as white brined cheese) is the most popular part of dairy products export of Turkey besides being the mostly consumed cheese type in Turkey (Temelli et al., 2007). It is a rindless, close textured, white-colored, semi-soft cheese with a salty acid taste, which is ripened in 12–14 % NaCl (brine) solution for a period of 1–3 months. Since it is usually produced under artisanal conditions and is handled at different stages of production, various microorganisms can enter to the cheese during its production and subsequent handling (Hayaloglu et al., 2002).

S. aureus grows in a wide range of environmental conditions. However; dairy products, especially cheeses, are the most frequent food type that are usually implicated in *S. aureus* contamination and also staphylococcal food poisoning since they offer a good substrate for *S. aureus* growth and enterotoxin production (Mercanoglu Taban et al, 2017). Despite the implementation of effective control procedures and the improvement of production facilities in dairy industries, it is still one of the leading pathogen that contaminates cheeses based on the quality of raw milk, the processing plant environment, and personnel hygiene throughout the production and subsequent extensive handling (Meyrand et al., 1998; Le Loir et al., 2003; Charlier et al., 2009). Therefore, the behaviour of *S. aureus* during production and ripening of some cheese varieties has been widely studied (Nunez et al., 1988; Erkmen, 1995; Meyrand et al., 1998). To overcome the problem of S. aureus contamination and to control this pathogen during cheese production, addition of purified or semi-purified bacteriocins as biopreservatives has been well documented (Abdalla et al., 1993; Cintas et al., 1998). On the other way, the use of bacteriocin-producing starter cultures in cheese production as a strategy to achieve biopreservation has gained an interest which accommodates consumers' preferences for foods including minimum levels of chemically-synthesized additives (Rilla et al., 2004). Besides, many papers have been published in combined use of various hurdles with either bacteriocins (Capellas et al., 2000; Al-Holy et al., 2012) or bacteriocin-producing cultures (Arques et al., 2005) to inhibit foodborne pathogens in cheeses. However; although the antilisterial potential of using bacteriocin-producing starter or adjunct cultures in cheese production has been evaluated by a number of research groups (Buyong et al., 1998; O'Sullivan et al., 2002; Rodriguez et al., 2005) yet little has been known about the efficacy of using bacteriocin-producing starter or adjunct cultures on the growth and survival of *S. aureus* in cheese production (Rodriguez et al., 2000; Favaro et al., 2005; Rodriguez et al., 2015), which may result in a more economic and better way to compete with this foodborne pathogen in addition to their essential contribution to the typical sensory characteristics and nutritional value of cheeses. Such studies have mostly been done with nisin-producing strains in cheeses. For example; nisin-producing dairy starters have been designed to specifically inhibit *S. aureus* in acid-coagulated cheeses (Rilla et al., 2004). Rodriguez et al. (2000) showed a reduction on *S. aureus* counts in a semi-hard cheese made with a nisin-producing starter.

Since bacteriocin-producing starter cultures with strong antimicrobial activities usually show poor technological performance in cheese production, the purpose of this study is to indicate distinct antimicrobial efficacy of use of autochthonous probiotic brevicin-like bacteriocin-producing *Lb. brevis* BG18 and pediocin-like bacteriocin-producing *P. pentosaceus* BH105 as adjunct cultures in white-pickled cheese production for controlling *S. aureus* growth and survival during production and ripening of white-pickled cheeses. Considering the risks for *S. aureus* contamination in cheese production, the impact of staphylococcal food poisoning on public health, and the increased demand for high-quality cheeses that are free of contaminating microorganisms, the use of anti-staphylococcal adjunct cultures in white-pickled cheese production like in this study, is a point of importance in respect of food technology as well as food safety.

Materials and methods

Milk, *S. aureus* and adjunct cultures

White-pickled cheeses were produced from bovine milk (supplied from Haymana Research and Experimental Farm, Faculty of Agriculture, Ankara University) which was pasteurized at 80–85 °C for 2–3 s at Pilot Dairy Processing Plant, Faculty of Agriculture, Ankara University. Initially, the raw milk was investigated for the presence of *S. aureus* by polymerase chain reaction (PCR) and no any *S. aureus* was detected in it. *S. aureus* ATCC 6538 (supplied from Dr. M. Akcelik, Biology Department, Faculty of Science, Ankara University) was propagated in tryptic soy broth (TSB; Merck, Germany) at 35–37 °C for 18–24 h and subcultured twice in sterile reconstituted skim milk before use in white-pickled cheese production. *Lb. brevis* BG18 and *P. pentosaceus* BH105 (supplied from Dr. M. Akcelik, Biology Department, Faculty of Science, Ankara University) which were isolated from traditional Turkish Tulum cheese and human faeces, respectively, were used as adjuncts to the starter culture in white-pickled cheese production. They were grown in MRS broth (De Man, Rogosa, & Sharpe) (Merck, Germany) at 35–37 °C for 18–24 h and subcultured twice in sterile reconstituted skim milk before use in white-pickled cheese production. The synthesis of brevicin-like bacteriocin (800 AU/mL) with glicolipid moiety by *Lb. brevis* BG18 and of pediocin-like bacteriocin (1600 AU/mL) with high pH and heat stability by *P. pentosaceus* BH105, were defined in previous studies, including their antimicrobial and probiotic potentials (Uymaz et al., 2009; Uymaz et al., 2011). In addition, these two strains were previously tested for whether they exhibited promising technological criteria as adjunct cultures rather than starters in cheese production (not published yet). All strains were maintained as frozen stocks in sterile reconstituted skim milk supplemented with 30 % glycerol at 80 °C. The initial numbers of each strain used to inoculate pasteurized bovine milk was determined by plating in a suitable medium and incubating at 35–37 °C for 18–24 h.

White-pickled cheese production protocol

The flow-sheet of production of white-pickled cheeses with adjunct cultures and of control group cheeses are summarized in Fig 1. White-pickled cheeses were made in three trials carried out on different weeks from pasteurized (at 80–85 °C for 2–3 s) and cooled (to 32–34 °C) bovine milk that was firstly clarified and standardized. Milk at 32–34 °C with 0.02 % $CaCl_2$ was transferred into four separate vats and commercial mesophilic homofermentative starter culture at a level of 1 % (CHR Hansen R-708, Denmark) was added to each vat. Since vat no:1 and vat no:3 were served as controls to determine the effect of production procedures for white-pickled cheese on the growth and survival of *S. aureus* added to milk at the start of the process, adjunct

Journal of Food Safety and Food Quality 70, Heft 1 (2019), Seiten 1–30

cultures of *Lb. brevis* BG18 and *P. pentosaceus* BH105 were only added separately to the vat no:2 and vat no:4, respectively. Then, vat no:1 and vat no:2 were inoculated with *S. aureus* to the mean level of 6.252 log MPN/mL, and vat no:3 and vat no:4 were inoculated with *S. aureus* to the mean level of 5.952 log MPN/mL. Next, liquid rennet (CHR Hansen Naturen® Mandra 175, Denmark) was added to all vats at a level of 14 mL/100 L. The coagulum in each vat was cut into 1–2 cm cubes 90 min after the rennet addition and the curds were allowed to rest in the whey for about 10 min. Whey in each vat was drained off and the curds were transferred to the stainless steel moulds. The surface of the cheeses were covered with cheese cloths, followed by a plate on which weights were placed to compact the curd. Then, pressure was applied until the whey drainage was stopped. After the weights were removed and the cheese cloths were opened, the cheese masses were divided into blocks of about 7 x 7 x 7 cm^3 and these blocks were salted in 13 % NaCl for 12–18 h at 18–20 °C. The brined blocks were then placed in tinned cans filled with 10 % NaCl and ripened at 12–15 °C for 92 days.

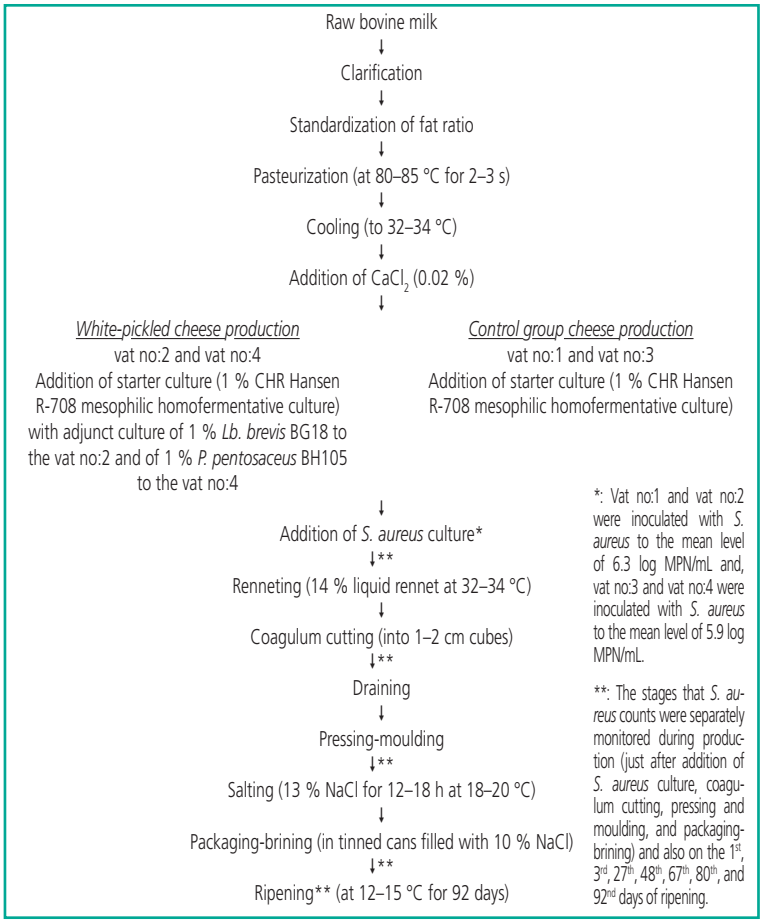

FIGURE 1: *The flow-sheet of production of white-pickled cheeses with adjunct cultures and of control group cheeses, including the stages that S. aureus counts were monitored during production and ripening.*

Enumeration of *S. aureus*

In each trial, all cheeses were sampled during production (just after addition of *S. aureus* culture, coagulum cutting, pressing-moulding, and packaging-brining) and at the days of 1, 3, 27, 48, 67, 80, and 92 of ripening. The *S. aureus* counts were monitored by BAM's recommended 5-tube MPN method. Both during production and ripening of cheeses, twenty five grams of each sample were mixed in sterile plastic bag for 1 min with 225 mL of 0.1 % Butterfield's phosphate buffer in stomacher (Stomacher 400, the UK). One mL portions of decimal dilutions of each sample homogenate was inoculated into 5 tubes of tryptic soy broth (TSB) (Merck, Germany) containing 10 % NaCl and 1 % sodium pyruvate (Merck, Germany) and these tubes were incubated at 35–37 °C for 48 h. One loopful from each tube showing growth (turbidity) was spreaded onto the surface of prepared Petri plates on duplicate with Baird-Parker agar (Merck, Germany) and all plates were incubated at 37 °C for 48 h. At least 1 colony suspected to be *S. aureus* from each plate showing growth, was transferred to TSB and was confirmed for S. aureus by PCR amplification of a 400 bp region of the *nuc* gene.

Confirmation of *S. aureus* by PCR

All suspensions were treated with a high-pure PCR template preparation (HPPTP) kit (Roche, Germany), and the DNA isolation procedure was performed as in the study of Mercanoglu and Aytac (2009). The resulting template DNAs were subjected to PCR. The PCR reaction mixture (total volume, 50 µL) contained the following: 4 mM $MgCl_2$ (with 1× PCR buffer, containing 10 mM Tris, 50 mM KCl, pH 8.3) (Roche, Germany), 200 µM of dNTP mix, each PCR primer at a concentration of 0.4 µM [based on the sequence of thermostable nuclease gene *(nuc)*, F166: (5'-AGT TCA GCA AAT GCA TCA CA-3') and R565: (5'-TAG CCA AGC CTT GAC GAA CT-3') Cremonesi et al. (2005)] (Roche, Germany), 0.04 µM 5 U/µL FastStart Taq DNA polymerase (Roche, Germany), 3 µL target DNA, and ultrapure water. The mixture was subjected to 35 cycles in a Primus 96 thermocycler (The MWG, Germany). The amplification reactions included an initial denaturation step at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s. A final elongation of 72 °C for 7 min was also applied. The PCR products were detected in 1 % agarose gel (that was stained with 1 mg/mL ethidium bromide solution), with a 100-bp GeneRuler DNA ladder plus (ready-to-use, Fermentas, Lithuania), in Trisborate EDTA buffer; bands were visualized by using the InGenius gel visualization and analysis system (Syngene, the UK). The purified DNA from *S. aureus* ATCC 6538 was used as a positive control and ultra-pure H_2O served as a negative control. The expected size of the *nuc* PCR product is 400 bp.

Statistical analysis

Data analyses were conducted by using SPSS 23.0 program (SPSS Inc., the USA). Comparison of means of *S. aureus* counts separately during production and during ripening by "paired-samples t test" was performed using the same program (Table 1 and Table 2). Comparison of mean reductions in *S. aureus* counts between *L. brevis* BG18 and *P. pentosaceus* BH105 in each trial during ripening by "independent-samples t test" was performed using the same program (Table 3). The comparative results were considered statistically significant when the significance value was $P < 0.05$.

Results and discussion

Survival of *S. aureus* during production and ripening of white-pickled cheeses

Pasteurized milk in vat no:1 and vat no:2 were inoculated with *S. aureus* to the mean level of 6.252 log MPN mL^{-1}, and in vat no:3 and vat no:4 were inoculated with *S. aureus* to the mean level of 5.952 log MPN/mL. The counts of *S. aureus* were monitored separately during two individual processes (during production and ripening). The count of *S. aureus* in pasteurized milk inoculated with 1 % adjunct culture of *Lb. brevis* BG18 in vat no:2 was firstly decreased by 0.4 log unit from pasteurized milk to packaged cheese [during production, t (h) = 22)] and then reduced by 0.7 log unit (to the mean level of 5.740 log MPN/mL) during ripening (92nd day) whereas it was increased 0.7 log unit from pasteurized milk to packaged control group cheese [during production, t (h) = 22)] and then was resulted in a slight inhibition of *S. aureus* with count of 0.4 log unit (to the mean level of 6.582 log MPN/mL) at the end of the ripening of control group cheese in vat no:1 (Table 1). On the other hand; the count of *S. aureus* in pasteurized milk inoculated with 1 % adjunct culture of *P. pentosaceus* BH105 in vat no:4 was firstly increased 0.5 log unit from pasteurized milk to packaged cheese [during production, t (h) = 22)] and then was resulted in a sharp inhibition of S. aureus with count of 1.8 log unit (to the mean level of 4.361 log MPN/mL) during ripening (92nd day) whereas it was increased 0.6 log unit from pasteurized milk to packaged control group cheese, and then was resulted in a slight inhibition of *S. aureus* with count of 0.09 log unit (to the mean level of 6.490 log MPN/mL) at the end of the ripening of control group cheese in vat no:3 (Table 2).

As a result of paired-sample t-test applied to the data in Table 1, there was a statistically significant increase in *S. aureus* counts during production [between t (h)= 0 and t(h) = 22] of control group white-pickled cheeses in vat no=1 ($P = 0.001 < 0.05$) by time whereas no statistically significant difference was found in white-pickled cheeses in vat no:2 during production [between t (h)= 0 and t(h) = 22] ($P = 0.082 > 0.05$) by time. On the other hand, no any statistically significant difference was found in *S. aureus* counts during ripening (between the 1st day and the 92nd days) of control group white-pickled cheeses in vat no=1 ($P = 0.055 > 0.05$) by time. However, statistical analysis revealed significant effect ($P = 0.013 < 0.05$) of *Lb. brevis* BG18 on the counts of *S. aureus* in white-pickled cheeses in vat no:2 during the ripening (between the 1st day and the 92nd days). In other words; *S. aureus* counts in white-pickled cheeses were influenced by addition of adjunct culture of *Lb. brevis* BG18 only during the ripening process.

As a result of paired-sample t-test applied to the data in Table 2, there was a statistically significant increase in *S. aureus* counts during production [between t (h)= 0 and t(h) = 22] of control group white-pickled cheeses in vat no=3 ($P = 0.000 < 0.05$) by time whereas no statistically significant difference was found in white-pickled cheeses in vat no:4 during production [between t (h)= 0 and t(h) = 22] ($P = 0.053 > 0.05$) by time. On the other hand, no any statistically significant difference was found in *S. aureus* counts during ripening (between the 1st day and the 92nd days) of control group white-pickled cheeses in vat no=3 ($P = 0.233 > 0.05$) by time. However, statistical analysis revealed significant effect ($P = 0.000 < 0.05$) of *P. pentosaceus* BH105 on the counts of *S. aureus* in white-pickled cheeses in vat no:4 during the ripening (between the 1st day and the 92nd days). In other words; *S. aureus* counts in white-pickled cheeses were influenced by addition of adjunct culture of *P. pentosaceus* BH105 only during the ripening process.

As a result of independent-samples t-test applied to the data in Table 3, there was a statistically different in

TABLE 1: *Survival of S. aureus (mean log MP/mL) in white-pickled cheeses produced with 1 % adjunct culture of Lb. brevis BG18 (vat no:2) and in control group cheeses (vat no:1).*

	***S. aureus* count in vat no:1 (mean log MPN mL^{-1})**	***S. aureus* count in vat no:2 (mean log MPN mL^{-1})**
during production		
Just after addition of *S. aureus* culture t (h) = 0	6.252 ± 0.056[a]	6.252 ± 0.056[d]
Just after coagulum cutting t (h) = 2	5.581 ± 0.036	6.342 ± 0.129
Just after pressing-moulding t (h) = 6	6.605 ± 0.029	7.015 ± 0.022
Just after packaging-brining t (h) = 2	6.912 ± 0.028[b]	5.851 ± 0.160[d]
during ripening		
1st day	6.954 ± 0.121[c]	6.423 ± 0.085[e]
3rd day	6.492 ± 0.085	6.023 ± 0.127
27th day	6.397 ± 0.068	5.562 ± 0.096
48th day	6.777 ± 0.053	5.954 ± 0.004
67th day	6.454 ± 0.028	5.898 ± 0.017
80th day	6.413 ± 0.082	6.329 ± 0.075
92nd day	6.582 ± 0.044[c]	5.740 ± 0.049[f]

[a, b, c, d, e,] and [f] values in the same column with different superscripts are significantly ($P = < 0.05$) different.

TABLE 2: *Survival of S. aureus (mean log MPN/mL) in white-pickled cheeses produced with 1 % adjunct culture of P. pentosaceus BH105 (vat no:4) and in control group cheeses (vat no:3).*

	***S. aureus* count in vat no:1 (mean log MPN mL^{-1})**	***S. aureus* count in vat no:2 (mean log MPN mL^{-1})**
during production		
Just after addition of *S. aureus* culture t (h) = 0	5.952 ± 0.025[a]	5.952 ± 0.025[d]
Just after coagulum cutting t (h) = 2	5.447 ± 0.119	6.031 ± 0.033
Just after pressing-moulding t (h) = 6	6.245 ± 0.022	6.904 ± 0.040
Just after packaging-brining t (h) = 22	6.518 ± 0.037[b]	6.444 ± 0.181[d]
during ripening		
1st day	6.577 ± 0.029[c]	6.169 ± 0.140[e]
3rd day	6.387 ± 0.027	5.424 ± 0.117
27th day	6.364 ± 0.083	5.439 ± 0.105
48th day	6.664 ± 0.038	5.424 ± 0.113
67th day	6.257 ± 0.063	5.454 ± 0.080
80th day	6.312 ± 0.009	5.095 ± 0.039
92nd day	6.490 ± 0.061[c]	4.361 ± 0.119[f]

[a, b, c, d, e,] and [f] values in the same column with different superscripts are significantly ($P = < 0.05$) different.

TABLE 3: *Statistical analysis of comparison of mean reductions in S. aureus counts between L. brevis BG18 and P. pentosaceus BH105 in each trial during ripening.*

		mean (log MPN/mL)	**Standard deviation**	**P**
during ripening				
Control group	*L. brevis* BG18	372.67	157.561	0,052
	P. pentosaceus BH105	87.00	89.017	
Adjunct cultures	*L. brevis* BG18	672.33	133.800[a]	0,000
	P. pentosaceus BH105	1808.00	21.794[b]	

[a] and [b] values in the same column with different superscripts are significantly ($P = < 0.05$) different.

Journal of Food Safety and Food Quality 70, Heft 1 (2019), Seiten 1–30

comparison of mean reductions in *S. aureus* counts between *L. brevis* BG18 and *P. pentosaceus* BH105 in each trial during ripening ($P = 0.000 < 0.05$) whereas no statistically significant difference was found in control group cheeses ($P = 0.052 > 0.05$).

The purpose of this study was to construct brevicin-like bacteriocin-producing *Lb. brevis* BG18 and pediocin-like bacteriocin-producing *P. pentosaceus* BH105 adjunct cultures and evaluate their antistaphylococcal potentials during production and ripening of white-pickled cheeses. The high counts of *S. aureus* in white-pickled cheeses, produced with autochthonous probiotic strains of *P. pentosaceus* and *Lb. brevis,* even at the end of ripening were due to the high inoculum levels (since the maximum tolerable limit is 3 log/g cheese for *S. aureus* in Turkish Food Codex's microbiological, these high inoculum levels were several log units above the levels which could be expected in naturally contaminated cheeses). As Rodriguez et al. (2005) stated, the effects of these adjunct cultures on lower levels of *S.aureus* might be inhibitorier than the effects found in our work. However, the differences in reduction of *S. aureus* counts between these two strains might be attributed to different susceptibility of *S. aureus* for those two bacteriocins or to the amount of bacteriocin that they produced. In other words; according to the results obtained in this study, unlike the pediocin-like bacteriocin produced by *P. pentosaceus* BH105, the amount of brevicin-like bacteriocin produced by *Lb. brevis* BG18 was probably insufficient enough for a sharp inhibition of high levels of *S. aureus* cells present in white-pickled cheeses during the ripening process since it has been known that *S. aureus* shows reduced sensitivity to bacteriocins in food systems (Abdalla et al., 1993). Besides, *P. pentosaceus* BH105 showed a broad inhibitory activity against all the tested indicator strains, including a remarkable inhibitory effect on *S. aureus* growth by the agar overlay assays in the study of Uymaz et al. (2009) as in this study. As a result, both strains were found to be statistically ineffective on *S. aureus* counts during the production process but were found to be statistically effective on *S. aureus* counts during ripening process however, it was observed that the inhibition of the strain of *Lb. brevis* BG18 was not effective as the other adjunct culture during ripening of white-pickled cheeses. This might be attributed to the fact that different physical and chemical parameters in white-pickled cheese matrix affecting mainly the brevicin-like bacteriocin production and the following interaction of it with *S. aureus*. This is also supported by the results obtained by Sarantinopoulos et al. (2002) who concluded that the complex environment of Feta cheese, which is very similar to white-pickled cheese, thoroughly interferes with bacteriocin production levels of bacteriocinogenic starter or co-cultures and there is no guarantee for their in situ antimicrobial efficiency.

Much of the studies on the use of bacteriocin-producing cultures during cheese production related to the nisin-producing lactococci for the retardation of late gas blowing in Swiss style cheeses (O'Sullivan et al., 2002). Although Abdalla et al. (1993) showed that *S. aureus* was not inhibited by nisin during production of white-pickled cheeses from pasteurized milk samples and Cintas et al. (1998) reported a very weak inhibition of *S. aureus* by nisin A and pediocin PA-1, non-bacteriocinogenic *Lc. lactis* ESI 153 and nisin-producing *Lc. lactis* ESI 515 which were also used as adjunct cultures in cheese production, were found to lower *S. aureus* counts by 0.36 and 0.64 log units, respectively, on the 30th day of cheese ripening whereas their pediocin-producing transformants of *Lc. lactis* CL1 and *Lc. lactis* CL2 were found to lower the counts of *S. aureus* by 0.98 and 0.40 log units, respectively (Rodriguez et al., 2005). However in our study, 0.7 log unit reduction on *S. aureus* count was achieved by using adjunct culture of *Lb. brevis* BG18 in white-pickled cheese at the end of ripening and 1.8 log unit reduction on *S. aureus* count was achieved by using adjunct culture of *P. pentosaceus* BH105 in white-pickled cheese at the end of ripening which might only be achieved by transformant strain *Lc. lactis* CL1 of Rodriguez et al. (2005) and by nisin-producing *Lc. lactis* TAB 50 of Rodriguez et al. (2000) since these cultures showed 0.98 and 0.82 log units reduction on *S. aureus* counts in cheeses only after 30 days of ripening, respectively. A previous study with nisin-producing transconjugants of *Lc. lactis* ssp. *cremoris* JS102 and *Lc. lactis* ssp. *lactis* NCDO 1404 as starters in Cheddar cheese production also showed significant reductions in numbers of *Clostridium sporogenes, L. monocytogenes,* and *S. aureus* (Zottola et al., 1994). Likewise in our study, Rodriguez et al. (2000) found that *S. aureus* count was firstly increased from pasteurized milk to 1-day-old semi-hard cheese, and then was decreased. It might be due to the slow synthesis rate of BLIS of the adjunct cultures during the production and the level of them might not be enough for an inhibition and thus the pathogen continued to multiply and grow.

On the contrary, El-Kholy et al. (2014) evaluated the inhibition capacity of probiotic strains of *Lb. acidophilus* La-5 and *Bifidobacterium longum* ATCC 15707 on the growth of *S. aureus* and *Escherichia coli* O157:H7 during Domiati cheese production and storage and found that *Lb. acidophilus* La-5 reduced *S. aureus* and *E. coli* O157:H7 populations in Domiati cheese by about 3 and 1.88 logs after 14 days of storage, respectively, whereas *B. longum* ATCC15707 reduced *S. aureus* and *E. coli* O157:H7 populations in cheese by about 1.7 and 0.88 logs after 14 days of storage, respectively, compared with the control cheeses. Hence, El-Kholy et al. (2014) showed more remarkable reduction levels of *S. aureus* with these probiotic cultures than the probiotic culture of *Lb. brevis* BG18, whereas obtained almost the same reduction level of *S. aureus* with the probiotic culture of *P. pentosaceus* BH105 used in our study. However, cheese production environment of Domiati cheese is completely different than the white-pickled cheese production.

Use of bacteriocin-producing starter cultures in dairy industry are of considerable interest for the preservation of cheeses which may be achieved by inhibiting the growth of foodborne pathogens such as *L. monocytogenes* and *S. aureus*. However, there has still been problems in using them as starters in the context of industrial cheese production due to their main drawback like poor technological characteristics such as acidifying, lypolytic and proteolytic activities (Sarantinopoulos et al., 2002; Favaro et al., 2015) and thus it is very hard to select suitable strains having both strong technological and antimicrobial activities as starters in cheese production. Therefore in this study, the use of BLIS-producing *Lb. brevis* BG18 and *P. pentosaceus* BH105 that previously satisfied technological criteria as adjunct cultures rather than as starters in cheese production, to control growth and survival of *S. aureus* during white-pickled cheese production and ripening was investigated. Since looking for antimicrobial compounds is crucial to provide an alternative to chemical additives in dairy industry, to the best of our knowledge, the observed antistaphylococcal activity of both BLIS-producing au-

tochthonous probiotic strains against a foodborne pathogen in this study, is a useful and new data in the potential application of it in safe cheese production as adjunct cultures besides their contribution to typical sensory characteristics and nutritional value of cheese. Besides, these strains are good candidates for further investigation to elucidate their inhibition potentials of the expression of virulence genes of *S. aureus* although they are being able to slightly inhibit *S. aureus* growth in white-pickled cheeses. In addition, the antimicrobial potential of these two strains could also be exploited in the control of other foodborne pathogens.

Acknowledgements

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors completed this study with their own means. The authors would like to thank to Mr. Cem Güzey for supervising the statistical analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdalla OM, Davidson DM, Christen GL (1993): Survival of selected pathogenic bacteria in white pickled cheese made with lactic acid bacteria or antimicrobials. J Food Protect 56: 972–976.

Al-Holy MA, Al-Nabulsi A, Osaili TM, Ayyash MM, Shaker RR (2012): Inactivation of *Listeria innocua* in brined white cheese by a combination of nisin and heat. Food Control 23: 48–53.

Arques JL, Rodriguez E, Gaya P, Medina M, Guamis B, Nunez M (2005): Inactivation of *Staphylococcus aureus* in raw milk cheese by combinations of high-pressure treatments and bacteriocin-producing lactic acid bacteria. J Appl Microbiol 98: 254–260.

Buyong N, Kok J, Luchansky JB (1998): Use of a genetically enhanced, pediocin-producing starter culture, *Lactococcus lactis* subsp. *lactis* MM217, to control *Listeria monocytogenes* in Cheddar cheese. Appl Environ Microbiol 64: 4842–4845.

Capellas M, Mor-Mour M, Gervilla R, Yuste J, Guamis B (2000): Effect of high-pressure combined with mild heat or nisin on inoculated bacteria and mesophiles of goat's milk fresh cheese. Food Microbiol 17: 633–641.

Charlier C, Cretenet M, Even S, Le Loir Y (2009): Interactions between *Staphylococcus aureus* and lactic acid bacteria: an old story with new perspectives. Int J Food Microbiol 131: 30–39.

Cintas LM, Casaus P, Fernandez MF, Hernandez PE (1998): Comparative antimicrobial activity of enterocin L50, pediocin PA-1, nisin A and lactocin S against spoilage and foodborne pathogenic bacteria. Food Microbiol 15: 289–298.

Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, Agnellini D, Caramenti G, Moroni P, Castiglioni B (2005): Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. Mol Cell Probes 19: 299–305.

El-Kholy AM, El-Shinawy SH, Meshref AMS, Korany AM (2014): Microbiological quality of Domiati cheese and the influence of probiotics on the behavior of *Staphylococcus aureus* and *Escherichia coli* O157:H7 in Domiati cheese. J Food Safety 34: 396–406.

Erkmen O (1995): Behavior of *Staphylococcus aureus* in Turkish Feta cheese during manufacture and ripening. J Food Prot 58: 1201–1205.

Favaro L, Lucia A, Barretto Penna AL, Todorov SD (2015): Bacteriocinogenic LAB from cheeses-Application in biopreservation? Trends Food Sci Technol 41: 37–48.

Hayaloglu AA, Guven M, Fox PF (2002): Microbiological, biochemical and technological properties of Turkish white cheese 'Beyaz Peynir'-Review. Int Dairy J 12: 635–648.

Le Loir Y, Baron F, Gautier M (2003): *Staphylococcus aureus* and food poisoning. Genet Mol Res 2: 63–76.

Mercanoglu Taban B, Akineden O, Karimihachehsoo S, Gross M, Usleber E (2017): Enterotoxigenic *Staphylococcus aureus* in brined cheese from weekly street markets in Ankara, Turkey. J Food Saf Food Qual 68: 117–123.

Mercanoglu Taban B, Aytac SA (2009): Application of magnetic immuno-polymerase chain reaction assay for detection of *Salmonella* spp. in chicken meats. Eur Food Res Technol 229: 623–628.

Meyrand A, Boutrand-Loei S, Gueniot RS, Mazuy C, Gaspard CE, Jaubert G, Perrin G, Lapeyre C, Vernozy-Rozand C (1998): Growth and enterotoxin production of **Staphylococcus aureus** during the manufacture and ripening of Camembert-type cheeses from goats' milk. J Appl Microbiol 85: 537–544.

Nunez M, Bautista L, Medina M, Gaya P (1988): *Staphylococcus aureus,* thermostable nuclease and staphylococcal enterotoxins in raw ewes milk Manchego cheese. J Appl Bacteriol 65: 29–34.

O'Sullivan L., Ross RP, Hill C (2002): Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie 84: 593–604.

Rilla N, Martinez B, Rodriguez A (2004): Inhibition of a methicillin-resistant *Staphylococcus aureus* strain in Afuega'l Pitu cheese by the nisin Z-producing strain *Lactococcus lactis* subsp. *lactis* IPLA 729. J Food Prot 67: 928–933.

Rodriguez E, Calzada J, Arques JL, Rodriguez JM, Nunez M, Medina M (2005): Antimicrobial activity of pediocin-producing *Lactococcus lactis* on *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* O157:H7 in cheese. Int Dairy J 15: 51–57.

Rodriguez E., Arques JL, Gaya P, Nunez M, Medina M (2000): Behaviour of *Staphylococcus aureus* in semi-hard cheese made from raw milk with nisin-producing starter cultures. Milchwissenschaft 55: 633–635.

Sarantinopoulos P, Leroy F, Leontopoulou E, Georgalaki MD, Kalantzopoulos G, Tsakalidou E, de Vuyst L (2002): Bacteriocin production by *Enterococcus faecium* FAIR-E 198 in view of its application as adjunct starter in Greek Feta cheese making. Int J Food Microbiol 72: 125–136.

Temelli S, Anar S, Sen C, Akyuva P (2006): Determination of microbiological contamination sources during Turkish white cheese production. Food Control 17: 856–861.

Uymaz B, Akkoc N, Akcelik M (2011): Partial characterization of bacteriocins produced by two *Lactobacillus* strains with probiotic properties. Acta Biol Hung 62: 95–105.

Uymaz B, Simsek O, Akkoc N, Ataoglu H, Akcelik M (2009): In vitro characterization of probiotic properties of *Pediococcus pentosaceus* BH105 isolated from human faeces. Ann Microbiol 59: 485–491.

Zottola EA, Yezzi TL, Ajao DB, Roberts RF (1994): Utilization of Cheddar cheese containing nisin as an antimicrobial agent in other foods. Int J Food Microbiol 24: 227–238.

Address of corresponding author:
Dr. Birce Mercanoglu Taban
Ankara University, Faculty of Agriculture
Dairy Technology Department, Diskapi
06110, Ankara
Turkey
btaban@ankara.edu.tr