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Functional and technological characterization of lactic acid bacteria isolated from Turkish dry-fermented sausage (sucuk)

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Abstract

In this study, 10 lactic acid bacteria were isolated from Turkish fermented sausage (sucuk) and identified as *5 Lactobacillusplantarum*, *1 Pediococcus acidilactici*, *1 Weissella hellenica*, *1 Lactobacillus pentosus*, *and 2 Lactobacillus sakei.* PCR screening of genes encoding plantaricin A and pediocin showed the presence of plantaricin A gene in 9 and pediocin gene in 3 of strains. All isolates showed antibacterial and antifungal effect on most of the tested microorganisms. g*ad* gene, encoding glutamic acid decarboxylase enzyme, was detected in all isolates except *Weisella hellenica* KS-24. Eight of isolates were determined as gamma-amino butyric acid (GABA) producer in the presence of 53 mM mono sodium glutamate (MSG) by HPLC and TLC analysis. DPPH scavenging activity was observed for all isolates. Additionally, isolates were able to produce exopolysaccharide in the presence of sucrose. The best exopolysaccharide (EPS) production was achieved with *L. plantarum* KS-11 and *L. pentosus* KS-27. As a result, this study characterized some techno-functional properties of LAB isolates from sucuk. It was concluded that the isolates studied have the potential to be used in obtaining functional products in meat industry, as well as strain selection may be effective in providing the desired properties in the product.

Keywords Lactic acid bacteria · GABA · EPS · Antioxidative · Sucuk

Introduction

Turkish fermented sausage locally known as sucuk is a fermented dry-cured meat product which contains sheep/beef or buffalo meat. It is produced by using meat /tail fat together with various spices [1]. Sucuk manufacturing is made in two different ways, traditionally and commercially. Traditional method is differed by spontaneous fermentation (fermentation is carried out without starter culture) (Gökalp, Kaya, & Zorba, 2002). Lactic acid bacteria (LAB) are the main group of microbiota in fermented sausages. *Lactobacillus* is the most common genera isolated from sucuk [2, 3] similar to dry fermented sausages [4, 5]. *Pediococcus*, *Weisella*, *Lactococcus*, and *Leuconostoc* were generally isolated the other LAB genera in sucuk [2, 3, 6, 7]. *Lactobacillus sakei*, *L. curvatus*, *L. plantarum*, *Lactobacillus pentosus*, *Lactobacillus casei*, *Pediococcus pentosaceus*, and *P. acidilactici* are the most commonly used species in commercially presented culture preparations, for meat products [8]. However, in order to provide the taste and aroma of sucuk produced with traditional methods in industrial scale, commercial starter cultures specific to sucuk are needed. Preparation of starter culture for sucuk can be achieved by using strains isolated from the product.

The technological properties of fermented sausages can be altered depending on the biochemical capabilities of LAB strains [3, 9–11]. Fermented sausages are primarily characterized by increased acidity and distinctive aroma provided by fermentation [12]. LAB is effective in providing product-specific aroma with their technological features, as well as highly effective on texture, shelf life, and safety of product. Lactic acid bacteria contribute to product safety with antibacterial and antifungal effects exhibited by bacteriocin/bacteriocin-like metabolites and organic acids etc. [13–15]. Moreover, some lactic acid bacteria have antioxidative effects and decrease the accumulation of reactive oxygen species (ROS), as functional properties [16]. In the recent years, interests have been drawn to the ability of lactic acid bacteria to produce gamma-amino butyric acid (GABA),

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which is a non-protein amino acid structure further supporting their functionality [17]. GABA has many structural roles known for human metabolism such as cardiovascular functions and neurotransmitter in the brain [18–20]. GABA can be produced by α-decarboxylation of glutamic acid in the presence of glutamic acid decarboxylase (GAD) enzyme [21, 22]. And also, lactic acid bacteria can produce exopolysaccharide (EPS) in various composition and functionality [23, 24]. Lactic acid bacteria exopolysaccharides enhanced oxidative stability, color [25], and textural properties [26] of sausages. EPS can be showed prebiotic activity that has beneficial effects on human health [27].

Several studies on the characterization of lactic acid bacteria from sucuk have been performed [2, 3, 7, 28, 29] but the techno-functional properties of LAB isolates have not yet been investigated and detailed till now.

In this study, 10 different lactic acid bacteria strains were isolated and identified from traditionally produced sucuk. While the functional aspects of these identified lactic acid bacteria such as GABA and EPS production capabilities and antioxidative properties were examined, their antimicrobial activities were also determined. This study has showed that lactic acid bacteria isolated from sucuk, a fermented meat product, have potential to provide important functional properties to the product.

Materials and methods

Isolation of lactic acid bacteria

A total of 10 sucuk samples were collected from butchers in Bayburt province in summer season and used for isolation and identification purposes of lactic acid bacteria. All manufacturers used traditional methods (without starter culture).

For isolation and identification purposes, 10 g of sample was initially taken into sterile stomacher bags under aseptic conditions and then 90 mL of sterile saline solution (0.85%) was added and homogenized. The same procedure was repeated for each sample. The spread plate technique on MRS (de Man, Rogosa and Sharpe) plates was used for proper dilutions and incubated at 30 °C for 48 h in anaerobic conditions. Following incubation, typical colonies were selected and growth in MRS broth at 30 °C for 24 h. Same procedures were continuously repeated until pure culture was obtained from single colonies. For the obtained colonies, various properties such as morphological structures, microscopic views, Gram staining, and catalase properties were determined. Considering that lactic acid bacteria are Gram positive, catalase negative, and cocci or rod-shaped, the cultures selected were stored at − 80 °C in tubes containing glycerol (30%), glass beads, and MRS broth for further diagnostic tests.

Genotypic characterization by RAPD-PCR analysis

For strain differentiation of 100 LAB isolates, RAPD-PCR analysis was performed. Colonies of isolates were used as templates (without DNA isolation). Each PCR mix contained 0.75 µl template, 25 pmol of primer M13 (GAG GGTGGCGGTTCT), 5XPCR buffer for Taq polymerase (Promega, UK), 2.5 mM of deoxyribonucleoside triphosphates (dNTPs) (Bioline, UK), and 1.5 U Taq polymerase (Promega, UK). For the amplification with primer M13, denaturation was performed at 94 °C for 60 s, binding at 40 °C for 20 s, and 2 min for extension at 72 °C. PCR products were separated by electrophoresis in 1% agarose gel for 90 V 1.5 h [30].

Bacterial identification by 16S rRNA gene sequencing

Genomic DNA of isolates were extracted according to Barış [31]. For 16 sRNA gene sequences, primers AMP_F and AMP_R [32] were used. PCR mix contained 25 µL PCR Master Mix (Thermo Scientific, cat. No. K0171), 1 µL template DNA, 10 µM AMP_F, and 10 µM AMP_R primers and water in 50 µL total volume. The PCR program was run as 95 °C for 12 min, 20 cycles of 95 °C for 30 s, 55 °C for 20 s, and 72 °C for 30 s and 72 °C for 5 min final extension. Electrophoresis of PCR products were performed in gel prepared with 1% agarose to confirm the presence of amplification. Sequence analysis of PCR products were performed by GEN Plaza (Turkey). The 16S rRNA gene sequences of isolates were arranged in Bioedit and compared with NCBI database for the final identification by using Blast program (http://blast.ncbi.nlm.nih.gov). The 16S rRNA gene sequences were deposited in GenBank under accession numbers MK694773-MK694781 and MK845561 and were aligned in MEGAX 10.1.7. Phylogenetic tree was constituted by using neighbor join (NJ) method with 1000 bootstrap replicates [33]. Phylogenetic tree analyses were done with MEGAX [34].

Molecular detection of glutamic acid decarboxylase gene (*gad*) in LAB strains

The molecular detection of glutamic acid decarboxylase in LAB strains performed using primers CoreF (5′-CCTCGA GAAGCCGATCGCTTAGTTCG-3′) and CoreR (5′ TCA TATTGACCGGTATAAGTGATGCCC-3′) [35]. PCR was performed with the following program: 10 min denaturation at 95 °C, followed by 20 cycles of 30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C. PCR products were run on a gel (1.5%) to check the amplicon size 600 bp.

Determination GABA production levels of LAB strains

LAB isolates were grown in MRS broth containing 53 mM monosodium glutamate (MSG) for 96 h at 30 °C. It was then centrifuged at 6000 g for 5 min and the resulting supernatant was analyzed for the presence of GABA using thin layer chromatography (TLC). For this purpose, Silicagel 60 F254 TLC plates (Merck, Darmstadt, Germany) and 1-butanol: acetic acid: distilled water (4: 1:1) as mobile phase were used. After 2 h running, the plate was sprayed with 0.2% (w/v) ninhydrin solution and heated at 105 °C for 2 min. GABA producing isolates were detected by the spot loaded with MSG and GABA [36].

For quantitative evaluation, supernatant of GABA producer isolates were filtered by cellulose acetate syringe filter (0.45 µm). The GABA content of 72-h cell supernatants was determined by high-pressure liquid chromatography (HPLC) analysis. GABA derivatization was accomplished with dansyl chloride. Inertsil ODS column (13.5 µm, 4.6*250 mm) and 1:1 methanol: H_2O (v:v) as mobile phase were used. The samples were separated at 40 °C and flow rate of 0.5 mL/min. The GABA content was given as "mM" based on standard curve prepared of GABA analytical standard (Sigma-Aldrich) [37].

Moleculer detection of bacteriocin encoding genes in LAB strains

The presence of gene encoding bacteriocin production was determined with different primer sets and reactions were performed at different annealing temperatures that were suitable for each primer sets (Table 1). PCR products were run on a gel (1% agarose) with electrophoresis at 100 V for 40 min to check the amplification. In the positive strains, appropriate amplicon sizes were detected.

Table 1 The oligonucleotides used for determination of the presence of the bacteriocin encoding genes

Bacteriocin encoding genes	Primer sets	Amplicon size (bp.)	Annealing temp. (°C)
*pln*A	F:5′- GTA CAG TAC TAA TGG GAG-3′ R: 5′-CTT ACG CCA ATC TAT ACG-3′	450	53
ped	F 5′-GGTAAG GCTACCACT TGCAT-3′ R: 5′-CTACTA ACGCTTGGC TGGCA-3′	332	55

Determination of antibacterial and antifungal activities of LAB strains

The antibacterial activities of the isolates against indicator pathogens (*Bacillus cereus* BC 6830, *Escherichia coli* BC 1402, *Staphylococcus aureus* ATCC 25,923, *Salmonella typhimurium* RSSK 95,091, and *Yersinia enterocolitica* ATCC 27,729) were determined according Schillinger, Lücke [38] by agar-spot and well diffusion tests. Briefly, 0.5 µl 24-h cultured isolates in MRS was dropped on a MRS agar plates with 4 dots at approximately 30 mm intervals. Plates were incubated under anaerobic conditions (Aneorocult A, Merck) at 30 °C for 24 h. The size of clear zones around the spots was measured. The isolates that gave positive results in the agar spot test incubated for 24 h at 30 °C in MRS broth. The cell free supernatant was separated by centrifugation at 7000 rpm for 10 min, and pH was adjusted to 6.6 by 1 N NaOH. Supernatant was filtered through a cellulose acetate filter (0.45 µm pore size) and used in the well diffusion test.

The antifungal activities of strains were determined against *Aspergillus niger* and *Penicillium chrysogenum* origined molded bread. Overnight LAB strain culture was spotted on MRS agar plates in 10 µl volume, under anaerobic conditions. After incubation at 30 °C for 24 h, the petri dishes were overlayed with PDA semi-solid medium (0.8% agar) containing 10^6 spores/mL. Spore-suspensions were prepared with sterile distilled water from fungal colonies incubated for 7 days in Potato Dextrose Agar (MERCK, Darmstadt) at 30 °C. The antifungal activity was determined by measuring the zone diameters observed in the petri plates that were incubated at 30 °C- 5 days, aerobically [39].

Determination of antioxidative activities of LAB strains

The strains were incubated in MRS medium at 30 °C for 24 h. Supernatant was removed after centrifugation at 2000 g for 5 min. Bacterial cells were re-suspended with distilled water. Antioxidative activity of cells was measured by 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. Two hundred µl of 0.2 mM DPPH solution (with methanol) was mixed with cell suspension. After incubation at dark conditions for 30 min, suspension was filtered through 0.45 µm pore syringe filter (Millipore). Absorbance value of samples was determined at 517 nm. Distilled water was used as blank. Scavenging activity was calculated as inhibition $\% = (1 - (A_{sample}/A_{blank})) * 100$ [16].

Determination of EPS production level

Isolates, previously growth in MRS broth, were inoculated at a volume of 1% into the 50 mL modified MRS/s medium

containing of 100 g/L sucrose [40]. After 72 h incubation, supernatants of the samples (centrifuged for 30 min at 6000 g) and an equal volume of cold ethanol were mixed. The supernatant-ethanol mixture was kept for one night at 4 °C. The mixture (supernatant-ethanol) was centrifuged at 2000 g for 15 min in order to obtain EPS. It was afterwards suspended again with twofold volumes of ethanol and precipitated by centrifuge (at 2000 g for 15 min). Precipitates were dried at 55 °C. The dried samples were diluted with ultrapure water and EPS levels were determined as mg glucose/L by phenol–sulfuric acid method [41].

Statistical analysis

Data analysis was conducted by Minitab.19 package program. The differences between means were tested by Duncan's multiple range test ($p < 0.05$). The results of statistical analysis were shown as mean value ± standard deviation in the tables.

Results and discussion

In this study, LAB strains were isolated from Turkish dry fermented sausage (sucuk). Sucuk samples were collected from 3 different producers. LAB counts of sucuk samples were determined and the results are shown in Table 2. The LAB counts of samples differed between 9.72 and 10.33 log cfu/g according to producers. There was no significant difference ($p > 0.05$) between producers. Total 100 lactic acid bacteria (Gram +, catalase −) isolates were selected for further analysis. With the use of RAPD-PCR analysis in genotypic identification, 10 isolates selected as a result of pre-screening were selected among 100 isolates for techno-functional characterization. Isolates were identified as *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Weissella hellenica*, *Lactobacillus pentosus*, *and Lactobacillus sakei* by 16S rRNA gene sequence analysis. In spontaneously fermented sucuks, *L plantarum* [3, 42], *Pediococcus acididilactici* [43], and *Lactobacillus sakei* [44] were isolated as major LAB species. While Lactobacilli are generally included in the major flora, *Weissella* was detected as minor genera in sucuk [6, 42, 43]. Lücke [11] reported that while *L. sakei* and *L. curvatus* were dominant in sausages ripened at low temperatures (20–22 °C), *L. plantarum* was dominant at high temperatures (> 25 °C). Considering that the samples were collected in summer season (the ambient temperature above 25 °C), it can be said that the high number of isolates, identified as *L. plantarum* is due to the high ripening temperatures.

Table 2 Lactic acid bacteria counts (log cfu/g) sucuk samples from different sources (means ± SD)

Source	LAB log cfu/g	Number of isolates
A	10.33 ± 0.01	1–29
B	10.09 ± 0.21	30–82
C	9.72 ± 0.42	83–100
Significance level	-	

SD standard deviation

The phylogenetic tree obtained by evaluating the 16 s rRNA gene of the isolates is presented in Fig. 1. The isolates constituted 6 subgroups. The cluster analysis showed that 16 s rRNA gene sequences of *L. plantarum* strains were close to *L. pentosus* KS-27 strain while *Pediococcus acidilactici*, *Weisella hellenica*, and *Lactobacillus sakei* strains placed separately from other isolates. *L. plantarum* isolates fell into different subgroups. While *L. plantarum* KS-12 and KS-25 were close to the *L. pentosus* KS-27, *L. plantarum* KS-3 and KS-17 formed different subgroup.

Genes responsible for the production of pediocin, plantaricin, and the gene responsible for GAD production were amplified by PCR with specific primers (Table 3.).

At least one of the genes encoding bacteriocin was found in all isolates. Pediocin and plantaricin genes were not amplified in *W. hellenica* KS-24. *L. plantarum KS-11*, *L. plantarum subp*, *plantarum KS-12*, *and L. pentosus KS-27* contained the gene responsible for the pediocin production. In the well diffusion test, antimicrobial activity was not detected with the neutralized supernatant. The antibacterial effects of isolates on Gram positive (*B. cereus*, *S. aureus*) and Gram negative (*S. typhimurium*, *Y. enterolitica*, and *E. coli*) food pathogens in agar-spot test are presented in Table 4. All isolates inhibited the test pathogens at different levels. In fact, *W. hellenica* KS-24 and *L. sakei* KS-30 isolates did not inhibit the growth of *S. aureus*. The highest inhibitions were observed against to Gram negative, *S. typhimurium*, *E. coli*, and *Y. enterocolitica* pathogens, while the effect on *B. cereus* and *S. aureus* was minor (negligible small). Similarly, Bartkiene et al. [45] stated in their study that *L. sakei* and *P. acidilactici* strains have antimicrobial effects on *E. coli*, *S. typhimurium*, *Y. enterolitica*, *B. cereus*, and *S. aureus* strains. Gao et al. [46] reported that many LAB strains have antagonistic effects against some food borne pathogens such as *Salmonella* species, *E. coli*, *Bacillus cereus*, and *S. aureus* strains. The inhibition effects of lactic acid bacteria on pathogens may be caused by organic acids, as well as by hydrogen peroxide and antimicrobial peptides such as bacteriocins [47]. By evaluating the presence of bacteriocin encoding genes and antimicrobial activity, it can be said that the antimicrobial activity in LAB isolates may be caused by bacteriocin. However, the lack of antimicrobial activity in neutralized supernatants suggested other bacteriostatic effects. It can be said that the antimicrobial activity detected with *W. hellenica* KS-24 was not due to pediocin or plantaricin.

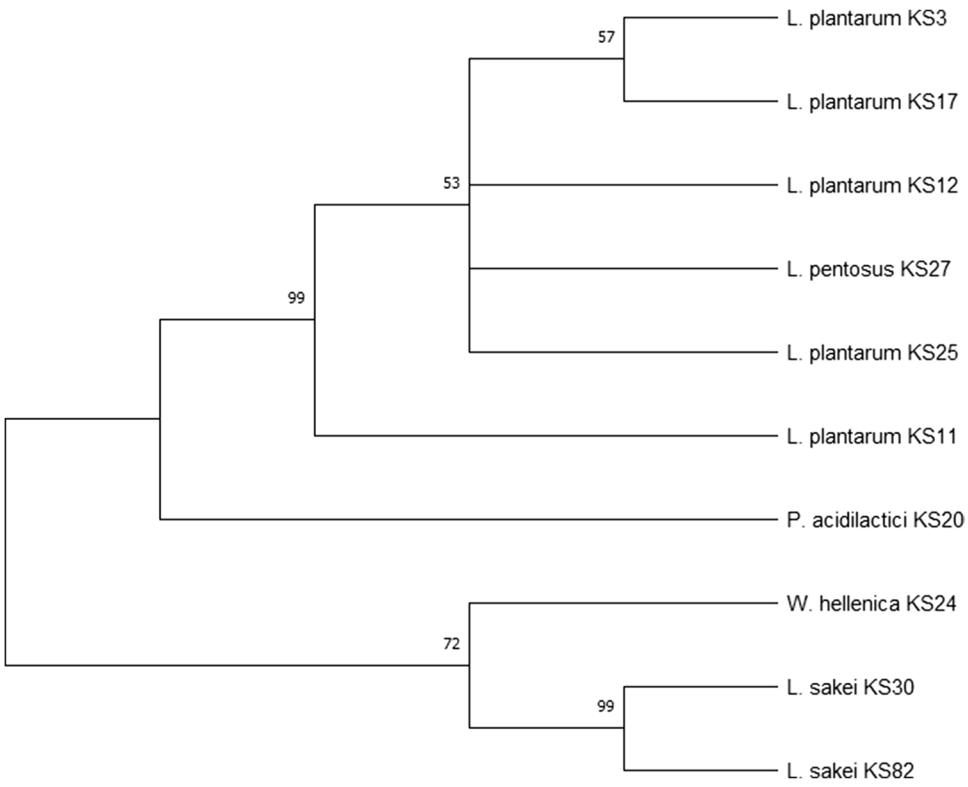

Fig. 1 A phylogenetic tree was constructed by using 16S rRNA gene sequences. Phylogenetic tree was created by Neighbor-Joining method (Saitou and Nei, 1987). Phylogenetic distances were calculated for 1200nt. The percentage of replicate trees (1000 replicates) was shown near the branches. Optimal tree with the sum of branch length = 0.20777641 is shown (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000)

Another functional feature desired in lactic acid bacteria is antifungal behavior. Fungal growth is an undesirable and frequently encountered problem in many fermented meat products such as sausages [48]. *Aspergillus* and *Penicillium* are fungal species that are frequently isolated from meat and meat products [49–51]. The antifungal activities of strains are presented in Table 5. *L. plantarum* KS-17, KS-25, and KS-27 had a strong antifungal effect on *A. niger* and *P. chrysogenum* and *L. sakei* isolates showed weak effect (Table 5). While *L. sakei* strains exhibited an inhibitory effect on fungal growth, other isolates inhibited both spore formation and mycelial development. Cizeikiene et al. [47] reported that *L. sakei* KTU05-6 strain inhibited *A. niger* MD-029 growth, inhibited spore formation of *P. acidilactici* KTU05-7 and did not affect *P. chrysogenum* 48-L growth. The antifungal effects of lactic acid bacteria can be caused by many compounds such as organic acids, reuterine, phenyl lactic acid, cyclic dipeptides, fatty acids, and proteinaceous substances [52]. Besides the antibacterial effects of the isolates, the presence of antifungal effects is promising in that they can be used as a protective culture.

Table 3 Screening gene encoding pediocin, plantaricin production, and GAD for GABA production

	Pediocin	Plantaricin A	GAD gene
L. plantarum KS-3	-	+	+
L. plantarum KS-11	+	+	+
L. plantarum subp. plantarum KS-12	+	+	+
L. plantarum KS-17	-	+	+
P. acidilactici KS-20	-	+	+
W. hellenica KS-24	-	-	-
L. plantarum KS-25	-	+	+
L. pentosus KS-27	+	+	+
L. sakei KS-30	-	+	+
L. sakei KS-82	-	+	+

Lactic acid bacteria can produce GABA by decarboxylation of glutamic acid. Core F and Core R primer sets were used in the isolates to determine the presence of GAD enzyme production ability that provides GABA accumulation. All tested strains except *W. hellenica* KS-24 harbored *gad* gene responsible for GABA production. It was amplified to approximately 540 bp with Core F and Core R primers. The GABA production capabilities of LAB strains in 53 mM MSG concentration were determined primarily by TLC (Fig. 2). TLC analysis showed KS-20, KS-24, KS-30, and KS-82 isolates did not produce GABA. The presence of GABA in samples obtained using the same medium was quantitatively determined by HPLC analysis. GABA production amounts of strains are given in Table 6. When evaluated by species, it was determined that *L. plantarum* strains produced high amounts of GABA. Contrary to other studies, it was reported that *P. acidilactici*

Table 4 Antibacterial activity of LAB strains

	E. coli	*B. cereus*	*S. typhimurium*	*Y.enterolitica*	*S. aureus*
L. plantarum KS-3	+ + +	+ +	+ + +	+ + +	+ +
L. plantarum KS-11	+ + +	+ +	+ + +	+ + +	+ +
L. plantarum subp. plantarum KS-12	+ + +	+ +	+ + +	+ + +	+ +
L. plantarum KS-17	+ + +	+ +	+ + +	+ + +	+ +
P. acidilactici KS-20	+ + +	+ +	+ + +	+ + +	+ +
W. hellenica KS-24	+ + +	+	+ + +	+ + +	-
L. plantarum KS-25	+ + +	+ +	+ + +	+ + +	+ +
L. pentosus KS-27	+ + +	+ +	+ + +	+ + +	+
L. sakei KS-30	+ + +	+	+ + +	+ + +	-
L. sakei KS-82	+ + +	+ +	+ + +	+ + +	+

-, no inhibition; +, inhibition zone ≤ 2 mm; + +, inhibition zone 3–4 mm; + + +, inhibition zone > 4 mm

Table 5 Antifungal activities of LAB strains

LAB	*Aspergillus niger*	*Penicillium chrysogenum*
L. plantarum KS-3	+ +	+ + +
L. plantarum KS-11	+ +	+ + +
L. plantarum subp. plantarum KS-12	+ +	+ + +
L. plantarum KS-17	+ + + +	+ + + +
P. acidilactici KS-20	+ + +	+ +
W. hellenica KS-24	+ +	+ +
L. plantarum KS-25	+ + + +	+ + + +
L. pentosus KS-27	+ + + +	+ + + +
L. sakei KS-30	+	+
L. sakei KS-82	+	+

The antifungal activity was determined as (+), delayed spore formation; (++), delayed spore formation and clear zone (small); (+++), delayed spore formation and clear zone (good); (++++), delayed spore formation and clear zone (define)

KS-20, *W. hellenica* KS-24, and *L.sakei* (KS-30, KS-82) were not capable of producing GABA in the presence of 53 mM L-glutamate in accordance with TLC. The highest GABA production was determined to be 1.657 mM with *L. plantarum* KS-25 ($p < 0.05$). Lactobacilli are already known as GABA producers [53]. Demirbaş et al. [39] found 4.92 mM GABA production in the medium containing 53 mM MSG with *L. plantarum* isolate. In another study, Zhang et al. [54] detected GABA production ranging from 3.5 to 14 mM with *L. plantarum* isolates in the presence of 97 mM MSG. *W. hellenica* KS-24, which is the only isolate among all the strains that does not contain the GAD gene, did not produce GABA as expected. However, *P. acidilactici* and *L. sakei* isolates contained GAD gene in their structures, they did not produce GABA. GABA production levels of the isolates are in similar intervals with other previous studies [55]. Recently, *L. plantarum* [54] *W. hellenica* (Barla et al., 2016), *P. acidilactici* [55], *L. sakei* [56], and *L. pentosus* [57] were isolated as GABA producer species from fermented foods. The presence of GABA production ability enables the isolates to be evaluated as functional starter cultures instead of classical starter cultures [55]. In this study, the production abilities were investigated with L-glutamate addition. However, the glutamic acid content of meat offers lactic acid bacteria the possibility of producing GABA. For example, Ratanaburee et al. [17] also showed the presence of GABA in samples that did not add monosodium glutamate in the production of "nham," a fermented meat product.

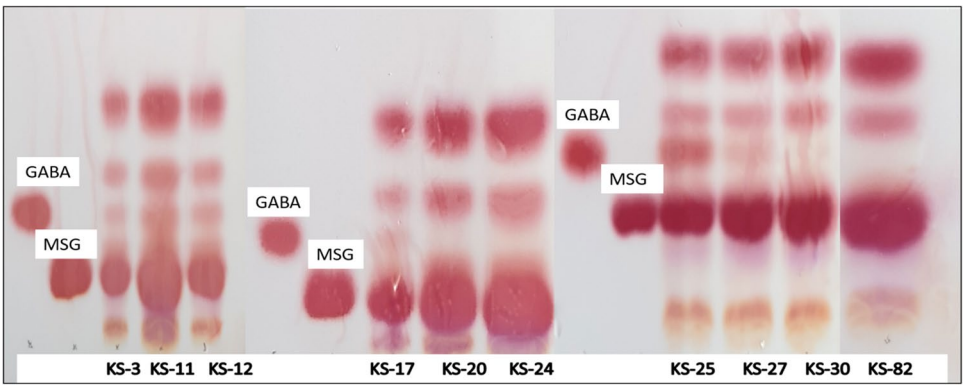

Fig. 2 Screening of GABA producing LAB isolates by TLC plates (all strains (*L.* plantarum KS-3, KS-11, KS-17, KS-25, *L. plantarum subp. plantarum* KS-12, *P. acidilactici* KS-20, *W.hellenica* KS-24, *L. pentosus* KS-27, L.sakei KS-30, KS-82) were grown in MRS broth containing 53 mM MSG for 96 h at 30 °C, *GABA* GABA standard, *MSG* MSG standard)

Table 6 GABA production at 53 mM MSG (means ± SD)

LAB	GABA content (mM)
L. plantarum KS-25	1.657 ± 0.151[a]
L. plantarum KS-11	0.315 ± 0.185[b]
L. pentosus KS-27	0.252 ± 0.123[b]
L. plantarum subp. plantarum KS-12	0.242 ± 0.151[b]
L. plantarum KS-17	0.237 ± 0.13[b]
L. plantarum KS-3	0.165 ± 0.055[b]
P. acidilactici KS-20	ND
W. hellenica KS-24	ND
L. sakei KS-30	ND
L. sakei KS-82	ND

a–b: Any two means in the column having the same letters are not significantly different at $P > 0.05$; *SD*, standard deviation; *ND*, not detected

One other biological function of lactic acid bacteria is their antioxidant activity. DPPH radical scavenging activity is one of the most preferred methods for determining antioxidative activity of lactic acid bacteria [58–61]. Ten LAB isolates were tested for antioxidative abilities. DPPH scavenging activities of supernatant free cells of strains are presented in Table 7. Radical scavenging abilities were not different from each other ($p < 0.05$). Regardless of species, all isolates showed approximately 20% DPPH scavenging activity. In previous studies, *L. plantarum* strains showed approximately 10–20% DPPH scavenging activity at 10^9 cfu/ml concentration [61], *P. acidilactici* strain showed approximately 40% inhibition at 10^9 cfu/ml concentration [62]. Contrary to these results, Meira et al. (2012) reported none of studied *Lactobacillus* species showed DPPH scavenging activity (60 µM). Considering the studies, it can be said that LAB isolates show a moderate antioxidant activity.

Table 7 DPPH scavenging activity of supernatant free cells (means ± SD)

LAB	% inhibition
L. plantarum KS-3	21.60 ± 1.13
L. plantarum KS-11	24.13 ± 3.50
L. plantarum subp. plantarum KS-12	22.49 ± 1.70
L. plantarum KS-17	21.94 ± 3.97
P. acidilactici KS-20	21.24 ± 0.58
W. hellenica KS-24	24.30 ± 0.45
L. plantarum KS-25	26.25 ± 0.50
L. pentosus KS-27	21.30 ± 2.07
L. sakei KS-30	24.01 ± 0.45
L. sakei KS-82	21.20 ± 0.52

SD standard deviation

Antioxidant activity demonstrated by LAB cells can be due to cell surface proteins [61] or exopolysaccharides (EPS) [63]. EPS can exhibit bioactive properties (antiviral, anticarcinogen, antioxidative, etc.) and can contribute to probiotic activity by providing colonization [64, 65]. There are many microorganisms reported as EPS producers [66–68]. Since most LAB species have been generally recognized as safe (GRAS) status [69], species that can produce EPS attract attention. The fact that EPS producer LAB isolates improve the texture properties of meat products [26] and delay color and oxidation [25] show that EPS production is also an important factor technologically. EPS production levels determined after 72 h incubation of strains in sucrose containing medium are indicated in Table 8. *L. plantarum* KS-11 (991.75 ± 94.81) and *L. pentosus* KS-27 (991.75 ± 37.55) exhibited the highest EPS production and lower EPS production levels were determined with KS-20, KS-24, KS-30, and KS-82 strains. *L plantarum* [70] and *L. pentosus* (Rodríguez-Carvajal et al., 2008) are lactobacilli that are commonly capable of producing EPS. *L. plantarum* strains provided higher EPS production in this study. Mıdık et al. [71] determined 512.81 mg/L EPS production with *L. plantarum* isolate. Their LABs show EPS production at low levels (50–400 mg/L) [72] as well as at higher levels (10.78 g/L) in optimized conditions such as *L. rhamnosus* strain [73]. EPS production is also found in the literature.

Conclusion

In this study, 10 lactic acid bacteria isolated from sucuk were genotypically identified. Furthermore, some functional properties of the isolates were evaluated. It was observed that some isolates are capable of producing GABA, which is known to have positive effects on human health. The highest

Table 8 EPS production of isolates (means ± SD)

LAB	EPS(as mg glucose/L)
L. plantarum KS-11	991.75 ± 94.81[a]
L. pentosus KS-27	991.75 ± 37.55[a]
L. plantarum KS-17	832.69 ± 89.23[ab]
L. plantarum subp. plantarum KS-12	806.14 ± 88.86[ab]
L. plantarum KS-3	755.4 ± 67.67[b]
L. plantarum KS-25	496.98 ± 15.99[c]
P. acidilactici KS-20	99.1 ± 12.27[d]
L. sakei KS-30	98.23 ± 15.13[d]
W. hellenica KS-24	90.05 ± 4.61[d]
L. sakei KS-82	74.51 ± 0.56[d]

[a–d]: Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$; *SD* standard deviation

GABA production was achieved with *L. plantarum* KS-25 strain. Additionally, it was determined that a significant part of the isolates is capable of producing EPS and all isolates show antioxidant activity. Most of the isolates inhibited five food-borne pathogens, while all isolates showed antagonistic effect on *A. niger* and *P. chrysogenum*. In these isolates, substantially antifungal, antibacterial, antioxidant activity, and ability to produce EPS and GABA have been determined, although it varies according to the strain representing some technological and functional properties of autochthonous LAB strains from sucuk. Further work will investigate these functional properties of LAB isolates in vitro conditions such as fermented sausages and meat products.

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