FOOD MICROBIOLOGY - RESEARCH PAPER

Technological properties of autochthonous *Lactobacillus plantarum* strains isolated from sucuk (Turkish dry-fermented sausage)

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Abstract

Purpose Five *Lactobacillus* strains isolated from sucuk (Turkish dry-fermented sausage) were studied for their genetic and technological properties.

Methods For genotypic identification, strains 16S rRNA gene sequences were used. To determine the antimicrobial activity of strains, seven foodborne pathogens were tested. Strains technological properties were characterized.

Results These strains were identified as *Lactobacillus plantarum* by 16S rRNA gene sequence analysis and the phylogenetic tree obtained by neighbor-joining method allowed grouping of these strains into three subgroups. *L. plantarum* strains showed antagonistic activities against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Micrococcus luteus* strains. PCR assay, using specific primers, showed the presence of bacteriocin (plantaricin) encoding genes in all *L. plantarum* strains tested. Antimicrobial metabolite production of these strains started at log phase and reached the maximum level at the end of the stationary phase. Regarding their technological properties, better growth was observed at 25 °C compared with 15 °C and 45 °C. The isolates which grown well within the pH scale pH 4.5–6.5 range additionally showed a decent growth at 6.5% salt concentration. It has been found that strains do not exhibit lipolytic and proteolytic activities nor have lysine, ornithine, and arginine decarboxylase activity. On the other hand, one strain showed weak nitrate reductase activity, and four strains produced acetoin from glucose. In addition, all strains were DL-lactic acid producers. Consequently, *L. plantarum* strains isolated exhibited some biochemical properties required for a starter culture in sucuk and similar products.

Conclusions All identified strains may be a protective culture in the production of fermented meat products. In particular, *L. plantarum* S51 was distinguished from other isolates due to the inability to form acetoin from glucose. Further work will be needed to characterize *L. plantarum* strains as starter culture.

Keywords *Lactobacillus plantarum* · Fermented sausage · Characterization · Antimicrobial activity

Introduction

Sucuk (Turkish dry-fermented sausage) is a very popular meat product in Turkey. It is a dry-fermented sausage variety produced traditionally and industrially. Traditional production is usually carried out in small-scale enterprises without the use

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of starter cultures, and sausages are ripened under natural conditions. In industrial production, starter cultures are used for controlled fermentation, and air conditioning units that can automatically control temperature, air current, and relative humidity for fermentation and drying processes.

Lactic acid bacteria (LAB) are the main bacteria group for sausage fermentation that improve the safety and sensorial properties of fermented sausages. The major role of this group is to provide rapid acidification by lactic acid production during sausage fermentation. They also produce other important products such as acetic acid, ethanol, volatile compounds, and antimicrobial metabolites. Some metabolic end products, like bacteriocins and bacteriocin-like inhibitory substance (BLIS), are responsible for antagonistic effects of LAB [1]. As the rate and extent of acid formation of LAB are effective, the antagonistic activity of the strains to be used against foodborne

pathogens is also important for ensuring product safety. In terms of standardized production, the use of starter cultures is also of great importance. In many countries, strains isolated and identified from traditional products have been used in starter cultivation and are still being explored. Traditionally produced fermented products are preferred for their sensory properties [2]. However, it is not always possible to achieve the same level of quality and safety in traditional products [3]. The use of autochthonous lactic acid bacteria as a starter culture permits to produce sausages with similar sensory properties to the traditional production [4]. Autochthonous starter cultures isolated from traditional fermented products are well adapted to fermentation conditions [5, 6]. Many LAB strains were isolated and identified from sucuk [7–13]. *Lactobacillus*, which is the largest group in the lactic acid bacteria, is quite common in traditional fermented products and industrial starter preparations [14, 15]. *Lactobacillus plantarum* and *Lactobacillus sakei* are two important species isolated from sucuk [7–13]. *L. brevis*, *L. curvatus*, *L. pentosus*, and *L. fermentum* are the other species isolated from sausage [7, 8, 11, 13, 16]. In fermented meat products, *L. plantarum* stand out in lactobacilli because of its reliability, ability to use different sugars, easy adaptation to various media, and probiotic properties. *L. plantarum* is isolated from fermented sausages at different rates depending on fermentation conditions. In similar dry-fermented Greek sausages, Papamanoli et al. [17] found that *L. plantarum* strains represent 9.4% of LAB isolates, while Drosinos et al. [18, 19] reported that this species reach 37.2 to 45.6% of LAB isolates in these meat products. Considering the case of sucuk, Özdemir [9] and Kaban and Kaya [11] reported that *L. plantarum* strains constitute 2.8 to 45.7% of LAB isolates. To the best of our knowledge, no studies appeared to test the technological characteristics of sucuk originated LAB at the level tested in this study. In addition, the genetic determinants behind the antimicrobial activity of the autochthonous sucuk strains were not investigated previously, and the antimicrobial activity of these strains might be important for the food safety.

The aim of the present work was to characterize and genotypically identify sucuk originated LAB isolates and determine their technological characteristics including antimicrobial activity against food-borne pathogens in order to test their potential as a starter culture in fermented meat products.

Materials and methods

Isolates and indicator microorganisms

In this study, among the 150 LAB strains phenotypically identified previously by Kaban [20], 5 isolates were selected considering the antagonistic activities. For antagonistic activity tests, microorganisms that given in Table 1 were used.

Table 1 Indicator microorganisms used in the study

Indicator microorganisms used in study	Source
Listeria monocytogenes ATCC 3970	ATCC
Listeria monocytogenes ATCC 7644	ATCC
Staphylococcus aureus ATCC 29213	ATCC
Staphylococcus aureus ATCC 25923	ATCC
Staphylococcus aureus ATCC 25922	ATCC
Bacillus cereus ATCC 11778	ATCC
Micrococcus luteus NCIMB 8166	NCIMB

ATCC, American Type Culture Collection; *NCIMB*, National Collection of Industrial Food and Marine Bacteria

LAB strains were grown in de Man, Rogosa, and Sharpe broth (Merck, Darmstadt, Germany) at 30 °C for 24 h. *Indicator* microorganisms were grown in Tryptic Soy Broth (TSB; Oxoid, New Hampshire, UK) at 37 °C for 24 h. All microorganisms were stored at −80 °C in stock solutions (40% glycerol concentration).

Determination of the antimicrobial activity of isolates

The antagonistic activities of the isolates against indicator strains listed in Table 1 were determined according to Schillinger and Lücke's [21] well diffusion method. For this methodology, the culture supernatant was first prepared. Strains were inoculated in MRS liquid medium and incubated at 30 °C for 24 h. Centrifugation was then performed at 25 °C, 5700g for 10 min, and the supernatant was recovered. The supernatant was passed through a cellulose acetate filter having a pore diameter of 0.45 μm and used for well diffusion test. For the test, 7 ml of semisolid (tryptone soy agar) (Oxoid CM0131) medium containing 100 μL of an overnight culture of indicator microorganism was poured onto TSA plates. After solidification of the semisolid agar, wells of 5 mm were realized on the plates. Then 50 μL of the cell-free supernatant were transferred to the wells and incubated at 37 °C for 24 h. At the end of the incubation, the clear zones larger than 0.5 mm were evaluated as positive. *Lactobacillus sakei* Lb 706 (Federal Meat Research Institute, Kulmbach, Germany) strain was used as a positive control. Three microliters of proteinase K (10 mg / ml) were dropped into well containing cell-free supernatant. The absence of inhibition zone indicates the protein nature of the antimicrobial compounds [22].

Identification of LAB isolates

Genomic DNA of the isolates was obtained according to Barış [23]. The genotypic identification of the isolates was done by selecting 16S rRNA coding region sequence from genomic DNA.

For each sample to be subjected to PCR, 7 µl of 10× PCR buffer (Sigma), 1.4 µl of dNTP (deoxynucleotide triphosphates: dATP, dGTP, dCTP, dTTP, 10 mM), 0.7 µl of 50 µM of primer 27F forward (5′-AGA GTT TGA TCC TGG CTC AG-3 ′), 0.7 µl of 50 µM primer 1492R (reverse 5′-GGT TAC CTT GTT ACG ACT T-3′), 2.8 µl dimethyl sulfoxide, 4.2 µl MgCl$_2$, 0.7 µl 5 units/µl Taq DNA polymerase, and 51 µl sterile distilled water were mixed to prepare a reaction mixture of 68.5 µl. Finally, 1.5 µl of template DNA was added to the final volume of 70 µl. Reaction mixtures were subjected to 2 min of initial denaturation at 95 °C and 36 cycle denaturation at 94 °C for 1 min, followed by 1 min at 53 °C and 2 min elongation at 72 °C and finalized with the elongation at 72 °C for 5 min. The electrophoresis of PCR products was performed in gel prepared with 0.7% agarose. Electrophoresis was carried out at 100 V for 45 min. After the run, the gel was visualized with a gel documentation system and analyzed with DNR BioImaging Systems Software. PCR products were sequenced by Macrogen Company (the Netherlands). The 16S rRNA gene sequences for all of the LAB strains were arranged in BioEdit version 7.2.5 and blasted on NCBI database using BLAST program (http://blast.ncbi.nlm.nih.gov). The 16S rRNA gene sequences for LAB species are regulated in MEGAX 10.1.7. Phylogenetic tree was obtained by using neighbor join (NJ) method with 1000 bootstrap replicates [24]. Phylogenetic tree analyses were performed with MEGAX [25].

Detection of bacteriocin encoding genes

Taking into account the bacteriocins that may be produced by the LAB identified, primers have been selected for genetic detection for bacteriocin production. All isolates were PCR tested for the presence of 13 plantaricin encoding genes with the primer sets given in Table 2. PCR mixes were prepared as described above with primer sets and reactions performed at different annealing temperatures suitable for each primer sets. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gels at 100 V for 40 min. In the positive strains, appropriate amplicon sizes were detected.

The growth curve and bacteriocin-like metabolite production during growth

MRS broth was inoculated with 1% of the 24 h culture of LAB isolate and then absorbance values (Aquamate Thermo

Table 2 The oligonucleotides used for determination of the presence of the bacteriocin encoding gene (Omar et al. [26])

Plantaricin coding genes	Primer	Amplicon size (bp)	Annealing temp. (°C)
plnA	F: GTA CAG TAC TAA TGG GAG	450	53
	R: CTT ACG CCA ATC TAT ACG		
plnB	F: TTC AGA GCA AGC CTA AAT GAC	165	51.5
	R: GCC ACT GTA ACA CCA TGA C		
plnC	F: AGC AGA TGA AAT TCG GCA G	108	49.5
	R: ATA ATC CAA CGG TGC AAT CC		
plnD	F: TGA GGA CAA ACA GAC TGG AC	414	53
	R: GCA TCG GAA AAA TTG CGG ATA C		
plnEF	F: GGC ATA GTT AAA ATT CCC CCC	428	53.2
	R: CAG GTT GCC GCA AAA AAA G		
plnI	F: CTC GAC GGT GAA ATT AGG TGT AAG	450	52.5
	R: CGT TTA TCC TAT CCT CTA AGC ATT GG		
plnj	F: TAA CGA CGG ATT GCT CTG 51475	475	51
	R: AAT CAA GGA ATT ATC ACA TTA GTC		
plnk	F: CTG TAA GCA TTG CTA ACC AAT C	246	52.9
	R: ACT GCT GAC GCT GAA AAG		
plnG	F: TGC GGT TAT CAG TAT GTC AAA G	453	52.8
	R: CCT CGA AAC AAT TTC CCC C		
plnN	F: ATT GCC GGG TTA GGT ATC G	146	51.9
	R: CCT AAA CCA TGC CAT GCA C		
plnNC8	F: GGTCTGCGTATAAGCATCGC	207	60
	R:AAATTGAACATATGGGTGCTTTAAATTCC		
plnS	F:GCCTTACCAGCGTAATGCCC	320	60
	R:CTGGTGATGCAATCGTTAGTTT		
plnW	F:TCACACGAAATATTCCA	165	55
	R:GGCAAGCGTAAGAAATAAATGAG		

electron corporation, England) at 600 nm were measured in 2 h intervals at 30 °C for 24 h of incubation. Growth curves were formed with obtained optical density (OD) values. For determining the relation between growth and antimicrobial metabolite production, antimicrobial effects against *L. monocytogenes* ATCC 7644 of the samples taken at different times during the 24 h incubation period were examined. Antimicrobial activity of the cell-free supernatant was determined according to Biswas et al. [27]. Activity was calculated as AU/ml (Arbitrary unit/ml) (AU/ml = Dilution Factor * 1 ml/50 μl). Antimicrobial activities of samples were compared with growth curve analysis [28].

Biochemical and technological characterization of isolates

Isolates were characterized by the following biochemical tests: growth at pH 4.5, 5, 5.5, 6, or 6.5 in MRS broth, tolerance to NaCl by growth in NaCl containing MRS broth at concentrations of 6.5 and 10%; growth at different temperatures at 4 °C for 7 days, 15 °C for 3 days, 25 °C for 2 days, and 45 °C for 3 days in MRS broth [29]; production of acetoin from glucose, lypolytic activity in trybutyrin agar, gelatinase activity in gelatinase agar, and calcium hydrolyze activity in caseinate agar were determined according to Harrigan [29]. To determine whether isolates form acetoin from glucose, 5 ml methyl-red Voges-Proskauer (Merck, Darmstadt, Germany) broth was used. MR-VP broth was inoculated with 24-h cell culture and left for 48 h incubation at 30 °C. At the end of the incubation, 1 ml was taken from the tubes and transferred to a sterile tube and 0.2 ml of 40% KOH solution and 0.6 ml of alpha-naphthol were added. After 15 min, the presence of colored ring changing from pink to bright red on the liquid surface was evaluated as a positive result. Trybutyrin agar (Merck, Darmstadt, Germany) was used to determine lipolytic activity of isolates. Twenty-four-hour culture was transferred to wells drilled in agar plates. After the incubation at 30 °C for 6 days, clear zones were evaluated as positive results. Gelatin agar (Merck, Darmstadt, Germany) and calcium caseinate agar (Merck, Darmstadt, Germany) were used to determine proteolytic activity. The medium was inoculated with 24-h culture, and the presence of clear zone was evaluated as positive after 48-h incubation at 30 °C. For nitrate reductase activity, 5 ml nitrate broth (Sigma-Aldrich 72548) medium were

inoculated with isolates, and after 24-h incubation at 30 °C, red color conversion was observed in the presence of N, N-dimethyl-1-naphtylamine as positive result [30]. Deamination of arginine, ornithine, and lysine were determined as follows:

Arginine, ornithine, and lysine amino acids were added to the Miller broth at a rate of 1%. The prepared media was filled into tubes of 3 ml each, and liquid paraffin was added to the tubes to ensure anaerobiosis [29]. Lactic acid configuration and quantities were detected enzymatically with D-lactic acid/L-lactic acid kit (Cat. No. 11112821035, Boehringer Mannheim/R-Biopharma, R-Biopharma GmbH, Darmstadt, Germany).

Results and discussion

In total, 150 LAB strains previously isolated from sucuk [20] were tested for antagonistic activity (Data not shown). According to antagonistic activity test, 5 strains (S50, S51, S72, S74, and S85) were selected for further technological and genotypic characterization. Comparison of 16S rRNA regions showed that S50, S51, S72, S74, and S85 are *L. plantarum* strains with 99% similarities. The accession numbers of LAB strains are given in Table 3.

Figure 1 represents the phylogenetic relationship between MEGA alignments of 16S rRNA genes of isolates. In phylogenetic analysis, 3 subgroups were formed among *L. plantarum* strains. The first group consisted of S51, S72 and S74, second group S50 and third group S85, respectively.

In fermented sausages such as sucuk, salami, and Rohwurst, LAB are important microorganisms for the inhibition of food pathogens such as *S. aureus* and *L. monocytogenes* [21, 33, 34]. As seen in Table 4, all of the strains showed antagonistic activity in the well diffusion test against seven indicator bacteria. Similar to our results, in previous studies, *L. plantarum* strains isolated from fermented sausages showed antimicrobial effects on several foodborne pathogens [17, 35–38]. Importantly using proteinase K during antimicrobial tests resulted in the loss of inhibition zones showing that the antimicrobial substances produced by the strains are of peptidic nature. Because of the protein-based antimicrobial activity, the presence of genes responsible for bacteriocin production in genomic DNA was investigated. Five *L. plantarum* strains were PCR screened for the 13

Table 3 Accession numbers of lactic acid bacteria isolates

Isolate number	Accession number	Isolates	Similarity (%)
S50	KR011000	*Lactobacillus plantarum* S50	99
S51	KR011001	*Lactobacillus plantarum* S51	99
S72	KR011010	*Lactobacillus plantarum* S72	99
S74	KR011011	*Lactobacillus plantarum* S74	99
S85	KR011017	*Lactobacillus plantarum* S85	99

Fig. 1 A phylogenetic tree was constructed with the MEGA version 10.1.7 program using 16S rRNA gene sequences. Phylogenetic tree was inferred by Neighbor-Joining method [24]. Phylogenetic distances were calculated based on 1313 nt of 16S rRNA gene. The optimal tree with the sum of branch length = 0.00489278 is shown. The robustness of the NJ tree was tested by bootstrapping with 1000 replicates of data, and percentages are shown next to the branches (only values above 50% are reported) [31]. The evolutionary distances were computed using the p-distance method [32]
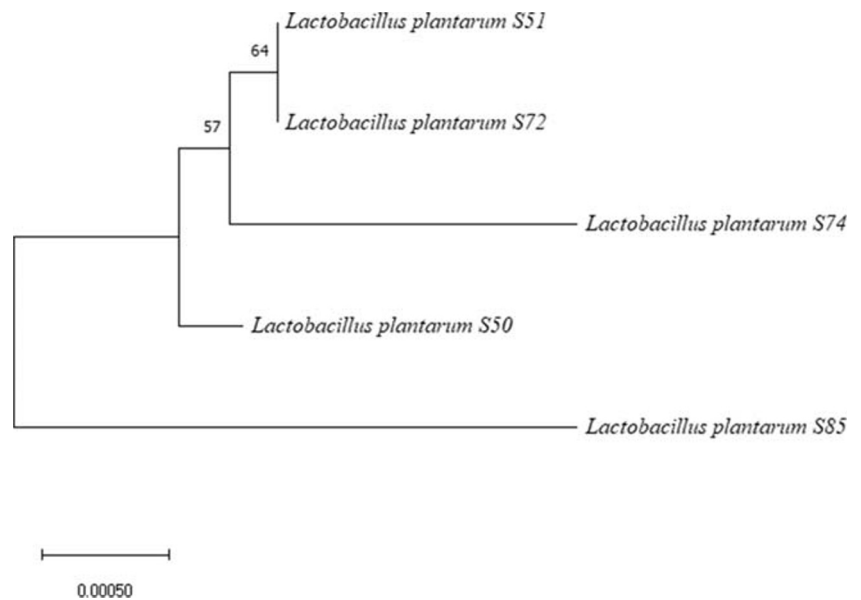
plantaricin genes (Table 5). Bacteriocins that produced from *L. plantarum* are known as plantaricins. Generally, many numbers of genetic determinants for plantaricin production are located in gene clusters. Among the studied 13 plantaricin genes, *pln*NC8, *pln*S, and *pln*W are structural genes, while others are plantaricin operons [26]. As a result of PCR products electrophoresis, amplicons were obtained with seven primer sets for *pln*B, *pln*C, *pln*D, *pln*I, *pln*J, *pln*K, and *pln*N genes. All these amplicons showed expected lengths given in Table 2. The obtained amplifications indicate the presence of genes responsible for bacteriocin production [39]. These results were suggesting that protein-based antimicrobial activity of strains is due to plantaricin.

The growth curves for *L. plantarum* S50, S51, S72, S74, and S85 reveal that the first 4 h of lag phase for all strains were evident (Fig. 2). Antimicrobial activity during 24-h incubation was detected against *L. monocytogenes* ATCC 7644. Antimicrobial activity was started at the log phase for all

strains and reached the maximum antimicrobial level at the stationary phase. Increasing cell numbers and critical nutrient level in the medium were thought to increase antimicrobial metabolite production. Similar results were given by Diep et al. [40]. Differences between strains were observed in terms of bacteriocin activity. The antimicrobial activities of *L. plantarum* S50 and S51 were found to be higher than those of S72, S74, and S85 strains. Antimicrobial activity showed similar changes in growth rate. We can say that the bacteriocin-like antimicrobial substance has the primary metabolite kinetic property like most of the plantaricins. Similar results were described in the previous study [41]. *L. plantarum* strains showed similar decreases in pH value after 24 h of incubation. The antimicrobial effect for all strains was started at pH 4.5–5.0, and the maximum antimicrobial activity was observed at pH 3.95–4.50. Similar results have been reported for the other bacteriocin producer *L. plantarum* strains [42–44].

The characteristics of *L. plantarum* strains were studied, and results are presented in Table 6; *L. plantarum* S50, S51, S72, S74, and S85 have optimum growth at 25 °C. Strains that grow at a temperature of 15 °C showed a poor growth at 45 °C. Schillinger and Lücke [45] reported that *L. plantarum* strains isolated from meat and meat products can grow at 45 °C. In accordance with sausage production conditions, *L. plantarum* is the usually dominant strain [11, 12]; while strains showed the best growth at pH 6 and 6.5, they showed medium level growth at low pH values (pH 4.5 and 5) and good growth at pH 5.5. These results suggested that the pH decreases to around 5 during fermentation, affecting the LAB growth otherwise. All of the strains grew at 6.5% salt concentration, whereas no growth was detected at 10% salt concentration. NaCl is an important additive for fermented sausages. It is used in 2–3% concentration for sausage batter [46]. The

Table 4 Antimicrobial activity of *L. plantarum* strains against selected foodborne pathogens

Indicator microorganisms	S50	S51	S72	S74	S85
	Inhibition zone (mm)				
S. aureus ATCC29213	+	+	+	+	+
S. aureus ATCC25923	+	+	++	+	+++
S. aureus ATCC25922	++	++	++	++	++
L. monocytogenes ATCC 3970	+	+	++	+	++
L. monocytogenes ATCC 7644	+++	++	+++	+++	++
Bacillus cereus ATCC 11778	+	+	++	+	+
Micrococcus luteus NCIMB 8166	+	+	+++	+	+

+, inhibition zone < 2 mm; ++, inhibition zone 3–4 mm; +++, inhibition zone >4 mm

Table 5 Detection of the presence of genes responsible for plantaricin production

Isolates	plnA	plnB	plnC	plnD	plnEF	plnI	plnJ	plnK	plnG	plnN	plnNC8	plnS	plnW
S50	−	+	+	+	−	+	+	+	−	+	−	−	−
S51	−	+	+	+	−	+	+	+	−	+	−	−	−
S72	−	+	+	+	−	+	+	+	−	+	−	−	−
S74	−	+	+	+	−	+	+	+	−	+	−	−	−
S85	−	+	+	+	−	+	+	+	−	+	−	−	−

Results reveal the PCR detection of the related gene; + and − represent the presence and absence of the corresponding gene

NaCl added to fermented sausages reduces the initial a_w value and encourages the growth of *Micrococcaceae* family and LAB, which are technologically important groups [47, 48]. In fermented meat products, especially in formulations using nitrate, nitrate reductase activity of microorganisms is necessary for the development and stability of red color. This activity is provided especially by the members of *Micrococcaceae* family such as *Staphylococcus xylosus*, *S. carnosus*, and *Kocuria varians* strains in these products. These catalase-positive strains are often found as mixed cultures with LAB [49]. On the other side, there are also lactic acid bacteria which show nitrate reductase activities. Paik and
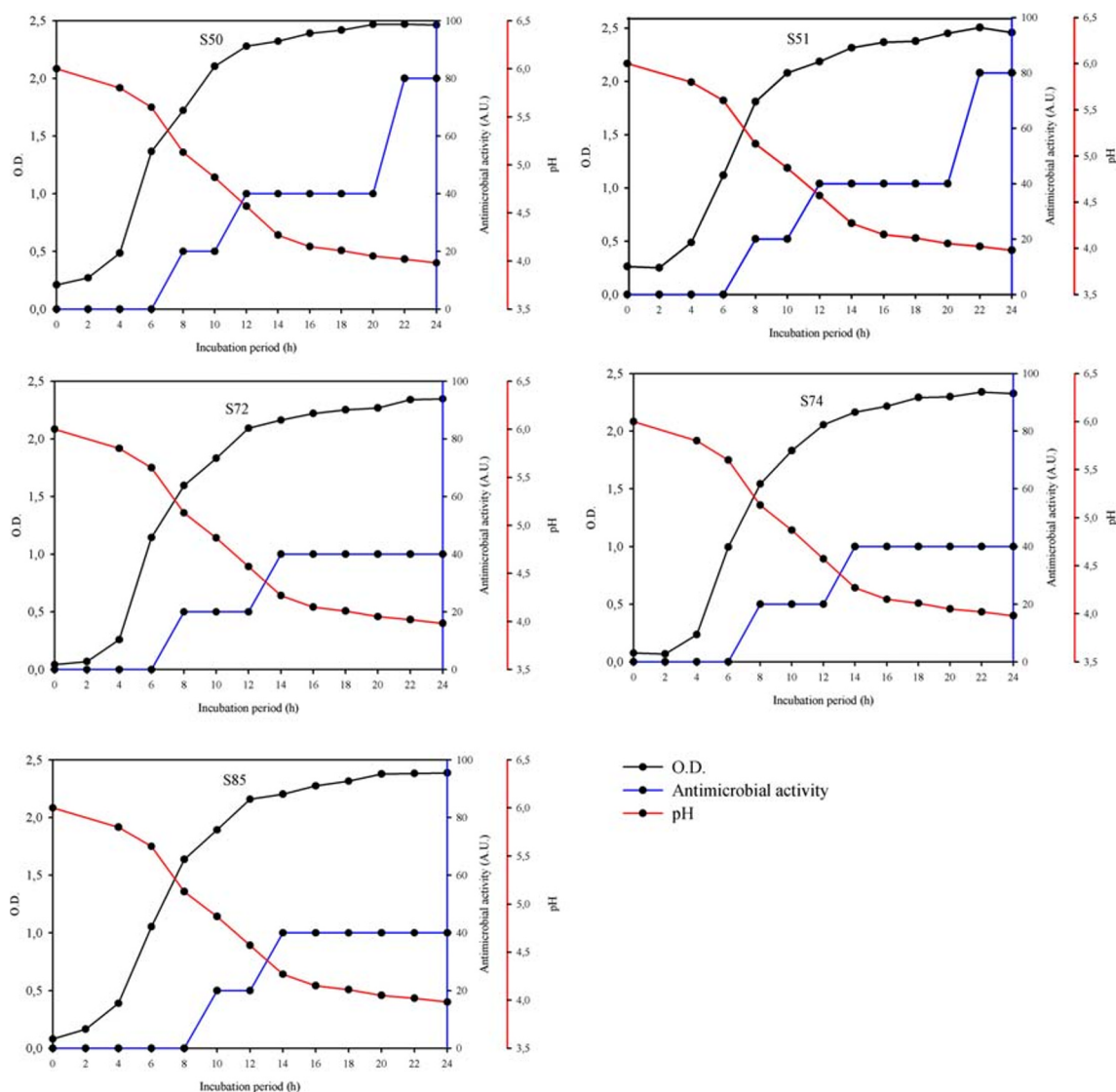
Fig. 2 Antimicrobial activity and pH of strains throughout the growth

Table 6 Technological and biochemical properties of *L. plantarum* strains

Technological and biochemical properties	S50	S51	S72	S74	S85
4 °C	–	–	–	*	–
15 °C	++	++	++	+	++
25 °C	+++	+++	+++	+++	+++
45 °C	+	+	+	*	+
pH 4.5	+	+	+	+	+
pH 5.0	+	+	+	+	+
pH 5.5	++	++	++	++	++
pH 6.0	+++	+++	+++	+++	+++
pH 6.5	+++	+++	+++	+++	+++
6.5% NaCl	++	++	++	++	++
10% NaCl	–	–	–	–	–
Nitrate reductase	–	–	+	–	+
Lysine decarboxylase	–	–	–	–	–
Ornithine decarboxylase	–	–	–	–	–
Arginine decarboxylase	–	–	–	–	–
Casein hydrolysis	–	–	–	–	–
Gelatin hydrolysis	–	–	–	–	–
Tributyrin hydrolysis	–	–	–	–	–
Acetoin production from glucose	+	–	+	+	+
D-Lactic Acid (g/L)	10. 54	6.47	5.70	6.73	8.97
L-Lactic Acid (g/L)	5.98	8.27	6.01	3.46	4.52

− not detected, * weak, + medium, ++ good, +++ very good

Lee [50] reported that *L. plantarum* strains isolated from kimchi show weak nitrate reductase activity. In this study, a weak nitrate reductase activity was also observed in two *L. plantarum* strains (S72, S85). According to phylogenetic tree (Fig. 1), S51, S72 and S74 were quite close in subgroups. Despite the closeness of strains, nitrate reductase activities of S72 and S85 indicated phenotypical differences of strains.

Biogenic amines are undesirable compounds in food. They may have toxic effect on human. Generally, biogenic amines are formed as a result of decarboxylation of amino acids by microbial enzymes. Lactobacilli can form different biogenic amines by decarboxylase activity depending on the species [50]. As a matter of fact, it has been determined that some strains of *Lactobacilli* produce biogenic amines [51, 52]. As can be seen in Table 6, all of the strains were negative for lysine, ornithine, and arginine decarboxylase activity. No strain hydrolyzed tributyrin, gelatin, and casein. These results showed that the tested strains did not show proteolytic and lipolytic activity. It has also been reported that *L. plantarum* strains do not have lipolytic activity [17, 53–56] and proteolytic activities [54].

L. plantarum, *Pediococcus pentosaceus*, and *P. acidilactici* are LAB used as starter cultures that can form acetoin. *L. sakei* is rare, while *L. curvatus* is very rare producer for this compound [47]. In the present study, except for *L. plantarum* S51, the others were positive in the Voges-Proskauer test. The formation of acetic acid or acetoin in very high amounts in fermented meat products is not desirable [48].

The stereochemistry of lactic acid produced by LAB is an important feature. Lactic acid isomer produced during fermentation varies according to the species. The ratio of D-lactic acid and L-lactic acid varies in the racemic mixture they produce based on lactate dehydrogenase (LDH) activities of LAB. The racemic mixture is not always uniformly formed. Since D (−)-lactic acid cannot be hydrolyzed by lactate dehydrogenase in humans, L (+)-lactic acid producing strains are preferred as starter cultures [56]. We determined that all *L. plantarum* strains tested produced DL-lactic acid. In two strains, L (+)-lactic acid production was more than 50% (*L. plantarum* S51, S72). *L. plantarum* strains differed from each other with their technological characteristics.

Conclusion

In conclusion, some technological properties of *L. plantarum* strains isolated from traditionally fermented sausage were determined. All strains showed good antagonistic activity against *Listeria monocytogenes*. The amplification of genes responsible for the production of plantaricin (*pln*B, *pln*C, *pln*D, *pln*I, *pln*J, *pln*K, and *pln*N) by PCR analysis supported this result. According to our findings, these strains may be

potential protective cultures. When considered in terms of biochemical and technological properties, *L. plantarum* strains exhibited some of the properties required for a starter culture in fermented sausage and similar products. The *L. plantarum* strain S51 did not produce acetoin from glucose, which is a major drawback for this strain. Montel et al. [57] showed that acetoin producer starter cultures can cause undesirable dairy-like product odor in dry sausages. In terms of the importance of acetoin level on product flavor, it is important to investigate the effect of aroma formation in model systems for other strains in detail. Further work will characterize the role of the identified *L. plantarum* strains as starter cultures in fermented meat products as well as their probiotic and functional properties will be investigated.

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