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Investigation of Bacteriocin and Probiotic Properties of Lactic Acid Bacteria from Pastrami

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ABSTRACT: The aim of this study was to determine the microbiological quality, LAB profile, phylogenetics of *Lactobacillaceae* and bacteriocin synthesis potential of pastrami isolates produced in Turkey, Kayseri (June 2019 – June 2020). The probiotic properties of bacteriocinogen strains were investigated in the second stage of the research. To identify the isolates, MALDI-TOF MS and 16S rRNA sequence analysis methods were used. The bacteriocinogenic properties of the strains were evaluated both phenotypically, using the well diffusion method, and at the gene level, using conventional and RT-PCR. To control the stability of cell-free supernatants of bacteriocinogen strains, tolerance tests for enzyme, temperature and pH were performed. To evaluate the probiotic properties of the strains, we investigated their tolerance to gastric juice and bile salts, susceptibility to antibiotics, and ability to synthesize EPSs. The identification process revealed that the dominant species in pastrami was *Latilactobacillus sakei*. Based on the analyses performed, it was determined that 15 strains (14.56%) exhibited phenotypic antimicrobial activity against at least one of the selected indicator bacteria. Five strains were found to be positive for at least one of the sakacin P and sakacin Q synthesis genes on molecular analysis, which were confirmed by sequence analysis. Cell-free supernatants of bacteriocinogen strains were found to be tolerant of low pH and different temperature conditions. In conclusion, it has been determined that the *Latilactobacillus sakei* 2 and *Latilactobacillus curvatus* 48 strains have significant potential in terms of probiotic and starter culture properties.

Keyword: Bacteriocin; LAB; *Latilactobacillus sakei*; Probiotic; Sakacin.

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INTRODUCTION

The need to preserve meat for a long time from past to present has led to the emergence of many meat products. Pastrami, which is the result of the application of several of these preservation methods together. Large-scale food production mobilisation has started to meet the increasing demand. Extended storage times have increased the need for additives to preserve food without spoilage (Castellano et al. 2017). As consumers become more aware, their sensitivity to the health problems caused by chemical additives continues to increase. This sensitivity has increased social interest in minimally processed and chemical additive-free products, and in parallel, a challenging and complex process has begun for food producers (da Costa et al. 2019). Today, to control the growth of undesirable microorganisms in foods, alternative preservation methods that can replace chemical additives have been sought. This situation has paved the way for the use of microbial-derived bio preservatives. The reflection of the awareness on the food sector has brought along the interest in fermented products. It is estimated that today's fermented food industry size has reached 30 billion dollars (Voidarou et al. 2020). The utilisation of probiotics and prebiotics has become a prevalent practice within numerous medical disciplines, including oncology, where their employment has been demonstrated to reduce the risk of carcinogenesis. Moreover, their benefits extend to the maintenance of gastrointestinal and urinary/vaginal tract, and oral health. Consequently, a comprehensive understanding of their risks and benefits is imperative. Studies have demonstrated the capacity of gut microbiota to modulate inflammation, adiposity, energy expenditure, and glucose metabolism (Voidarou et al., 2020). The capacity of these bacteria to produce and respond to neurochemicals lends support to the notion that probiotics have the potential to influence psychological health and general behaviour (Hsiao et al., 2013).

LAB contribute to the product in terms of shelf life by producing various antimicrobial compounds as well as providing advantages such as aroma, taste, and stability. These natural antimicrobial compounds consist of organic acids, hydrogen peroxide, acetoin, diacetyl, reuterin, antifungal compounds and bacteriocins (Castellano et al. 2017). Bacteriocins are peptide-structured compounds that are synthesised from ribosomes and can be easily inactivated by proteases during digestion, which do not pose any threat to eukaryotic cells and are resistant to high temperature and low pH values, especially against foodborne pathogenic and spoilage-causing saprophytic bacteria (Gontijo et al. 2020). Bacteriocins are not affected by antibiotic resistance due to their different inhibitory mechanisms of action. The fact that some bacteriocin synthesis genes are encoded by plasmids allows molecular manipulations. In addition, the encoding of bacteriocin by plasmids is very important in terms of transferring the synthesis ability to strains that do not show bacteriocinogenic properties (da Costa et al. 2019; Albedwawi et al. 2021).

In Turkey, research on pastrami is generally designed to determine microbiological quality (Öz et al., 2017; Ertekin et al., 2023). Although there is no use of starter culture in any part of the production stage, the presence of LAB in the meat microbiota has been determined in many studies (Topcu et al., 2020). However, there are a limited number of studies examining the probiotic and bacteriocinogenic properties of the identified LAB. In addition, the phylogenetic relationship, and the potential of the isolated bacteria to produce antimicrobial compounds have not been fully elucidated. The main motivation and the first stage of this study was to determine the phylogenetics and bacteriocin production potential of *Latilactobacillus* spp. which constitute an important part of the pastrami microbiota. In the second stage, the probiotic properties of bacteriocinogenic strains were focused. Probiotics can affect almost all physiological functions of the host organism, including nutritional status, metabolism, immunity, mental health, and mood. It is very important to elucidate the mechanism of action of LAB and to develop criteria for the selection of suitable probiotic strains (Castellano et al., 2017; Novik and Savich, 2020). The first of these criteria is the correct identification of the probiotic candidate strain. Phenotypic methods for the identification of LAB are both difficult and time consuming. Biochemical tests (such as API CHL50) used for the identification of LAB have been shelved with the development and diversification of molecular-based diagnostic techniques (Dec et al., 2014). The most preferred of these molecular diagnostic techniques is 16S rRNA sequence analysis (Gontijo et al., 2020). Due to the difficulties experienced in species differentiation of bacteria belonging to the genus Lactobacillus, it has been stated that the use of 16S rRNA sequence analysis in parallel with other diagnostic methods is

necessary for more reliable results (Dec et al., 2014). One of these alternative methods, MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionisation time of flight, Mass Spectrometry) is a high-throughput identification method based on the analysis of bacterial cell proteins. Over 300 species in 7 genera and 2 families of LAB were reclassified into one family, the *Lactobacillaceae*, with 31 genera including *Lactobacillus*, *Paralactobacillus*, *Pediococcus*, *Weissella*, *Fructobacillus*, *Convivina*, *Oenococcus*, *Leuconostoc*, and 23 new genera that comprise organisms formerly classified as *Lactobacillus* species in 2020 (Zheng et al., 2020). Recognizing the advantages of the new taxonomy, government agencies, including the US Food and Drug Administration, Health Canada, and the European Food Safety Authority, have started to use the current nomenclature (Qiao et al., 2022).

Phenotypic and genotypic methods are preferred to determine the bacteriocinogen potential of the strains. Phenotypic antimicrobial activity tests are performed with CFSs (cell-free supernatant) obtained from bacteriocinogen candidate strains and indicator bacteria by methods such as agar spot-on lawn, well diffusion, triple-agar-layer (Todorov et al., 2013). PCR methods using specific primers targeting one or more gene regions are preferred to determine the bacteriocin profile of bacteriocinogen strains showing genotypic antimicrobial activity (Macwana and Muriana, 2012). Several studies have been carried out in different countries to investigate the bacteriocinogen potential of LAB isolated from meat and meat products (da Costa et al., 2019). The size of the probiotic industry is estimated to reach 77 billion dollars in 2025 (Voidarou et al., 2020). It is very important to reveal the mechanism of action of LAB and to develop criteria for the selection of suitable probiotic strains (Novik and Savich, 2020). The tolerance of probiotic strains to gastric fluid and bile salt is essential for their effectiveness (Novik and Savich, 2020). Despite their GRAS status, existing antibiotic resistance genes can be transferred to pathogenic bacteria and/or commercial starter culture strains. Therefore, it is very important to determine the antibiotic susceptibility of probiotic candidate bacteria. Synthesis of compounds with antimicrobial activity, exopolysaccharide (EPS) production ability and immune response enhancing effect are other features sought in probiotic candidate bacteria (Dobson et al., 2012; Moradi et al., 2020; Voidarou et al., 2020).

In Turkey, research on pastrami is generally designed to determine microbiological quality (Öz et al., 2017; Ertekin et al., 2023). Although there is no use of starter culture in any part of the production stage, the presence of LAB in the meat microbiota has been determined in many studies (Topcu et al., 2020). However, there are a limited number of studies examining the probiotic and bacteriocinogenic properties of the identified LAB. In addition, the phylogenetic relationship, and the potential of the isolated bacteria to produce antimicrobial compounds have not been fully elucidated. The main motivation and the first stage of this study was to determine the phylogenetics and bacteriocin production potential of *Latilactobacillus* spp. which constitute an important part of the pastrami microbiota. In the second stage, the probiotic properties of bacteriocinogenic strains were focused.

MATERIALS AND METHODS

The study analysed 50 pastrami samples obtained from retail sales centres in Kayseri throughout the year (June 2019- June 2020). Isolation and identification are carried out according to ISO 15214 standard. MALDI-TOF MS (Bruker, Germany) method was preferred for the first stage of identification. The final identification of the isolates identified as *Lactobacillus* spp. by MALDI-TOF MS was carried out by amplification of the 16S rRNA gene region and sequence analysis the 27F and 1492R primers were preferred based on literature searches (Ma et al., 2017; Lee et al., 2018). PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) was used for genomic DNA extraction according to the product`s instructions. DNA concentrations for PCR were adjusted to 100 ng/µL using a Qubit™ 3 Fluorometer (Invitrogen™- Thermo Fisher Scientific, USA). After purification of the bands, 16S rRNA sequence analysis was performed by Macrogen (Netherlands). Sequence results were edited with Geneious Prime (Biomatters LTD, New Zealand) and base sequences were determined. The sequence data obtained were subjected to BLAST (Basic Local Alignment Search Tool) and the final identification of the strains was completed. MEGA X (www.megasoftware.net) software was used for phylogenetic analysis and Maximum Likelihood and GTR (General Time Reversible) were preferred as method and model, respectively (Stecher et al., 2020).

Cell free supernatants were obtained as described by de Souza Barbosa et al (2015). The possible an-

timicrobial activity of the obtained CFSs was tested by well diffusion method on indicator bacteria *E. coli* ATCC 25923, *S. aureus* ATCC 25922, *E. faecium* NCTC 12202, *E. faecalis* ATCC 51299, *L. monocytogenes* NCTC 7144 and *B. cereus* NCTC 9946. The antimicrobial activities of the isolates were determined according to the zone diameters (mm). CFSs obtained from *Pediococcus acidilactici* SAFEPRO® B-LC-20 (Chr. Hansen, Denmark) strain, which is known to have antagonist effect against *Listeria monocytogenes*, was used as positive control. Since bacteriocins are proteins and can be degraded by proteolytic enzymes in the mammalian gastrointestinal system. Therefore, the enzymes pepsin (pH 7.0 Merck, Germany), catalase (pH 7.0 Merck, Germany), trypsin (pH 7.0 Merck, Germany) and proteinase K (pH 7.0 Merck, Germany) were added to the cell-free supernatants of LAB isolates showing antimicrobial activity at final concentrations of 1 mg/ml. The effect of the experiment on the antibacterial activity was determined using the well diffusion method (Chen et al., 2014). As a positive control, cell-free supernatants without enzyme addition were used. To see the response of antimicrobial activity at different pHs and the effect of different temperatures on antimicrobial activity, the methods mentioned by Chen et al (2014) were applied.

This study investigates the presence of curvacin A, sakacin (P, Q, G, and X), mesenterocin Y and mesenterocin B genes in *L. sakei*, *L. curvatus* and *L. mesenteroides* strains that exhibit antimicrobial activity. The investigation is carried out using primers that bind to specific bacteriocin gene regions (Cocolin and Rantsiou, 2007; Todorov et al., 2011; Macwana and Muriana, 2012). After electrophoresis, the purified bands were subjected to sequence analysis by Macrogen (Netherlands). Sequence results were edited with Geneious Prime (Biomatters LTD, New Zealand) and base sequence was determined. After BLAST screening of the sequence results, the final presence of bacteriocin synthesising genes was determined. The same base sequences were compared in the BAGEL4 database based on the hidden Markow model designed for bacteriocins (Gontijo et al., 2020). To determine the acid tolerance of bacteriocinogenic LAB strains, the instructions of Son et al (2018) were followed. *L. plantarum* ATCC 8014 strain was used as a positive control in the experiment. For the evaluation of gastric acid tolerance, appropriate dilutions were prepared, and the instructions of Jeon et al (2016) were traced. Modified Ruthenium Red Agar was preferred to observe the EPS synthesis potential of bacteriocinogenic LAB strains (Yildiran et al., 2019). White and sticky colonies were considered as EPS producers and pink colonies as non-producers. Antibiotic susceptibility of bacteriocinogen LAB strains was evaluated according to CLSI (2022) guidelines.

STATISTICAL ANALYSES

All the variables obtained because of the experiments carried out for low pH and resistance to bile salts were analysed by means of the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances, before proceeding to the tests of significance. One-way analysis of variance (ANOVA) was used to statistically control for differences between variables. The Duncan test was used as a post-hoc test for variables where the difference between groups was significant. The SPSS 23 package (IBM SPSS Statistics, USA) was used for the analyses.

RESULTS

Gram-positive, catalase-negative, and oxidase-negative rod/cocobacilli-shaped 103 different colonies were obtained. MALDI-TOF MS was used to identify the 103 pure isolates transferred to blood agar, resulting in 17 different species from six different genera (*Lactobacillus*, *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus*, and *Brevibacilus*). The dominant genus in the pastrami microbiota was *Lactobacillus* spp. (52.42 %), followed by *Leuconostoc* spp. (22.33 %). The remaining LAB were *Weissella* spp. (13.59%), *Pediococcus* spp. (9.70%), *Brevibacillus* spp. (0.97%) and *Lactococcus* spp. (0.97%). Upon analyzing the genera isolated from pastrami by species, it was observed that *Latilactobacillus sakei* was the most prevalent species. The score distribution and discrimination power were also evaluated. 57 out of 103 strains (55.33%) were within the high probability correct identification score range (2.30-3.0) at both genus and species level. Additionally, 35 strains (33.98%) were ranked at a safe genus and probable species identification score range of (2.0-2.29). All 11 remaining strains (10.67%) were ranked within the possible range for genus identification (1.70- 1.99). None of the isolates scored below 1.69, indicating that this method is suitable for identifying LAB. Based on the results of 16S rRNA sequence analysis, 49 out of 103 LAB isolated from pastrami were identified as *Lactobacillus* spp. (47.57%). The dominant biota consisted of 30 strains of *L. sakei* (61.22%), followed by *L. curvatus* (26.50%) with 13 bacteria. The remaining six bacteria were identified

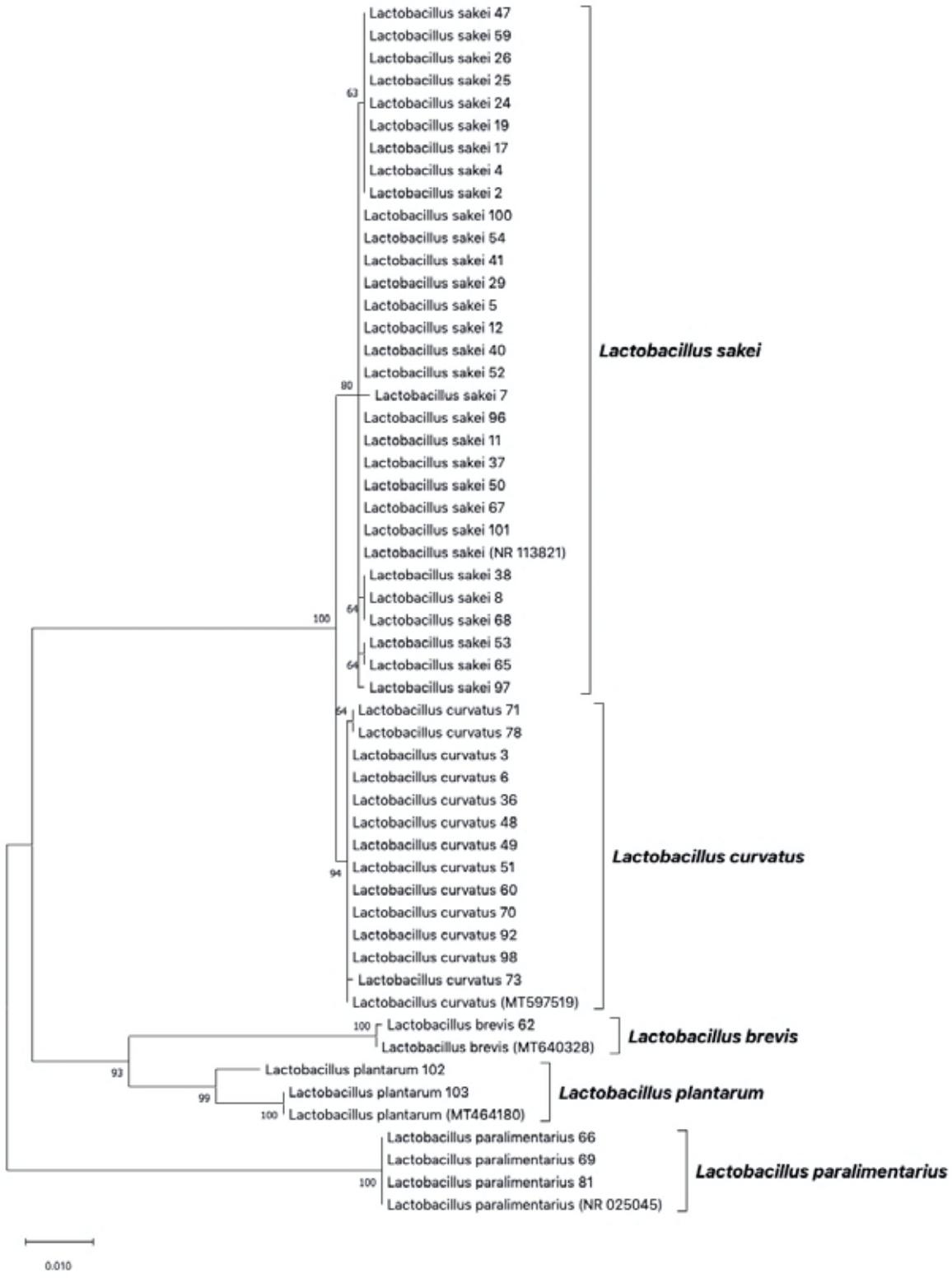

Figure 1. Phylogenetic relationship of Lactobacillus spp. strains.
The analysis of the 16S rRNA gene was conducted using the Maximum Likelihood method and General Time Reversible model. The phylogenetic tree bootstrapping was performed with 1000 replicates, and only values above 60% were displayed on the nodes. The scale bar represents a deviation of 0.01% (Figure 1).
Latilactobacillus sakei subsp. sakei, Latilactobacillus curvatus, Lactiplantibacillus plantarum subsp. plantarum, Levilactobacillus brevis, Companilactobacillus paralimentarius

as three *L. paralimentarius* (6.12%), two *L. plantarum* (4.08%), and one *L. brevis* (2.04%).

In this study, 16 bacteriocinogenic strains (15.53%) were detected. The bacteriocinogenic strains included *L. curvatus* 49 strain, which carries the sakacin Q synthesis gene (*sppQ*), despite its lack of antimicrobial activity against the selected indicator bacteria. None of the strains that exhibited antimicrobial activity demonstrated any antagonistic effects against *L. plantarum* ATCC 8014 (Table 1).

The cell-free supernatants of 4 strains (except *L. curvatus* 49), which were found to carry the bacteriocin synthesis gene, were identified and their responses to different pH, temperature and enzyme treatments were taken into consideration to determine their stability under various conditions. In the

Table 1. Antimicrobial activity of the bacterocinogenic strains

Strains	*E. coli* ATCC 25923	*S. aureus* ATCC 25922	*E. faecium* NCTC 12202	*E. faecalis* ATCC 51299	*L. monocytogenes* NCTC 7144	*B. cereus* NCTC 9946	*L. plantarum* ATCC 8014
***L. sakei* 2**	-	-	-	-	**+ 11 mm**	-	-
***L. curvatus* 6**	-	-	-	-	**+ 11 mm**	-	-
L. mesen 18	-	-	-	-	-	+ 11 mm	-
L. curvatus 38	-	-	+ 12 mm	+ 12 mm	-	-	-
L. sakei 47	+ 11 mm	-	-	-	-	-	-
***L. curvatus* 48**	-	-	-	-	**+ 12 mm**	-	-
***L. curvatus* 49**	-	-	-	-	-	-	-
L. sakei 67	+ 12 mm	-	-	-	+ 12 mm	-	-
L. curvatus 70	+ 10 mm	-	-	-	+ 11mm	-	-
***L. curvatus* 78**	-	-	-	-	**+ 13 mm**	-	-
P. acidi 87	+ 13 mm	-	-	-	-	-	-
P. pentosa 91	+ 10 mm	-	-	-	-	-	-
L. sakei 97	+ 12 mm	-	-	-	-	-	-
L. sakei 101	+ 12 mm	-	-	-	-	-	-
L. plant 102	+ 14 mm	-	-	-	-	-	-
L. plant 103	+ 15 mm	-	-	-	-	-	-
SAFEPRO® B-LC-20	-	-	+ 16 mm	+ 16 mm	+ 23 mm	-	-

susceptibility tests, *L. monocytogenes* NCTC 7144 strain, which all CFSs can show antagonist effect, was preferred as indicator test bacteria. The antimicrobial activity of bacteriocinogen strains showed stability against pH changes. No change was observed in the antimicrobial activity zone diameters of the strains, indicating that they were resistant to different temperature applications. The response of bacteriocins, which are known to have peptide character, to proteolytic enzymes is a valuable indicator to determine the source of inhibition. Proteolytic enzymes terminated the antimicrobial activity of the strains, while catalase enzyme had no effect, indicating that the inhibitory effect was not caused by hydrogen peroxide (Supplemantary figures 11- 12).

The analysis revealed that the (*sppQ*) gene was present in strains *L. sakei* 2, *L. curvatus* 48, *L. curvatus* 6, *L. curvatus* 49, and *L. curvatus* 78, in relation to sakacin Q. PCR procedures were performed to detect sakacin P and the (*sppA*) gene was found to be present in *L. curvatus* 6 and *L. curvatus* 78 strains. The *L. curvatus* 78 strain was the only one to test positive for the sakacin X (*sppX*) gene. No strains were found to contain curvacin A or sakacin G bacteriocins. As there was no positive control, the bands detected by conventional PCR underwent sequence analysis after purification. The obtained sequence data were blasted, and the identified possible bacteriocin gene regions were confirmed. While the similarity rate for the *sppQ* gene varied between 97.37% and 98.23%, this rate was determined as 98.54% in the two strains carrying the *sppA* (*sakP*) gene. When the sequence of the *sppX* gene was searched in the Genbank system, no similarity rate with any previously reported sakacin X synthesis gene region was detected (Genbank Blast results and classical PCR images are included in Supplementary figures 1-7). The gDNAs of bacteriocinogen strains were also used for Real-Time PCR detection of bacteriocin synthesis genes. Peaks and melting curves of sakacin genes were found to support the results of conventional PCR (Supplemantary figures 8-10). After comparing the OD values of the strains incubated in pH 2.5 medium for 24 hours with the OD values in MRS broth without pH change, it was found that all strains except *L. curvatus* 49 showed more than 70% acid tolerance. The strain *L. plantarum* ATCC 8014, known to have high acid tolerance, was used as a positive control in the experiment. The ability of bacteriocinogen strains to synthesise EPS was determined by colour and colony consistency. It was found that all bacteriocinogen strains except *L. curvatus* strain 78 were able to produce EPS in mRRA (Supplemantary figure 13). The strains were susceptible to ampicillin (AMP 10), clindamycin (DA 2) and tetracycline (TE 30) and resistant to vancomycin (VA 30), streptomycin (S 10) and kanamycin (K 30). Except for *L. curvatus* strain 6, which was resistant to erythromycin (E 10), all other strains were susceptible to this antibiotic (Table 2).

While determining the time parameters of the experiment, the average time that the foods spent in the stomach environment was taken into consideration. The gastric fluid and bile salt resistance results of the strains showing antimicrobial activity are shown in Table 3.

DISCUSSION

The nutritional significance of meat is attributable to its high biological value as a protein, which contains all the essential amino acids. Additionally, meat is highly bioavailable in terms of minerals, vitamins and micronutrients, including iron, selenium and zinc. Meat is a substantial source of micronutrients as well as macronutrients, providing essential amino acids, essential fatty acids, minerals and vitamins necessary for human development. Pastrami is an ethnic meat product produced by dry curing, drying, pressing and coating all the muscles of cattle and buffaloes with a paste called ‘cemen’ made with

Table 2. Acid tolerance, EPS synthesis, and antibiotic susceptibility results of the strains

Strains	MRS $_{(OD)}$	pH 2.5 $_{(OD)}$	Tolerance (%)	EPS	AMP 10	E 15	TE 30	K 30	DA 2	S 10	VA 30
L. sakei 2	0,9626	0,8235	85,54	+	19	>30	25	0	20	0	14
L. cur 6	0,8588	0,6699	78,0	+	20	**0**	25	0	20	0	0
L. cur 48	1,0574	1,0207	96,52	+	15	>30	20	0	20	8	0
***L. cur* 49**	1,0197	0,6413	**62,89**	+	16	>30	20	0	20	0	0
L. cur 78	1,5005	1,2252	81,65	-	20	>30	25	0	20	0	0
Control	1,0521	0,9937	**94,44**	+	-	-	-	-	-	-	-

Table 3. Gastric fluid and bile salt tolerance

Strains	Counts in pH 2.5 and 0.3% pepsin medium (log cfu/ml)		Resistance (%)	Counts in the presence of 0.3% bile salt (log cfu/ml)		Resistance (%)
	0. hour	3. hours		0. hour	24. hours	
2	8,25 ± 0,04[b]	7,03 ± 0,06[b]	85,21	8,44 ± 0,04[b]	8.59 ± 0,06[b]	101,77
6	7,11 ± 0,03[d]	5,62 ± 0,17[d]	79,04	8,90 ± 0,02[a]	8,92 ± 0,02[a]	100,22
48	8,62 ± 0,03[a]	8,22 ± 0,04[a]	95,35	7,49 ± 0,04[c]	7,61 ± 0,05[c]	101,60
49	5,57 ± 0,06[e]	3,50 ± 0,09[e]	62,83	6,65 ± 0,06[d]	5,40 ± 0,09[e]	81,20
78	7,50 ± 0,06[c]	6,15 ± 0,16[c]	82,00	7,44 ± 0,03[c]	7,47 ± 0,03[d]	100,40

Different letters in the same column indicate $p<0.05$.

special spices. The quality of the final product is directly proportional to the quality of the raw meat. The meat used in the production of pastrami is obtained from well-matured carcasses that comply with beef slaughter standards. The Turkish Food Codex stipulates limits of 50% moisture, 10% salt (in dry matter) and 6.0% pH for pastrami, thus classifying it as an ethnic dry cured meat product. It is acknowledged that the employment of conventional methodologies during the fabrication of pastrami may result in disparities in its microflora, contingent on the specific producer.

Among the LAB isolated from pastrami, *L. sakei* was the dominant species. *L. sakei* strains show high adaptability to meat and fermented production conditions (Janßen et al., 2020). Considering the drying temperature applied in conventional pastrami production, it is not surprising that *L. sakei* dominates the microbiota. When the literature data are examined, many studies indicating that *L. sakei* is the host species in meat and meat products stand out (Xu et al., 2015; Janßen et al., 2020). Conventional identification methods based on morphological and biochemical characteristics require molecular validation due to the similar reactions of many genera and species-based differences, as well as the potential for subjective evaluation. The MALDI-TOF MS method was preferred for identifying LAB in our study. This method compares protein fingerprints and provides highly reliable genus and species results compared to systems such as API 50 CHL and VITEK 2 (Lee et al., 2018). In addition, genotypic confirmation of the strains identified by MALDI-TOF MS using 16S rRNA sequence analysis was preferred. The agreement between MS and 16S rRNA analysis was 90.74% at the genus level, and 81.81% at the species and subspecies level. LAB were isolated from cheeses collected from different regions of Turkey by conventional methods and 381 probable LAB were isolated. After 184 strains showing antimicrobial activity were identified by VITEK 2 and API 50 CHL, 16S rRNA sequence analysis was preferred for genotypic confirmation. The results of 16S rRNA sequence analysis were compared with the VITEK 2 system used in the identification of LAB and 75.49% agreement was observed between the two methods. When the API 50 CHL test used for the identification of *Lactobacillus* spp. was compared with 16S rRNA sequence analysis, a 67.53% match was obtained (Özlü and Atasever, 2019). MALDI-TOF MS was found to be more effective than both tests in LAB identification.

The experiments revealed that five strains contained at least one of the synthesis genes of sakacin P, Q and X. Furthermore, the strains *L. curvatus* 6 and *L. curvatus* 78 were found to be multi-bacteriocinogenic, producing both sakacin P and Q. In strain *L. curvatus* 78, although the gene for sakacin X synthesis (*sppX*) was detected by both conventional PCR and Real-time PCR, it did not yield a positive result in BLAST analysis. Similar issues have been reported by other researchers (Todorov et al., 2017). In conclusion, four strains of bacteriocinogens were isolated and found to exhibit antimicrobial activity against the *L. monocytogenes* NCTC 7044 strain. In a study in which LAB showing antimicrobial activity were isolated from Italian salami, CFS of *L. curvatus* MBSa2 and *L. curvatus* MBSa3 strains showed a strong inhibitory effect against *L. monocytogenes* Scott A indicator strain (de Souza Barbosa et al., 2015). As a result of PCR experiments performed with specific primers for bacteriocins sakacin A, T, Q, P, G1 and G2, *L. curvatus* MBSa2 and MBSa3 strains were found to be positive for sakacin P (186

bp). Another study found that these strains also carried genes for sakacin Q synthesis (Cocolin and Rantsiou, 2007). Previous studies have reported a close relationship between the bacteriocins sakacin Q and P (Mathiesen et al., 2005). Some researchers have reported the existence of strains that do not show antimicrobial activity despite carrying bacteriocin synthesis genes (Todorov et al., 2017). It has been reported that some plasmids affect the 'quorum sensing' metabolism of the bacterium, which may inhibit the expression of bacteriocin genes encoded by chromosomes (Cocolin and Rantsiou, 2007). The absence of problems in the expression of sakacin P and Q in *L. curvatus* L442 strain was attributed to the absence of any plasmid affecting the synthesis. This information provided a clue to the *L. curvatus* 49 strain, which did not phenotypically exhibit antimicrobial activity against selected indicators in our study despite carrying the bacteriocin synthesis gene (*sppQ*).

It is very important that bacteriocins remain stable under different environmental conditions. It was determined that the CFSs obtained retained their antimicrobial activity in the pH 2-10 range. There are many studies indicating that bacteriocin or CFS stability persists in a similar pH range (Todorov et al., 2011; de Souza Barbosa et al., 2015). Another parameter affecting bacteriocin stability is temperature variation. The fact that temperature treatments such as pasteurisation, cooking and smoking do not affect bacteriocin stability is valuable for successful application. The CFSs obtained in this study were found to be temperature tolerant. Bacteriocin studies with similar results are available in the literature (Todorov et al., 2011; de Souza Barbosa et al., 2015; Todorov et al., 2017). In this study, except for *L. curvatus* 49 strains (62.83%), the other bacteriocinogens were found to be tolerant to low pH by more than 70%. In addition, all the strains showed over 80% resistance to bile salt. After evaluating the probiotic properties of LAB strains isolated from the traditional fermented product kimchi in Korea, it was determined that LABs showed high tolerance to low pH (2.5) and bile salt (0.3%) (Jeon et al., 2016; Won et al., 2020). After analysing the probiotic properties of 80 LAB isolated in another pastrami study, it was determined that a significant portion of the strains showed tolerance to high acidity (Topcu et al., 2020).

Antimicrobial resistance has become a serious public health threat. Son et al (2018) determined the antibiotic susceptibility of four probiotic candidate strains by disc diffusion method according to CLSI 2010. Won et al (2020) determined the antibiotic resistance profile of the probiotic candidate *L. sakei* ADM14 strain in a similar way and our data were found to be compatible with these two studies. *Lactobacillus* spp. species carry a non-transmissible internal resistance to aminoglycoside group (streptomycin, kanamycin) antibiotics and that this would not pose a problem for public health. Some researchers stated that erythromycin, tetracycline and vancomycin resistance, which may pose a public health problem, should be investigated at the molecular level (Todorov et al., 2017). In our study, all bacteriocinogen strains were resistant to streptomycin, kanamycin and vancomycin. However, according to literature data, it has been stated that this resistance is a natural feature of the species and cannot be transferred (Shokryazdan et al., 2014; Ma et al., 2017; Albedwawi et al., 2021). When the whole genome sequence analysis of the vancomycin resistance, vancomycin-resistant probiotic candidate *W. cibaria* CH2 strain was performed, it was stated that the resistance was not encoded by plasmids and was not portable (Albedwawi et al., 2021). There are also studies indicating that it is not necessary to look for vancomycin resistance in heterofermentative *Lactobacillus* spp. species (Shokryazdan et al., 2014). As a result, the fact that the strains identified in our study did not show an antibiotic resistance profile that may prevent them from being probiotic candidates is positive, but the fact that one of the strains obtained (*L. curvatus* 6) showed erythromycin resistance is worrying, and this resistance should be investigated at the molecular level.

Nowadays, social concern about chemical additives has increased the demand for natural polymers. The search for compounds that can replace chemical additives in industrial applications has led to extracellular polymeric substance research (Wang et al., 2019). EPSs of LAB have become the focus of these studies, especially due to their GRAS status. In our study, the EPS synthesis ability of *L. sakei* and *L. curvatus* strains showing bacteriocinogenic properties was phenotypically determined using modified Ruthenium Red agar. Except for *Latilactobacillus curvatus* strain 78, other strains were found to be able to produce EPS in the prepared medium composition. There are many studies in the literature determining that *L. sakei* and *L. curvatus* strains produce EPS (Prechtl et al., 2018; Wang et al., 2019). *L. sakei* TMW 1.411 strain isolated from traditionally produced sauerkraut in Germany was

phenotypically determined with modified MRS agar to produce dextran (Prechtl et al., 2018). It was determined that medium composition, incubation temperature, pH and initial inoculum volume are very important in EPS production by LAB (Wang et al., 2019). It is thought that *Latilactobacillus curvatus* 78 strain, which cannot synthesise EPS in modified Ruthenium Red agar, may be able to produce EPS in the medium containing different carbon source. However, the final decision should be made after the presence of EPS synthesis genes is determined by molecular methods.

CONCLUSION

The fact that *L. curvatus* 49 does not show antimicrobial activity despite carrying the *sppQ* gene, *L. curvatus* 72 is phenotypically unable to synthesise EPS, *L. curvatus* 6 shows erythromycin resistance and the determination of its transmissibility remains a mystery and needs further genomic studies. In the light of the analyses carried out, it was determined that *L. sakei* 2 and *L. curvatus* 48 strains from pastrami have an important potential in terms of probiotic and starter culture properties.

Author contribution

All authors have carried out the experiments and discussed the results and contributed to the final manuscript. **H.B.D**.: formal analysis, investigation, resources, data curation, writing, review, and editing. **Z.G**.: methodology, review, and project administration.

Declarations

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