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KÜBRA FETTAHOĞLU

KÜBRA ÇİNAR

MÜKERREM KAYA

GÜZİN KABAN

TÜBİTAK

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Biodiversity and characterization of gram-positive, catalase-positive cocci isolated from pastırma produced under different curing processes

Kübra FETTAHOĞLU[1], Kübra ÇINAR[2], Mükerrem KAYA[1], Güzin KABAN[1,*]

[1]Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum, Turkey
[2]Department of Food Engineering, Faculty of Engineering, Bayburt University, Bayburt, Turkey

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Abstract: The aim of this study was to determine the effects of different curing temperatures (4 °C or 10 °C) and agents (KNO_3 or $NaNO_2$) on microbiological and physicochemical properties of pastırma and diversity of gram-positive, catalase-positive cocci (GCC^+) and also to investigate some technological properties of the strains. Four different groups of pastırma were produced applying different curing temperatures and agents. After production, GCC^+ isolated from pastırma were subjected to phenotypic and genotypic (16S rRNA sequencing) identification. Genotypically characterized strains were evaluated for their technological properties. In all groups, a_w and pH were observed to be under 0.90 and over 5.5, respectively. In genotypic identification, 45 isolates were identified as *Staphylococcus vitulinus*, while other isolates identified were *S. equorum* (n = 15), *S. saprophyticus* (n = 15), and *S. xylosus* (n = 12). All of the strains could grow at different salt concentrations (8% and 15%) and temperatures (4 °C, 10 °C, and 37 °C). Four strains of *S. saprophyticus* showed strong lipolytic activity while nine strains of *S. vitulinus* had strong proteolytic activity. This study showed that different curing temperatures and agents are particularly effective on the microflora in pastırma; however, these factors do not cause a high diversity in GCC^+ in terms of species.

Key words: *Staphylococcus*, pastırma, catalase positive cocci, curing temperature, nitrite, nitrate

1. Introduction

Pastırma is a traditional Turkish dry-cured meat product obtained by the curing and drying of the meat pieces obtained from certain parts of beef or buffalo carcasses and then drying again after covering them with çemen, a paste made from *Trigonella foenum-graecum* flour, fresh garlic, red pepper, and water (1). In the curing process, the first stage of pastırma production, nitrate is usually used as a curing agent along with salt (2,3). However, nitrite may be used alone or with nitrate in the process as a curing agent. In order to observe the expected effects of nitrate, nitrate should be converted to nitrite by microorganisms that have nitrate-reductase activity (4). The curing process for pastırma production can take a few days and curing temperature can range between refrigerator temperature and about 10 °C (1).

Gram-positive, catalase-positive cocci (GCC^+), particularly coagulase-negative staphylococci (CNS), are technologically important in cured meat products and play an important role in the color formation and stabilization, delaying the oxidation, aroma formation, and lipolytic and proteolytic activities (5,6). These microorganisms, which are susceptible to acid, can show good development due to the suitable pH value (>5.5) of pastırma (4). GCC^+ counts in pastırma range between 10^4 and 10^7 cfu/g and these microorganisms constitute the dominant microflora very often (2,3,7,8). It must be noted that high diversity has been observed within GCC^+. Such species as *Staphylococcus saprophyticus*, S. *xylosus*, *S. epidermidis*, S. *simulans*, S. *intermedius*, S. *auricularis*, *S. cohnii*, *S. equorom*, *S. arlettae*, *S. kloosii*, *S. warneri*, *S. gallinarum*, *S. haemolyticus*, and *S. hominis*, along with *Micrococcus varians* (*Kocuria varians*), *M. luteus*, *M. nishinomiyaensis*, *M. lylae*, and *Kocuria rosea*, were isolated in studies for isolation and identification of catalase-positive cocci and it was indicated that a significant portion of the catalase-positive cocci consists of CNS (9–11).

To the best of our knowledge there are no studies on the determination of the effects of different curing conditions on GCC^+ diversity in pastırma. The primary aim of this study was to assess the effects of different curing temperatures (4 °C or 10 °C) and curing agents (potassium nitrate or sodium nitrite) on microbiological and physicochemical properties of the product and

* Correspondence: gkaban@atauni.edu.tr

diversity of GCC+. Another aim was to investigate some technological properties of the isolated strains, thereby providing a starting point for further research on the usability of these strains as starter cultures.

2. Material and methods

2.1. Pastırma production

M. longissimus thoracis et lumborum obtained from beef carcasses that were aged for 24 h after slaughtering was used as raw material in pastırma production. The muscles (right and left) obtained from each carcass were divided into two pieces, and four pieces of meat were obtained from a beef carcass. In the production, two different carcasses were used as raw material and the production was carried out twice.

The meat strips were subjected to the incision process called "şaklama". After that, they were taken to the curing process, and 5% salt and 0.3% saccharose by weight of the meat were used in curing. In the production 300 mg/kg potassium nitrate or 150 mg/kg sodium nitrite was used as a curing agent, and the curing process was carried out at two different temperatures of 4 °C and 10 °C. The meat strips, treated with curing mixtures, were cured for 48 h at 4 ± 1 °C or 10 ± 1 °C. Following the curing process, meat strips were hung for the 1st drying process and dried at 15 ± 1 °C for 6 days with 80 ± 2% relative humidity in a climate chamber (Reich, Germany). The pressing process was applied to cured and partially dried meat strips at 7 ± 1 °C for 20 h (1st pressing, cold press). In the pressing process, 15 kg of weight was calculated for 1 kg of meat. After this process, meat strips were hung again and dried for 5 days in the chamber with 70 ± 2% relative humidity at 20 ± 1 °C. After this stage, which is called the second drying, a hot pressing (2nd pressing) process was applied at 25 ± 1 °C for 7 h. In the pressing process, 15 kg of weight was taken as a basis for 1 kg of meat. After this treatment, meat strips were subjected to the 3rd drying process and dried at 20 ± 1 °C for 5 days at 70 ± 2% relative humidity.

After pressing and drying processes, pastırma was kept waiting in çemen paste (500 g *Trigonella foenum-graecum* flour, 350 g smashed fresh garlic, 150 g red pepper, and 1200 mL water were used in the preparation of çemen paste) at 7 °C for 1 day, and the process of drying with çemen was initiated after the çemen on the surface had been thinned out to be 2–3 mm. The process of drying with çemen was carried out at 20 ± 1 °C for 10 days at 70 ± 2% relative humidity. After the çemen was removed from the surface of pastırma samples, samples were subjected to analyses.

2.2. Determination of pH, a_w, and residual nitrite

Ten grams of sample was weighed for determining the pH value, and after having added 100 mL of distilled water to it, it was homogenized for 1 min using an Ultra-Turrax (IKA Werk T 25, Germany). The pH value was measured using a pH meter (Thermo, Orion Star, Singapore). The a_w value of the samples was measured at 25 °C using a Novasina device (Novasina TH-500 a_w Sprint, Switzerland). Five different calibration salts were used in the calibration of the device. Residual nitrite values were determined by the method of Tauchmann (12).

2.3. Microbiological analyses

For microbiological analyses, 25 g of sample was transferred to a sterile Stomacher bag and 225 mL of sterile physiological saline (0.85% NaCl) was added. After homogenizing for 1.5 min in the Stomacher (Lab Stomacher Blender 400-BA 7021, Seward, UK), serial decimal dilutions were prepared and appropriate dilutions were spread on selective agar plates. Lactic acid bacteria were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid, Basingstoke, UK) under anaerobic conditions (AneorocultA, Merck, Darmstadt, Germany) for 48 h at 30 °C, *Micrococcus/Staphylococcus* on Mannitol Salt Phenol-Red agar (MSA, Oxoid) for 48 h at 30 °C, Enterobacteriaceae on Violet Red Bile Dextrose agar (VRBD, Merck) for 48 h at 30 °C under anaerobic conditions (AneorocultA, Merck), and molds-yeasts on Rose Bengal Chloramphenicol (RBC Agar, Merck) for 5 days at 25 °C. The counts were given as colony-forming units per gram of sample (cfu/g).

2.4. Isolation and phenotypic and genotypic identification of catalase-positive cocci

MSA agar was used for isolation. For each sample, 10 to 15 colonies were randomly selected and purified by streaking on tryptone soya agar (TSA, Oxoid). After incubation at 30 °C for 48 h, isolated colonies were tested for morphology, Gram reaction, and catalase. The isolates were stored at –80 °C in BHI broth (Oxoid) containing glycerol (30%) and glass beads.

A total of 87 isolates were submitted to phenotypic characterization using the VITEK 2 GP kit (bioMérieux, France). The evaluation was carried out with the BioMérieux VITEK2 Compact device (bioMérieux, USA).

After all GCC+ isolates were grown overnight on BHI, isolation of genomic DNA was carried out with a High Pure PCR template preparation kit (Roche, Indianapolis, IN, USA) according to the manufacturer's protocol. The 16S rRNA coding region sequence was selected and amplified by PCR (TC-4000 Techne, UK). In PCR, the FastStart High Fidelity PCR System dNTPack kit (Roche) was used. Universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were used to amplify the 16S rRNA gene. The amplification program was initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1.5 min; and a final extension step at 72 °C for 7 min. Cycle sequencing reaction products

were purified with a Sephadex column. 16S rRNA sequence analysis of PCR products was carried out by Macrogen (the Netherlands). The sequence results obtained were aligned with the NCBI database using the BLAST program (http://blast.ncbi.nlm.nih.gov).

2.5. Technological properties of catalase-positive cocci

Before determining technical properties, all strains were subjected to coagulase test (BD BBL coagulase plasma, rabbit with EDTA, USA). Identified strains were analyzed for nitrate reductase (13), proteolytic and lipolytic activities, and growth at different salt concentrations (8% and 15%) and at different temperatures (4 °C, 10 °C, and 37 °C) (14).

2.6. Statistical analysis

In the study, different curing agents (150 mg/kg $NaNO_2$ or 300 mg/kg KNO_3) and different curing temperatures (4 °C and 10 °C) were taken as factors. The experiments were carried out as two replications according to a randomized complete block design. The analysis of variance was applied to the data of pH, a_w, and residual nitrite values as well as microbiological counts. The averages of the main sources of variation found significant were evaluated with the Duncan multiple comparison test by SPSS 20 statistic software.

3. Results

Overall effects of curing temperature and curing agent on physicochemical and microbiological properties of pastırma are given in Table 1. The curing temperature had a significant effect ($P < 0.05$) on the pH value of pastırma. The average pH value was determined as 5.88 ± 0.02 at 4 °C curing temperature and as 5.85 ± 0.01 at 10 °C temperature. However, the curing agent and the interaction of curing temperature × curing agent did not show a significant effect on pH value ($P > 0.05$). The water activity of pastırma samples was not affected by the curing temperature, curing agent, or the interaction of curing temperature × curing agent ($P > 0.05$). The water activity dropped below 0.90 in all groups (Table 1). On the other hand, the main sources of variation had no significant effect on residual nitrite level, and residual nitrite level was observed below 10 mg/kg in all groups (Table 1).

The lactic acid bacteria count was determined below the detectable level (<2 log cfu/g) in the presence of nitrite as well as at 4 °C curing temperature. *Micrococcus/Staphylococcus* is one of the major microbial groups in pastırma. In this group, selected factors caused some differences. Although the curing temperature, one of the main sources of variation examined in this study, did not show a significant effect ($P > 0.05$) on the number of *Micrococcus/Staphylococcus*, the curing agent showed a very significant ($P < 0.01$) effect on its count. The mean count of *Micrococcus/Staphylococcus* was determined as 6.38 ± 0.34 log cfu/g in pastırma produced using nitrate, while the average number (5.50 ± 0.63 log cfu/g) was found lower in the groups in which nitrite was used (Table 1). On the other hand, curing temperature had no significant effect on yeast-mold count ($P > 0.05$). However, pastırma samples cured with nitrate showed a higher mean count of yeast-mold than those of nitrite-cured samples (Table 1). Moreover, Enterobacteriaceae count was found to be lower than the detection limit (<2 log cfu/g) in all groups.

Genotypic and phenotypic identification results of GCC^+ isolated from pastırma produced using two different curing agents (sodium nitrite or potassium nitrate) and

Table 1. Overall effects of curing temperature and curing agent on physicochemical and microbiological properties of pastırma (values are means ± SD).

Factors	a_w	pH	Residual nitrite (mg/kg)	Lactic acid bacteria (log cfu/g)***	*Micrococcus*/*Staphylococcus* (log cfu/g)	Yeast-mold (log cfu/g)
Curing temperature (CT)						
4°C	0.844 ± 0.022a	5.88 ± 0.02a	4.26 ± 1.64a	<2	6.32 ± 0.32a	4.51 ± 1.11a
10°C	0.826 ± 0.017a	5.85 ± 0.01b	5.04 ± 2.35a	3.91 ± 0.27	6.07 ± 0.53a	3.92 ± 0.57a
Significance	NS	*	NS	-	NS	NS
Curing agent (mg/kg) (CA)						
Nitrate	0.840 ± 0.021a	5.86 ± 0.03 a	4.39 ± 2.55a	2.96 ± 1.04	6.38 ± 0.34a	4.81 ± 0.68a
Nitrite	0.830 ± 0.022a	5.87 ± 0.02 a	4.90 ± 1.38a	<2	5.50 ± 0.63b	3.62 ± 0.71b
Significance	NS	NS	NS	-	**	**
CT × CA	NS	NS	NS	-	NS	NS

a-b: Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, NS: not significant, SD: standard deviation, ***: not statistically evaluated.

curing temperatures (4 °C or 10 °C) are given in Table 2. According to the phenotypic and genotypic identification results, all of the 87 isolates obtained were identified as coagulase-negative *Staphylococcus*. The species determined phenotypically were *Staphylococcus vitulinus* (45 isolates), *S. xylosus* (16 isolates), *S. saprophyticus* (15 isolates), *S. equorum* (8 isolates), and *S. gallinarum* (3 isolates). Four different species of *Staphylococcus* were determined by genotypic identification. *S. vitulinus* (45 isolates) was the dominant coagulase-negative cocci species, followed by *S. saprophyticus* (15 isolates), *S. equorum* (15 isolates), and *S. xylosus* (12 isolates). Three isolates that were identified phenotypically as *S. gallinarum* were genotypically identified as *S. xylosus*. Moreover, seven phenotypic isolates of *S. xylosus* were genotypically identified as *S. equorum*.

Distribution of species differed according to curing agent and curing temperature factors (Table 3). In the case of using nitrate at 4 °C curing temperature, 15 of 24 isolates (62.5%) were identified as *S. vitulinus*. In the case of presence of nitrite at the same curing temperature, 12 of 20 isolates (60%) were identified as *S. vitulinus*. Under 10 °C/nitrate combination, 15 of 22 isolates (68.18%) were also identified as *S. vitulinus*. On the other hand, the dominant species for the 10 °C/nitrite combination was identified as *S. saprophyticus* (10 strains, 47.62%). *S. xylosus*, widely used as a starter culture in meat products, was isolated in higher proportions in the presence of nitrate at 4 °C and in the presence of nitrite at 10 °C. Another species, *S. equorum*, which is also considered as a potential starter culture, showed an isolation rate between 9.52% and 22.73%, depending on curing conditions (Table 3).

Some technological properties of the strains are given in Table 4. The nitrate reductase activity was tested at three different temperatures, and it was determined that all strains, except for 10 strains of *S. saprophyticus*, showed this activity at all three temperatures. All of the strains isolated from pastırma showed good growth in the presence of 8% and 15% NaCl. The fact that the same strains can develop at 4 °C and 10 °C has particular importance.

As seen from Table 4, only one strain of 12 *S. xylosus* showed weak proteolytic activity. Three *S. xylosus* strains showed weak lipolytic activity. Proteolytic activity was observed in 43 of *S. vitulinus* strains, which are commonly found in pastırma groups. Nine of these strains had strong proteolytic activity. Lipolytic activity was also observed in 24 strains of these species. Only one of these 24 strains was determined to have a strong lipolytic activity. Four of the *S. saprophyticus* strains showed weak proteolytic activity and six of them had lipolytic activity. Although all of the *S. equorum* strains had no proteolytic activity, five strains exhibited lipolytic activity.

4. Discussion

The pH value is usually 5.5 or higher in pastırma, a traditional dry-cured meat product processed as whole pieces (2,3,7,10,15). In the present study, the pH value was found to be over 5.5 in all pastırma samples. Similarly, the absence of true lactic fermentation has been observed in other cured meat products such as French dry-cured hams (16) and dry-cured lacon (17,18). Contrary to pH, water activity is an important hurdle effect in pastırma. Salt added in the initial phase of pastırma production reduces water activity and inhibits microbial growth. Furthermore, salt increases protein solubility and regulates proteolysis and lipolysis reactions. Through these mechanisms salt has an effect on taste and texture (4). Water activity decreases depending on salt amount used in the production and the drying process applied (2,19). Pastırma can be identified as a shelf-stable product because of the low level of water activity and high salt concentration of the final product. In this study, the water activity dropped below 0.90 in all groups. Similar results were also observed in pastırma studies by Kaban and Kaya (10), Kaban (2), and Akköse et al. (15). An important quality criterion in pastırma is the amount of residual nitrite. According to the 2002 Turkish Standard TS 1071, the highest residual nitrite level in pastırma must be 50 mg/kg. As can be seen from Table 1, the amount of residual nitrite is below this limit in all pastırma samples. It is thought that this resulted from nitrate and/or nitrite reductase activities of the catalase-positive cocci that are present in pastırma flora (20). These microorganisms, which grow at high salt concentrations and at low water activity values, generally constitute the dominant microflora in pastırma (2,4,15). In the present study, GCC^+ (*Micrococcus/Staphylococcus*) constituted the dominant microflora in all groups as well. Moreover, they showed better growth in the presence of nitrate (Table 1). Nitrate is used more widely in production in comparison with nitrite (3). CNS, known as halotolerant and one of the dominant microbiota of salted/dried/fermented meat products (21,22), can be evaluated as microorganisms with technological importance for pastırma. They are also effective in the development of aroma with their lipolytic and proteolytic activities (23). Furthermore, they play an important role in the delay of oxidative processes through superoxide dismutase and catalase activities (24), and in the formation and also the stabilization of color by allowing the conversion of nitrate to nitrite with nitrate reductase activities (25). These microorganisms also contribute to the reduction of the amount of nitrite residue through nitrite reductase activities (20).

Lactic acid bacteria in pastırma can display a great variation (2,7,8,26). Production conditions (nitrate, nitrite, salt ratio, curing temperature, drying temperature, etc.) and house flora play a particularly important role in

Table 2. Genotypic and phenotypic identification results of GCC+ isolated from pastırma produced under different curing temperatures and curing agents.

Isolate no.	Genotype		Phenotype		Isolate no.	Genotype		Phenotype	
	Strain	% Ident.	Strain	% Ident.		Strain	% Ident.	Strain	% Ident.
1	*S. equorum*	98	*S. xylosus*	97	71	*S. vitulinus*	100	*S. vitulinus*	96
2	*S. vitulinus*	100	*S. vitulinus*	98	72	*S. vitulinus*	100	*S. vitulinus*	96
3	*S. saprophyticus*	99	*S. saprophyticus*	99	73	*S. vitulinus*	100	*S. vitulinus*	93
4	*S. saprophyticus*	97	*S. saprophyticus*	99	74	*S. vitulinus*	99	*S. vitulinus*	94
5	*S. equorum*	99	*S. equorum*	96	75	*S. vitulinus*	99	*S. vitulinus*	96
6	*S. vitulinus*	99	*S. vitulinus*	97	76	*S. vitulinus*	100	*S. vitulinus*	99
7	*S. vitulinus*	99	*S. vitulinus*	96	77	*S. vitulinus*	100	*S. vitulinus*	97
8	*S. vitulinus*	100	*S. vitulinus*	96	78	*S. vitulinus*	100	*S. vitulinus*	86
10	*S. equorum*	99	*S. equorum*	93	79	*S. vitulinus*	100	*S. vitulinus*	96
12	*S. equorum*	98	*S. xylosus*	97	80	*S. equorum*	97	*S. equorum*	88
17	*S. xylosus*	99	*S. gallinarum*	86	81	*S. vitulinus*	100	*S. vitulinus*	99
18	*S. xylosus*	99	*S. gallinarum*	91	82	*S. vitulinus*	100	*S. vitulinus*	96
18 A	*S. vitulinus*	98	*S. vitulinus*	97	84	*S. equorum*	99	*S. equorum*	95
18C	*S. vitulinus*	100	*S. vitulinus*	96	85	*S. vitulinus*	100	*S. vitulinus*	96
19	*S. vitulinus*	99	*S. vitulinus*	89	86	*S. vitulinus*	100	*S. vitulinus*	96
20	*S. vitulinus*	100	*S. vitulinus*	89	87	*S. equorum*	99	*S. xylosus*	97
21	*S. xylosus*	100	*S. gallinarum*	Low ident.	88	*S. saprophyticus*	99	*S. saprophyticus*	99
22	*S. vitulinus*	99	*S. vitulinus*	93	89	*S. equorum*	99	*S. xylosus*	97
24	*S. vitulinus*	100	*S. vitulinus*	Low ident.	90	*S. vitulinus*	100	*S. vitulinus*	93
25	*S. xylosus*	100	*S. xylosus*	99	92	*S. saprophyticus*	100	*S. saprophyticus*	99
31	*S. xylosus*	99	*S. xylosus*	97	93	*S. vitulinus*	100	*S. vitulinus*	97
34	*S. equorum*	99	*S. xylosus*	97	94	*S. saprophyticus*	99	*S. saprophyticus*	99
35	*S. xylosus*	100	*S. xylosus*	98	95K	*S. saprophyticus*	100	*S. saprophyticus*	99
36	*S. xylosus*	100	*S. xylosus*	97	95B	*S. xylosus*	99	*S. xylosus*	99
39	*S. xylosus*	100	*S. xylosus*	98	96	*S. saprophyticus*	100	*S. saprophyticus*	99
40	*S. equorum*	99	*S. xylosus*	97	97	*S. vitulinus*	100	*S. vitulinus*	99
44	*S. xylosus*	100	*S. xylosus*	97	98	*S. saprophyticus*	99	*S. saprophyticus*	99
46	*S. vitulinus*	100	*S. vitulinus*	96	99	*S. saprophyticus*	99	*S. saprophyticus*	91
49	*S. xylosus*	99	*S. xylosus*	97	100	*S. saprophyticus*	99	*S. saprophyticus*	Low ident.
53	*S. equorum*	98	*S. equorum*	87	101	*S. vitulinus*	100	*S. vitulinus*	97
54	*S. vitulinus*	100	*S. vitulinus*	98	102	*S. saprophyticus*	100	*S. saprophyticus*	99
55	*S. vitulinus*	99	*S. vitulinus*	98	103	*S. saprophyticus*	98	*S. saprophyticus*	99
56	*S. vitulinus*	100	*S. vitulinus*	95	104	*S. saprophyticus*	99	*S. saprophyticus*	99
57	*S. vitulinus*	100	*S. vitulinus*	97	107	*S. vitulinus*	100	*S. vitulinus*	99
58	*S. saprophyticus*	99	*S. saprophyticus*	99	108	*S. equorum*	99	*S. equorum*	93
60	*S. xylosus*	100	*S. xylosus*	97	109	*S. saprophyticus*	99	*S. saprophyticus*	99
61	*S. vitulinus*	100	*S. vitulinus*	96	110	*S. equorom*	100	*S. equorum*	93
62	*S. vitulinus*	99	*S. vitulinus*	95	111	*S. vitulinus*	100	*S. vitulinus*	96
63	*S. vitulinus*	99	*S. vitulinus*	96	112	*S. vitulinus*	99	*S. vitulinus*	96
66	*S. vitulinus*	100	*S. vitulinus*	98	113	*S. equorum*	98	*S. equorum*	93
67	*S. vitulinus*	100	*S. vitulinus*	96	114	*S. vitulinus*	100	*S. vitulinus*	96
68	*S. vitulinus*	99	*S. vitulinus*	98	115	*S. vitulinus*	100	*S. vitulinus*	96
69	*S. vitulinus*	99	*S. vitulinus*	96	119	*S. vitulinus*	100	*S. vitulinus*	99
70	*S. equorum*	99	*S. xylosus*	97					

Table 3. Diversity and prevalence of staphylococci isolated and genetically identified from pastırma produced under different curing temperatures and curing agents.

	Curing temperature							
Isolated staphylococci	4 °C				10 °C			
	Nitrate		Nitrite		Nitrate		Nitrite	
	Isolates	%	Isolates	%	Isolates	%	Isolates	%
S. vitulinus	15	62.5	12	60	15	68.18	3	14.29
S. xylosus	4	16.67	2	10	0	-	6	28.57
S. saprophyticus	1	4.17	2	10	2	9.09	10	47.62
S. equorum	4	16.67	4	20	5	22.73	2	9.52
Total	24		20		22		21	

Table 4. Some technological properties of *Staphylococcus* strains isolated and genetically identified from pastırma produced by using different curing temperatures and curing agents.

Strains	Isolate number	Nitrate reductase (°C)			NaCl (%)		Temperature (°C)			Proteolytic activity		Lipolytic activity	
		4	10	37	8	15	4	10	37	Weak	Strong	Weak	Strong
S. vitulinus	45	45	45	45	45	45	45	45	45	34	9	23	1
S. xylosus	12	12	12	12	12	12	12	12	12	1	0	3	0
S. saprophyticus	15	5	5	5	15	15	15	15	15	4	0	2	4
S. equorum	15	15	15	15	15	15	15	15	15	0	0	4	1

this variation. In this study, lactic acid bacteria count at 4 °C or in the presence of nitrite, which is a combination rarely applied in traditional production, was found under the detectable level. The 4 °C/nitrite combination is rather used in industrial production. Lactic acid bacteria count was also found under the detectable level at 10 °C or in the presence of nitrite (Table 1). These results show that use of nitrite during the curing phase inversely affects lactic acid bacteria growth. Similarly, a better development of yeast-mold can be seen in the presence of nitrate (Table 1). Enterobacteriaceae, which include many foodborne pathogens and undesirable microorganisms, generally cannot survive in pastırma (2,7,26). In the present study, Enterobacteriaceae count was also found to be under the detectable level (<2 log cfu/g) due to decreasing water activity in the production process.

In the present study, all of the 87 isolates were identified as *Staphylococcus*. On the contrary, Erol et al. (9) reported that 73.3% of isolates belonged to *Staphylococcus* after phenotypic identification of catalase-positive cocci in samples from different firms. Kaban (10), on the other hand, reported that 95.4% of catalase-positive cocci isolates belonged to *Staphylococcus*. Although catalase-positive cocci showed remarkable diversity in both studies, *S. saprophyticus* and *S. xylosus* were the predominant species in these studies (9,10). A higher prevalence of staphylococci than micrococci may be due to the fact that they are resistant to high salt concentration and have a low redox potential (27). Also, *Staphylococcus* species have tolerance to high concentrations of NaCl as a common feature (28).

In the present study, lower diversity was observed in comparison with the previous studies conducted on samples obtained from the market (9,11) and four species (*S. vitulinus*, *S. saprophyticus*, *S. equorum*, and *S. xylosus*) were genetically identified. *S. vitulinus*, which was determined as the dominant species from three different pastırma groups (4 °C/nitrate, 10 °C /nitrate, and 4 °C / nitrite), is one of the members of the *S. sciuri* group. This group was also isolated from meat and meat products and dairy and dairy products as well as various farm animals and domestic animals (29). It was reported that this microorganism will be the second-generation starter culture (30). In this study, 45 isolates were identified as *S. vitulinus* both phenotypically and genotypically. This species was isolated in ready-to-eat-meat samples by

Fijalkowski et al. (31) at a rate of 16%. It was also reported that *S. vitulinus* can be used as an autochthonous starter culture in order to limit the formation of undesired compounds in traditional fermented sausages as well as to protect typical sensory properties of the product (32).

Among pastırma groups, the maximum number of *S. saprophyticus* isolates was obtained from the group cured with nitrite at 10 °C. This microorganism draws attention as the dominant species in this group. It was reported that *S. saprophyticus* has a high prevalence in the environments of production facilities (33). While Kaban (11) identified this microorganism as the dominant species in pastırma, Erol et al. (9) and Kaban and Kaya (10) isolated *S. saprophyticus* from pastırma samples. This species was also isolated and identified from the dry-cured meat products (5,34–36).

S. equorum was identified in all pastırma groups. This species was also found in other meat products that were processed as whole pieces (5,6,18,34–36). However, this species can be confused with *S. xylosus* when phenotypic and biochemical methods are used for identification (6,27). A similar situation was experienced in this study, as seven isolates identified phenotypically as *S. xylosus* were genotypically identified as *S. equorum* (Table 2).

S. xylosus, which is an important starter culture used in meat products, was isolated from all pastırma groups except the group with 10 °C/nitrate combination. The maximum number of isolates was obtained from the 10 °C/nitrite combination. *S. xylosus* has also been isolated from pastırma (9–11). It was reported that *S. xylosus* was the dominant species in Spanish-type dry cured ham (6), dry cured ham (37), Iberian ham (5,34), samples taken from the surface of dry cured lacon (35), and kadidde, which is a traditional meat product specific to Tunisia (36).

CNS is important in aroma formation because of their proteolytic and lipolytic activities, color formation through their nitrate reductase activity, and prevention of oxidation and color stability through their nitrate catalase activity (23). Presence of nitrate reductase activity is quite important in pastırma production, particularly in processes where nitrate is used since conversion of nitrate to nitrite can only be possible with the presence of nitrate reductase. In the present study, all of the *S. vitulinus*, *S. xylosus*, and *S. equorum* strains showed nitrate reductase activity while only one-third of the *S. saprophyticus* strains showed nitrate reductase activity. Likewise, Mainar et al. (24) also reported that *S. saprophyticus* strains are often nitrate reductase-negative. Strains grow at high salt concentrations such as 8% and 15% and low temperatures of 4 or 10 °C. Considering that salt can be used up to a proportion of 10% and curing temperature must be 10 °C or lower in pastırma production, it is inferred that the above-mentioned strains can be good potential starter cultures for pastırma production. Presence of proteolytic and lipolytic activities determined in some strains in this study increases the possibility of using these strains as starter cultures. In this respect, some *S. vitulinus* strains particularly stand out with both their proteolytic and lipolytic activities.

In conclusion, CNS constitute the dominant flora in pastırma. These microorganisms show good growth in the presence of nitrate. Different curing temperatures and curing agents are particularly effective on the microflora in pastırma; however, they do not cause high diversity in CNS in terms of species. *S. vitulinus* was found more frequently in 4 °C/nitrate, 4 °C/nitrite, and 10 °C/nitrate combinations, while *S. saprophyticus* was found more frequently in 10 °C/nitrite. *S. vitulinus*, *S. saprophyticus*, *S. equorum*, and *S. xylosus* isolated from pastırma are technologically important species.

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