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Isolation and identification of lactic acid bacteria from fermented meat products and evaluation of their antimicrobial effect

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Abstract: In this study, 30 lactic acid bacteria (LAB) were isolated from 20 different fermented meat products obtained from the Afyonkarahisar province (Turkey). Molecular identification of these isolates was performed by amplification of the 16S rDNA gene using the polymerase chain reaction (PCR) method. LAB isolated from 'sucuk' (spicy and fermented sausage) samples were identified as *Enterococcus faecalis* (2 isolates), *Enterococcus durans* (1 isolate), *Lactobacillus sakei* (3 isolates), *Lactobacillus curvatus* (2 isolates), *Weissella viridescens* (3 isolates), *Weissella cibaria* (2 isolates) and *Weissella hellenica* (1 isolate). LAB, isolated from salami samples, were identified as *W. viridescens* (1 isolate), *E. durans* (3 isolates), *Leuconostoc mesenteroides* (4 isolates), *Carnobacterium maltaromaticum* (1 isolate), *Macrococcus caseolyticus* (1 isolate). Also, LAB, isolated from sausages samples, were identified as *E. faecalis* (1 isolate), *E. durans* (4 isolates), *Lactobacillus plantarum* (1 isolate). Both agar spot and agar well diffusion assay methods were used to determine the antimicrobial activity of the LAB isolates. Isolates of LAB showed higher antimicrobial activity against *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* NRRL B 4420, *Pseudomonas aeruginosa* ATCC 11778, *Streptococcus faecalis* NRRL B 14617 than against *Escherichia coli* ATCC 35218 and *Bacillus subtilis* NRS 744. Results showed that the LAB isolates produced antimicrobial substances that have a potential for different industrial and biotechnological uses.

Keywords: antimicrobial activity; bacteriocin; fermented meat products; identification; isolation; lactic acid bacteria

Foodborne illnesses are a major international problem and an important cause of reduced economic growth. The contamination of the food supply with the pathogenic microorganisms and its persistence, growth, multiplication and/or toxin production have emerged as an important public health concern (Paiva de Sousa 2008). Low temperature or heat treatment, packaging methods, preservative chemical additives and salt addition are used in food preservation. Demand for health-safe and quality food products of natural origin and modern technologies has increased in recent years (Dinçer et al. 2010; Sehrawat et al. 2019).

Bacteriocins are ribosomally synthesised peptide toxins elaborated by bacteria and designed to kill ot-

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her bacteria specifically. Toxic peptides are one of the inherent defence system weapons of bacteria that kill other narrow-spectrum or broad-spectrum microorganisms (Cotter et al. 2005; Yang et al. 2014). Recent studies have shown that various bacteria produce bacteriocins (López-Cuellar et al. 2016) especially lactic acid bacteria (LAB) are one of the most studied bacteria (Moshood and Tengku 2013; Masalam et al. 2018). LAB have many antimicrobial activities in fermented foods and produce a wide variety of antimicrobial substances. Most LAB are generally recognised as safe (GRAS) organisms and do not pose any health risks for people. Bacteriocins of LAB have been used in food preservation and other various applications (Silva et al. 2018). Although antibiotics are used as antimicrobial substances against bacteria, antibiotic treatments have some problems such as antibiotics resistance in pathogens, scarcity of new families of antibiotics to replace the ineffective existing antibiotics (Blaser 2011; White 2011). For this reason, bacteriocins and bacteriocin-like substances may become potential drug candidates for replacing antibiotics (Yang et al. 2014; Newstead et al. 2020). The aim of this study is to isolate and identify LAB from different traditional fermented meat products (fermented sausage 'sucuk', salami, sausage) in the Afyonkarahisar province (Turkey) and evaluate the antimicrobial effect of these isolates.

MATERIAL AND METHODS

Isolation and purification of LAB. In this study, 20 different fermented off-brand meat products (fermented sausage 'sucuk', sausage, salami), preferably produced by natural methods, were obtained from the Afyonkarahisar province (Turkey). In order to isolate LAB from the fermented meat samples, 10 g of each meat sample was homogenised into the flasks that contain 90 mL of physiological solution (0.85% NaCl, 0.1% peptone). A sequential 1/10 dilution solution was prepared for each sample under aseptic conditions. From every sample, diluted cell suspensions 10^{-4} (0.1 mL) and 10^{-5} (0.1 mL) were inoculated on cultivation agars: M17 agar (Merck, Germany) (pH 7.2 ± 0.2), Man Rogosa and Sharpe agar (MRS) agar (Oxoid, United Kingdom) (pH 5.7 ± 0.2), Nutrient agar (NA) (Merck, Germany) (pH 7.0 ± 0.2) and Kanamycin aesculin azide agar (Merck, Germany) (pH 7.0 ± 0.2). The reason was the isolation bacteria of the genera *Lactococcus, Lactobacillus* and *Enterococcus*. They were incubated at 37 °C for 48 h in both aerobic and anaerobic conditions (incubator; Thermo Fisher Scientific, USA). After incubations, colonies with the following morphological features were selected: cream-coloured, matte smooth-edged colonies were selected as lactobacilli, bright colonies with white smooth edges were selected as lactococci, and small, white or pale-coloured colonies with smooth edges were selected as enterococci (Azadnia and Khan-Nazer 2009). And also, black zone forming colonies on the Kanamycin Aesculin Azide agar were selected as enterococci. Selected isolates were passaged to MRS agar, and pure isolates were obtained. Microscopic appearance, Gram staining and catalase activity of these isolates were analysed (Başbülbül et al. 2015).

Genomic DNA isolation from LAB. The high Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Life Scienses, United Kingdom) was used in order to isolate genomic DNA from LAB. Purity and amount of DNA have been determined spectrophotometrically by use of the Thermo Scientific-Nanodrop 2000c device (Thermo Fisher, USA). The isolated DNA was standardised to the final concentrations from 20 ng at 50 µL.

Identification of LAB by the 16S rDNA PCR method. The Taq DNA Polymerase Kit (Sigma-Aldrich, USA) was used for the identification of the LAB isolates by use of the PCR method. During the PCR reaction, the 16S rDNA gene region was amplified in the PCR Thermal Cycler (Blue Ray Biotech, Taiwan) using the 20F primer (5'-AGA GTT TGA TCC TGG CTC AG-3') and the 1390R primer (5'-GAC GGG CGG TGT GTA CAA-3'). The Fermentas Gene Ruler TM marker (Germany) was used for base size determination. The PCR conditions for amplification of desired gene region have been programmed as follows. Pre-denaturation at 94 °C for 5 min, followed by the 35-cycle amplification (denaturation at 94 °C for 30 s, binding at 55 °C for 30 s, elongation at 72 °C for 1 min 30 s) with the final elongation at 72 °C for 15 min. The final PCR product was visualised on a 1.0% agarose gel (Merck, Germany) by using the Quantum Gel Visualization System (Vilber Lourmat, France). Sequence analysis, based on Sanger dideoxy sequence method, was performed by the ABI3730XL automatic sequence analyser (Altıgen Bio Company, Turkey).

Determination of antibacterial effect of LAB. Antibacterial activity of LAB isolates was determined by the agar spot test and by the agar well diffusion test. *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* NRRL B 4420, *Pseudomonas aeruginosa* ATCC 11778, *Streptococcus faecalis* NRRL B 14617, *Escherichia coli* ATCC 35218

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and *Bacillus subtilis* NRS 744 were used as test microorganisms. Single colonies of each LAB isolates were inoculated into MRS broth (Oxoid, United Kingdom) and incubated at 37 °C, for 24 h, under aerobic/anaerobic conditions. Fresh cell suspensions of individual isolates were inoculated (2 µL) on MRS agar by smear technique with subsequent incubation of Petri plates at 37 °C, for 24 h, under aerobic/anaerobic conditions. The indicator strains were cultivated on Nutrient broth (NB) agar (Merck, Germany) at 37 °C, for 24 h, under aerobic/anaerobic conditions. After that, cell suspensions of indicator strains (500 µL) were inoculated (5 mL) into soft agar (0.5% agar) in Petri plates and at 37 °C, for 24 h, under aerobic/anaerobic conditions (Schillinger and Luke 1989). The diameters of created inhibition zones were measured, and arithmetic means were calculated. The antimicrobial agent 10 µg amikacin (Merck, Germany) was used as a positive control. Also, each isolate of LAB was inoculated into MRS broth (10 mL), then cultivated at 37 °C, for 24 h, under aerobic/anaerobic conditions, for the agar well diffusion test. Then cell suspensions of LAB isolates were centrifuged at 8 000 rpm for 10 min (centrifuge; Nuve, Turkey). Supernatants were transferred to sterile Falcon tubes (Isolab, Turkey) and adjusted to pH 6.0 (by 1 M NaOH/HCl solutions) and filtered through a membrane filter (0.2 µm; Sigma-Aldrich, USA). By a sterile cork borer (with a diameter of 6 mm; Sigma-Aldrich, USA), wells into agar in Petri plates were done. Single colonies of each test strain were inoculated in NB medium and cultivated at 37 °C, for 24 h, under aerobic/anaerobic conditions. From these fresh cultures, cell suspensions (0.5 McFarland) were prepared by diluting with sterile distilled water. Each indicator strain was planted into NB agar using sterile cotton swabs. Into cut wells, cell-free supernatants (100 µL) of individual LAB isolates were added. After plates were incubated at 37 °C for 24 h, under aerobic/anaerobic conditions, created inhibition zones were measured (in diameters) and compared with the control group.

RESULTS AND DISCUSSION

In this study, 58 pure bacterial cultures were isolated from 20 different fermented meat products, which were purchased in the Afyonkarahisar province (Turkey). Thirty isolates characterised as Gram-positive and catalase-negative have been subjected to identification using the PCR method. The final PCR products, obtained after amplification of the 16S rRNA gene, generated about 1.5 kb fragments (Figure 1). These isolates were able to grow and replicate under aerobic and ana-

Figure 1. Electrophoresis gel with the PCR final products in connection to tested LAB isolates

LAB – lactic acid bacteria; PCR – polymerase chain reaction; SC – 'sucuk'; SL – salami; SS – sausage

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erobic conditions easily. Nucleotide sequence analysis results of PCR products were compared using the Nucleotide-nucleotide BLAST program at the Gen Bank, and homologies in the gene bank were determined for each isolate. The sequences of the LAB isolates were deposited to the NCBI Genbank, and the Genbank Accesssion numbers are shown in Table 1. According to the results of the 16S rDNA gene sequencing analysis of the isolates, the LAB isolates of 'sucuk' samples were identified as *E. faecalis* (2 isolates), *E. durans* (1 isolate), *L. sakei* (3 isolates), *L. curvatus* (2 isolates), *W. viridescens* (3 isolates), *W. cibaria* (2 isolates) and *W. hellenica* (1 isolate). LAB isolates of salami samples were identified as *W. viridescens* (1 isolate), *E. durans* (3 isolates), *L. mesenteroides* (4 isolates), *C. maltaromaticum* (1 isolate) and *M. caseolyticus* (1 isolate). Also, the LAB isolates of sausages samples were identified as *E. faecalis* (1 isolate), *E. durans* (4 isolates) and *L. plantarum* (1 isolate).

The results of our study show similarity to some results of previous studies (Aymerich et al. 2003; Kaihei et al. 2011; Samuel et al. 2011). Due to the work of Aymerich et al. (2003), 6 species of LAB and 6 species of Gram-positive and catalase-positive cocci obtained

Table 1. Results from the 16S rRNA PCR analysis of LAB isolates

Isolate of LAB (Genbank Accession No.)	Bacterial species (16S rRNA PCR analysis)	Identity (%)
SC1 (MT126411)	*Enterococcus faecalis,* strain FC1377	99.12% (NR_113901.1)
SC2 (MT129133)	*Weissella viridescens,* strain SF4	99.90% (NR_040813.1)
SC3 (MT129143)	*Weissella viridescens,* strain SF5	99.89% (NR_040813.1)
SC4 (MT128721)	*Weissella viridescens,* strain SF2	99.81% (NR_040813.1)
SC5 (MT129501)	*Enterococcus durans,* strain SF6	99.39% (NR_113257.1)
SC6 (MT129504)	*Lactobacillus sakei,* strain SF7	99.90% (NR_113821.1)
SC7 (MT129527)	*Lactobacillus sakei,* strain SF8	100.00% (NR_113821.1)
SC8 (MT129528)	*Lactobacillus curvatus,* strain SF9	99.56% (NR_113334.1)
SC9 (MT129530)	*Lactobacillus curvatus,* strain SF10	99.73% (NR_113334.1)
SC10 (MT129532)	*Weissella cibaria,* strain SF11	99.55% (NR_036924.1)
SC11 (MT129659)	*Weissella cibaria,* strain SF12	98.87% (NR_036924.1)
SC12 (MT129675)	*Lactobacillus sakei,* strain SF13	99.82% (NR_113821.1)
SC13 (MT128719)	*Weissella hellenica,* strain SF1	99.91% (NR_113775.1)
SC14 (MT129794)	*Enterococcus faecalis,* strain SF14	99.90% (NR_113901.1)
SL1 (MT130205)	*Weissella viridescens,* strain SF15	99.91% (NR_040813.1)
SL2 (MT130398)	*Enterococcus durans,* strain SF16	99.09% (NR_113257.1)
SL3 (MT130500)	*Enterococcus durans,* strain SF21	99.33% (NR_040813.1)
SL4 (MT130429)	*Leuconostoc mesenteroides,* strain SF18	98.07% (MK680145.1)
SL5 (MT130466)	*Enterococcus durans,* strain SF19	98.74% (MF424830.1)
SL6 (MT130501)	*Leuconostoc mesenteroides,* strain SF20	99.49% (NR_074957.1)
SL7 (MT130713)	*Leuconostoc mesenteroides,* strain SF22	99.64% (NR_113901.1)
SL8 (MT132331)	*Carnobacterium maltaromaticum,* strain SF23	97.17% (NR_044710.2)
SL9 (MT132365)	*Macrococcus caseolyticus,* strain SF24	99.10% (NR_119262.1)
SL10 (MT135179)	*Leuconostoc mesenteroides,* strain SF24	99.82% (NR_074957.1)
SS1 (MT135186)	*Enterococcus faecalis,* strain SF25	99.39% (NR_113257.1)
SS2 (MT135189)	*Enterococcus durans,* strain SF26	99.49% (NR_113257.1)
SS3 (MT135201)	*Enterococcus durans,* strain SF27	99.78% (NR_113257.1)
SS4 (MT135202)	*Lactobacillus plantarum,* strain SF29	99.27% (NR_104573.1)
SS5 (MT135520)	*Enterococcus durans,* strain SF30	99.78% (NR_113257.1)
SS6 (MT135518)	*Enterococcus durans,* strain SF31	98.90% (MF424830.1)

LAB – lactic acid bacteria; PCR – polymerase chain reaction; SC – 'sucuk'; SL – salami; SS – sausage

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from low-acid fermented sausages were assessed using the PCR method. In tested samples, *L. sakei* and *L. curvatus* were detected out of 100% *E. faecium* was detected out of 11.8% in the sausages. In another study Kaihei et al. (2011) isolated LAB from local meat products and identified them as *E. durans, E. faecalis, E. faecium, E. hirae, L. citreum, L. mesenteroides*, *Pediococcus pentosaceus* and *W. cibaria.* In the study of Samuel et al. (2011), 91 LAB strains were isolated from 50 beef samples and 10 different LAB species of LAB were identifi-

Table 2. Inhibition effect of LAB isolates on the pathogenic test bacteria using the agar spot test

Isolate of LAB	*Listeria monocytogenes* ATCC 19115	*Escherichia coli* ATCC 35218	*Bacillus subtilis* NRS-744	*Staphylococcus aureus* ATCC 25923	*Klebsiella pneumoniae* NRRL B 4420	*Pseudomonas aeruginosa* ATCC 11778	*Streptococcus faecalis* NRRL B 14617
SC1	+++	++	+	++	+++	+++	++
SC2	+++	++	+	+++	+++	+++	++
SC3	+++	+	++	+++	+++	+++	+++
SC4	+++	+	++	+++	+++	+++	+++
SC5	+++	++	+	+++	+++	+++	+++
SC6	+++	+	++	+++	+++	+++	+++
SC7	+++	+	++	+++	+++	+++	+++
SC8	+++	++	++	+++	+++	+++	+++
SC9	+++	++	++	+++	+++	+++	+++
SC10	+++	++	++	+++	+++	+++	+++
SC11	+++	++	++	+++	+++	+++	+++
SC12	+++	++	++	+++	+++	+++	+++
SC13	+++	+++	+++	+++	+++	+++	+++
SC14	+++	++	++	+++	+++	+++	+++
SL1	+++	++	++	+++	+++	+++	+++
SL2	+++	++	+++	+++	+++	+++	+++
SL3	+++	++	++	+++	+++	+++	+++
SL4	+++	++	++	+++	+++	+++	+++
SL5	+++	+++	++	+++	+++	+++	++
SL6	+++	+	++	+++	+++	+++	+++
SL7	+++	+	+++	+++	+++	+++	+++
SL8	+++	++	+++	+++	+++	+++	+++
SL9	+++	++	++	+++	+++	+++	+++
SL10	+++	+	+++	+++	+++	+++	+++
SS1	+++	++	++	+++	+++	+++	+++
SS2	+++	++	+++	+++	+++	+++	+++
SS3	+++	++	++	+++	+++	+++	+++
SS4	+++	++	++	+++	+++	+++	+++
SS5	+++	++	+++	+++	+++	+++	+++
SS6	+++	+++	+++	+++	+++	+++	++
Positive control (10 µg amikacin)	+++	+++	+++	+++	+++	+++	+++

+ – diameter of inhibition zone < 5 mm; ++ – diameter of inhibition zone 5–10 mm; +++ – diameter of inhibition zone 15–2020 mm; LAB – lactic acid bacteria; SC – 'sucuk'; SL – salami; SS – sausage

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ed as: *Enterococcus gilvus*, *E. faecium*, *E. casseliflavus*, *E. faecalis*, *E. malodoratus*, *E. devriessei*, *L. sakei*, *C. divergens*, *C. maltaromaticum* and *L. mesenteroides*. In the work of Çetin and Tuncer (2016) was written that *M. caseolyticus* strains were isolated from the 'sucuk' samples, which were produced without using a starter culture. Yüceer and Tuncer (2015) isolated LAB from 'sucuk' and determined their antibiotic resistance. Isolates of LAB were identified as *P. acidilactici*, *E. faecium*, *L. sakei* ssp. *carnosus*, *L. sakei* ssp. *sakei*, *P. pentosaceus*,

Table 3. Antimicrobial activity of LAB isolates on pathogenic test bacteria using the agar well diffusion test (mean ± SD; n = 3)

Isolate of LAB	*Listeria monocytogenes* ATCC 19115	*Escherichia coli* ATCC 35218	*Bacillus subtilis* NRS-744	*Staphylococcus aureus* ATCC 25923	*Klebsiella pneumoniae* NRRL B 4420	*Pseudomonas aeruginosa* ATCC 11778	*Streptococcus faecalis* NRRL B 14617
SC1	15 ± 0.8	8 ± 0.3	10 ± 0.4	11 ± 0.9	24 ± 1.5	18 ± 1.4	16 ± 0.9
SC2	20 ± 1.2	7 ± 0.4	11 ± 0.6	12 ± 0.8	20 ± 1.4	19 ± 1.4	19 ± 1.2
SC3	20 ± 1.2	8 ± 0.3	12 ± 0.9	13 ± 1.1	21 ± 1.5	18 ± 1.4	20 ± 1.2
SC4	22 ± 1.3	8 ± 0.3	11 ± 0.4	13 ± 1.0	21 ± 1.5	18 ± 1.4	19 ± 1.2
SC5	17 ± 0.9	12 ± 0.9	8 ± 0.3	13 ± 1.1	18 ± 1.3	21 ± 1.6	18 ± 0.9
SC6	20 ± 1.5	8 ± 0.4	8 ± 0.3	16 ± 1.2	18 ± 1.3	16 ± 0.9	20 ± 1.1
SC7	19 ± 1.5	9 ± 0.7	9 ± 0.3	17 ± 1.1	18 ± 1.3	16 ± 1.0	20 ± 0.9
SC8	20 ± 1.2	11 ± 0.9	9 ± 0.4	12 ± 0.9	20 ± 1.2	23 ± 1.2	13 ± 0.9
SC9	17 ± 0.8	11 ± 0.9	10 ± 0.5	13 ± 0.9	18 ± 1.5	24 ± 1.7	14 ± 1.1
SC10	16 ± 0.8	9 ± 0.5	12 ± 0.5	14 ± 0.9	20 ± 1.5	24 ± 1.3	14 ± 1.1
SC11	16 ± 0.7	9 ± 0.5	11 ± 0.5	15 ± 1.1	21 ± 1.1	23 ± 1.2	15 ± 1.2
SC12	19 ± 0.9	8 ± 0.4	8 ± 0.3	17 ± 1.1	18 ± 1.2	17 ± 1.1	20 ± 1.5
SC13	18 ± 1.0	15 ± 0.5	15 ± 1.2	12 ± 0.8	17 ± 1.3	28 ± 1.7	17 ± 1.2
SC14	20 ± 1.3	8 ± 0.3	8 ± 0.3	13 ± 0.7	21 ± 1.4	19 ± 1.4	19 ± 1.4
SL1	20 ± 1.3	9 ± 0.4	9 ± 0.3	14 ± 0.7	20 ± 1.4	19 ± 1.2	20 ± 1.5
SL2	17 ± 1.1	12 ± 0.8	10 ± 0.8	13 ± 0.8	23 ± 1.5	18 ± 1.1	17 ± 1.2
SL3	18 ± 0.9	12 ± 0.8	10 ± 0.9	14 ± 0.7	20 ± 1.2	22 ± 1.2	14 ± 1.2
SL4	16 ± 0.7	11 ± 0.9	10 ± 0.8	14 ± 0.7	18 ± 1.1	23 ± 1.5	15 ± 1.1
SL5	19 ± 0.9	16 ± 0.9	13 ± 1.1	18 ± 0.9	17 ± 1.2	24 ± 1.5	12 ± 0.9
SL6	16 ± 0.5	12 ± 0.8	9 ± 0.3	15 ± 1.1	18 ± 1.2	22 ± 1.5	15 ± 0.8
SL7	18 ± 1.2	12 ± 0.7	10 ± 0.9	15 ± 0.9	18 ± 1.2	22 ± 1.2	15 ± 0.9
SL8	16 ± 0.9	14 ± 1.2	19 ± 1.2	15 ± 0.8	15 ± 1.1	17 ± 0.9	14 ± 1.0
SL9	17 ± 1.1	13 ± 1.0	11 ± 1.0	14 ± 1.1	19 ± 1.6	23 ± 1.7	14 ± 0.7
SL10	18 ± 1.3	14 ± 1.2	10 ± 0.8	14 ± 0.9	22 ± 1.6	19 ± 1.6	16 ± 1.0
SS1	20 ± 0.8	9 ± 0.3	9 ± 0.3	13 ± 0.9	21 ± 1.5	19 ± 1.6	19 ± 1.1
SS2	17 ± 0.5	9 ± 0.3	10 ± 1.2	12 ± 0.7	20 ± 1.5	18 ± 1.4	15 ± 1.4
SS3	17 ± 0.6	12 ± 1.1	10 ± 0.9	14 ± 0.8	19 ± 1.2	24 ± 1.8	14 ± 0.8
SS4	20 ± 0.9	9 ± 0.3	9 ± 0.4	13 ± 0.5	21 ± 1.4	19 ± 1.2	20 ± 1.2
SS5	16 ± 0.9	12 ± 1.4	10 ± 0.5	13 ± 0.5	22 ± 1.4	18 ± 1.2	19 ± 0.9
SS6	17 ± 0.5	10 ± 0.6	10 ± 0.5	14 ± 0.5	23 ± 1.5	22 ± 1.2	15 ± 0.8
Positive control (10 µg amikacin)	20 ± 0.5	21 ± 0.6	22 ± 0.5	20 ± 0.4	24 ± 0.5	20 ± 0.6	20 ± 0.5

LAB – lactic acid bacteria; SC – 'sucuk'; SD – standard deviation; SL – salami; SS – sausage

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E. faecalis and *W. viridescens* using the sequencing 16S rDNA method. As in work, we identified some LAB isolates as *L. sakei, E. faecalis* and *W. viridescens.*

Antibacterial activity of LAB isolates was determined using the agar spot test (Schillinger and Luke 1989) and agar well diffusion test (Pringsulaka et al. 2012). Results from the agar spot test are shown in Table 2. The diameters of the inhibition zones were evaluated as: (+) < 5.0 mm, (++) 5.0–10.0 mm, (+++) 15.0–20.0 mm. According to results obtained from the agar spot test, LAB isolates showed high antimicrobial effects against *L. monocytogenes* ATCC 19115, *K. pneumonia* NRRLB 4420 and *P. aeruginosa* ATCC 11778. Relatively, a high antimicrobial effect was detected against *Str. faecalis* NRRL B 14617 and *S. aureus* ATCC 25923. Isolates of LAB showed a lower antimicrobial effect against *E. coli* ATCC 35218 and *B. subtilis* NRS 744.

In the study of Çınar et al. (2018), LAB isolated from 'sucuk' samples were identified as *L. plantarum, L. paraplantarum*, *L. sakei*, *P. acidilactici* and *P. pentasaceus* using the 16S rDNA gene-sequencing method. Similar to our study, all strains showed good or very good antibacterial activity against *L. monocytogenes* using the agar spot test. In another study, Çon and Gökalp (2000) investigated bacteriocin-like metabolites produced by LAB isolated from the 'sucuk' samples. Isolates of LAB were identified as: *L. plantarum, L. curvatus, P. pentosaceus, P. acidilactici, L. pentosus, L. sakei, L. delbrueckii* and *L. rhamnosus.* Similar to our study, *L. curvatus* and *L. sakei* were isolated from the 'sucuk' samples and showed bactericidal activity against tested pathogenic bacteria. In order to determine the antimicrobial effect of LAB isolates, the agar well diffusion test was also performed. The results of antimicrobial effects of LAB isolates against indicator bacteria are shown in Table 3. Isolates of LAB showed a different antimicrobial effect on tested pathogenic bacteria. High antimicrobial activity was determined against *L. monocytogenes* ATCC 19115, *K. pneumoniae* NRRLB 4420 and *P. aeruginosa* ATCC 11778. In the study of Barbosa et al. (2013) was reported, that the *L. sakei* MBSa1 strain, isolated from Turkish-type fermented sausages, showed a strong antimicrobial activity against *L. monocytogenes*. These scientists also found low antimicrobial activity against *Salmonella* spp., *E. coli*, *Bacillus cereus* and *S. aureus.*

CONCLUSION

In conclusion, 30 LAB isolates were isolated from 20 different traditional fermented meat products sold in the Afyonkarahisar province (Turkey). Molecular identifications of the isolates were performed using the 16S rDNA PCR method. The agar spot test and the agar well diffusion test were used in order to estimate the antimicrobial activities of LAB isolates. The isolates of LAB showed a higher antimicrobial effect against *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 25923, *K. pneumoniae* NRRLB 4420, *P. aeruginosa* ATCC 11778, *Str. faecalis* NRRL B 14617 strains than to *E. coli* ATCC 35218 and *B. subtilis* NRS 744 strains. The results of this study showed that LAB isolates produced antimicrobially active substances. Bacteriocin or bacteriocin-like substances of the LAB isolates should be isolated, purified and characterised in further studies. Antimicrobially active compounds or the LAB isolates could be used in food and health sectors in the future.

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