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Potential of *Lactiplantibacillus plantarum*, *Levilactobacillus brevis* and *Lactococcus lactis* strains as functional cultures for dairy industry

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Abstract

The functional properties of foodborne lactic acid bacteria (LAB) are related to their metabolites. In this study, the most important functional properties of LAB isolates isolated from different fermented foods were investigated. These include, primarily EPS production capabilities and the properties of the EPS structures they produce, antimicrobial activities, and resistance to the simulative gastrointestinal system, which is one of the important tests for the concept of potential probiotic isolates, were investigated. Among the isolates, the BL4, CL6 and KL2 belonging to the *Lactiplantibacillus plantarum* species, the EL1 belonging to the *Levilactobacillus brevis* species, and CL3 belonging to the *Lactococcus lactis* species were determined to be strong EPS producers. When the EPS structures produced by the strains were examined, especially EPS-EL1 obtained from the *Lv. brevis* EL1 showed shear thinning behavior and moreover, it was stated that it could provide significant rheological contribution for dairy industry applications. Moreover, this strain showed high antimicrobial activity against the bacterial pathogen *E. coli* ATCC 25922 and high resistance against gastrointestinal simulation medium along with CL6. On the other hand, high antimicrobial activity of the *Lp. plantarum* BL4 and the *Lc. lactis* CL3 strains was determined against the yeast pathogen *C. albicans* ATCC 10131. This reveals that the *Lv. brevis* EL1 strain is especially likely to be used for different functions such as co-culture and adjuvant culture in the dairy industry. As a result, it was revealed that these 5 strains have different supportive properties that can be used in the dairy industry, for various fermentation processes.

Keywords: exopolysaccharide; antimicrobial; *Levilactobacillus brevis*; *Lactiplantibacillus plantarum*; *Lactococcus lactis*; potential probiotic candidate

Introduction

Recently, significant advancements have been made in understanding the importance of a naturally balanced and healthy diet in all societies. When naturalness and health are considered together, fermented foods are the first products that come to mind. Fermented foods, such as cheese, yoghurt, tarhana, and vinegar, are distinct from their raw materials. They are produced by some bacteria and/or yeast strains using various ingredients, such as milk, dough, meat, and some vegetables and fruits, under specific fermentation conditions by some bacteria and/or yeast strains (Özdemir et al., 2019; Zehir-Şentürk et al., 2020).

Lactic acid bacteria (LAB) constitute the most important group of microorganisms involved in fermented foods and are generally recognized as safe (GRAS) (Yılmaz et al., 2014). Some of these microorganisms can produce functional metabolite products (short-chain fatty acids, functional peptides, oligosaccharides etc.) with different activities such as antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, cholesterol lowering, and antihypertensive (Wang et al., 2025; Iliev et al., 2025). In addition, they can produce agents such as thickener, viscosity-increasing agents, binders or acidifier (Mazzoli, 2020).

One of the LAB metabolites is an exopolysaccharide (EPS) structure. EPSs are in polysaccharide structure in terms of their chemical structures. EPS can consist of sugar or sugar derivatives such as glucose, galactose, rhamnose, glucuronic acid, fucose, N-acetyl glucosamine and N-acetyl galactosamine, as well as other organic and inorganic molecules (Ruas-Madiedo et al., 2002). The EPSs produced by bacteria are not a source of energy for the producing microorganism. They can perform functions such as protection from stress conditions, prevention of water loss, protection against phagocytosis and phage attacks, antibiotic and toxic compounds, attachment to surfaces, colonization in different ecosystems (Ruas-Madiedo et al., 2002; Akarca and Janseli Denizkara, 2024; Zhang et al., 2025). EPS can be used as thickener, gelling agent, and an emulsifier in foods, improving the texture and mouth feel. In addition, these ingredients are used in areas such as biomedical, biopharmaceutical and cosmetics outside the food sector (Pan et al., 2010). For example, fructan type EPS produced by *Fructilactobacillus sanfranciscensis* was reported to positively affect dough rheology and bread texture (Brandt et al., 2014).

Another important functional metabolites produced by LAB are compounds with antimicrobial activity. Most of the structures of the antimicrobial compounds of LAB are peptide structures that can have a lethal or inhibitory effect on pathogenic microorganisms (Rençber et al., 2024). Biochemical properties, spectrum of action and genetic determinants of antimicrobial components may differ. These compounds are generally cationic proteins with high isoelectric point and amphiphilic characters and are heat resistant. Therefore, it is useful for industrial applications due to its antimicrobial activities, can be used as a bio-preservative in food engineering or other fields: such as medicine, material engineering) (De Vuyst and Leroy, 2007; Khan-Mohammadi et al., 2023; Iliev et al., 2025).

In this study, different functional and technological properties of LAB strains isolated from Turkish traditional fermented foods such as tarhana (instant soup made from sourdough), cheese and pickle, especially their antimicrobial activities and their ability to produce EPS, were investigated. The aim was to highlight the potential functionality of these strains in order to be used in the dairy industry, which has a wide variety of fermentation processes.

Material and methods

Materials

In this study, the twenty LAB isolates that were kept in the Culture Collection of the Biotechnology Laboratory of Ondokuz Mayis University, Department of Food Engineering were used. These isolates had been isolated from Turkish fermented foods such as tarhana, cheese and pickles. Active cultures for 18 hours were stored at -80 °C in an environment containing 30 % glycerol in MRS (de Man, Rogosa and Sharpe) broth medium. These strains had been identified in a study by Şimşek et al. (2007).

Screening for exopolysaccharide producing-LAB isolates

LAB isolates were activated for 18 hours at 30 °C using MRS broth, and active cultures (MC-Farland OD600=0.5) were spot-seeded (20 µL) on MRS-sucrose agar (containing 4 % sucrose instead of 2% glucose) (5×10^7 log CFU mL^{-1}). After the petri dishes incubated at 30 °C for 48 h, the isolates with ropy or mucoid features (elongation of 5 mm or more should be observed when touching and pulling the colony with a loop) were selected as EPS producers (Bounaix et al., 2009)

Screening for LAB isolates with antimicrobial activity

The antimicrobial activity of isolates was tested against six microorganisms. The strains included four bacteria: *Escherichia coli* ATCC 25922, *Bacillus cereus* NRRL-B209 *Staphylococcus aureus* ATCC 33862 and *Listeria monocytogenes* ATCC 7644 and two yeast: *Candida albicans* ATCC 10131 and *Saccharomyces cerevisiae* ATCC 9763. Antimicrobial activities of the isolates were tested using the agar-spot and well-diffusion assay method, respectively. Firstly, the nutrient agar plate was inoculated with one of the indicator strains. The plates

were incubated at 35 °C for 20 h for bacteria and at 25 °C for 72-96 h for yeast. Antimicrobial activity defined as clear and measurable (mm) zone of inhibition formed around the isolates.

Secondly, to eliminate the effects of pH, neutral-cell-free supernatant (CFS) of the LAB isolates was prepared, adjusted to a pH value of 6.0 treated with catalase enzyme (Catalase from bovine liver, Sigma-Aldrich, Missouri, USA). Then, 10 µL of each of the prepared neutral-CFSs was spotted on a nutrient agar plate inoculated with one of the indicated pathogenic strains. Incubation conditions (at 35 °C for 20 h for bacteria and at 25 °C for 72-96 h for yeast) were applied similarly. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters (Reuben et al., 2020).

Identification of selected LAB isolates

In a previous study by Şimşek et al. (2017), the isolates selected because they produced EPS and/or showed antimicrobial activity were identified by 16S rRNA gene sequencing (Table 1A).

Exopolysaccharide isolation and purification

EPS was isolated by ethanol precipitation which also included the TCA precipitation and dialysis to remove any proteins and impurities presented in the EPS sample, respectively (Dertli et al., 2016) Isolated purified EPSs were lyophilized and stored at -20 °C for further studies.

Physico-chemical characterization of EPS

The main functional groups of EPS were analyzed by Fourier transform-infrared (FTIR; Nexus 5 DXC FTIR, Thermo Nicolet, Madison, WI, USA), which were used according to the method by Saravanan and Shetty (2016). IR-spectra of the EPS were used with a Fourier transform infrared spectrophotometer (Nexus 5 DXC FTIR, Thermo Nicolet, Madison, WI, USA). The purified EPS was ground with potassium bromide (KBr) powder and then pressed into 1 mm pellets for FTIR measurement in the 4000–400 cm^{-1} frequency range.

The sugar composition of the purified EPS (20 mg/mL) was analyzed, 218 µL 72 % formic acid (CH_2O_2) was added

Table 1. *The identification results with 16S rDNA sequence and pheS gene sequence (A), EPS production and antibacterial activity of LAB isolates from tarhana (B), Monosaccharide profiles of the EPSs (C)*

A) (Özel, 2012)	**Isolate Name**	**Homology %**		**Genebank accession number**
Isolate code		**16S rDNA**	**pheS gene**	
BL4	*Lactiplantibacillus plantarum*	100	100	KT285581
CL6	*Lactiplantibacillus plantarum*	99	100	KT285588
KL2	*Lactiplantibacillus plantarum*	100	100	
EL1	*Levilactobacillus brevis*	99	99	KT285592
CL3	*Lactococcus lactis*	99	-	KT285585

B)	**EPS production**	**Indicator strains***					
Isolates		***B. cereus* NRRL-B 209**	***E. coli* ATCC 25922**	***S. aureus* ATCC 33862**	***L. monocytogenes* ATCC 7644**	***C. albicans* ATCC 10131**	***S. cerevisiae***
Lp. plantarum BL4	-	++	+++	+	+	++	+
Lp. plantarum CL6	+	+	++	-	-	+	+
Lp. plantarum KL2	+	++	++	+	+	++	++
Lv. brevis EL1	+	++	++	+	-	+++	++
Lc lactis CL3	+	++	+	++	+	+++	++

C)		**Sugar composition of EPS (mg mL^{-1})****			
Isolates		**Disaccharide**	**Monosaccharide**		
		Maltose	**Glucose**	**Galactose**	**Fructose**
Lc. lactis CL3		ND	1.31 ± 0.01^{dB}	4.53 ± 0.02^{aA}	ND***
Lp. plantarum CL6		ND	2.55 ± 0.02^{cB}	3.55 ± 0.01^{bA}	ND
Lv. brevis EL1		0.58 ± 0.01^{bC}	15.87 ± 0.01^{bA}	1.75 ± 0.02^{cB}	0.38 ± 0.03^{aC}
Lp. plantarum KL2		0.61 ± 0.01^{aB}	19.71 ± 0.02^{aA}	0.17 ± 0.01^{dC}	0.10 ± 0.01^{bC}

**"-","+","++" and "+++" representing inhibition zone diameter of "0 mm" 11 mm≤DIZ≤15 mm, 15 mm≤ DIZ≤20 mm, and strong DIZ ≥21 mm*
*** A lower case "a-d" represents the statistically significant for a single column. An uppercase "A-C" represents the statistically significant for a single row. ***ND not determined.*

onto 800 µL EPS solution and kept in a water bath at 95 °C for 2 hours, then neutralized with 5M potassium hydroxide (KOH), by Ermiş et al. (2020) The monosaccharide composition of the EPS sample was analyzed using Shimadzu HPLC (Shimadzu, Manchester, UK) equipped with an Aminex HPX-87C ion-exchange Column (300x7.8 mm; Bio-Rad Hercules, CA) and coupled with refractive index (RI) detector (10A, Shimadzu, Manchester, UK). The monosaccharides were analyzed using the following parameters: injection volume: 20 µL, column temperature: 85 °C, mobile phase: ultra-pure water, flow rate: 0.4 mL min^{-1}, time: 30 min.

Rheological properties of EPS

The rheological properties of lyophilized EPS were analyzed using the method described by Yılmaz et al. (2014). Briefly, 40 mg mL^{-1} lyophilized EPS was dissolved in dH2O and rheological properties of EPS solutions were determined. It was performed using a rheometer (HAAKE MARS III, Thermo Scientific, USA) at 4 °C for the shear rate of 0.1-100 s^{-1} for 120 seconds. Flow-behavior index values were calculated by applying the Herschel-Bulkley model for shear stress values depending on the shear rate

Herschel-Bulkley modeli: $\tau=\tau_0+K(\dot{\gamma})n$

τ_0 - the yield stress (Pa)
K - the consistency index (Pa sn)
γ - shear rate(s^{-1})
n - the flow behavior index

Eq:1

A frequency sweep test was applied to determine the dynamic vibration shear properties of EPS. The frequency sweep test was conducted at 4 °C temperature between 0.1 Pa and 0.1-100 Hz (0.628-628.3 rad s^{-1}) for 120 seconds. The elastic modulus (G´) and viscosity modulus (G˝) were obtained and calculated using the following equations for dynamic vibration shear.

$$G' = K'(\omega)n'$$
$$G'' = K''(\omega)n''$$

K′ ve K″- constants (Pa s^{-1})
n′ ve n″ - slope values
ω- the angular frequency (s^{-1})

Eq:2

Temperature-dependent behavioral properties of lyophilized EPS were analyzed according to the method of Ayyash et al. (2020). The temperature sweep test was performed in the range of 10-80 °C, with a temperature increase rate 3 °C/min, at a constant cutting speed of 20 s^{-1} for 22 minutes. Shear pressure values were obtained against the shear rate, and flow behavior index values were calculated by applying the Arrhenius model:

Arrhenius modeli: $\eta=\eta_0 e^{\frac{E_a}{RT}}$

K′ ve K″- constants (Pa s^{-1})
n′ ve n″ - slope values
ω- the angular frequency (s^{-1})

Eq:3

Antibiotic susceptibility testing of selected LAB strains

Sensitivities of selected LAB strains against seven different antibiotics (AMC; Amoxicillin/clavulanic acid: 20/10 µg; E_{15}-Erythromycin: 30 µg, AM-Ampicillin: 10 µg, DA-clindamycin: 2 µg, MET- Metronidazole: 5 µg, TE-Tetracycline: 30 µg, and VA-Vancomycin: 30 µg; purchased from Liofilchem®, Teramo Italy) were tested. For this purpose, 18-hour cultures were set to 10^7 CFU/mL and 100 µL of these cultures were spread on the surface of the Mueller-Hinton agar in Petri plates. Then, the antibiotic discs were placed in each petri dish and the petri dishes were incubated at 30 °C for 24-48 hours. After incubation, microbial inhibition was determined by measuring the diameter of the clear zone of inhibition (DZI) of growth around each antibiotic disc (Reuben et al., 2020; Mohammed and Çon, 2020).

Test of growth of selected LAB strains under different conditions

For the growth test at different salt concentration values, the selected strains were incubated at 30 °C for 7 days (where the development of the strains was observed from the first day to the seventh day. Early development shows high resistance and adaptation to the environment in MRS broth with four different salt concentrations (3.0 %, 5.0 %, 7.0 % and 9.0 % NaCl). For the growth test at different temperatures, the selected strains inoculated into MRS broth media were incubated for 7 days at 6, 15, 30, 37, 40 and 45 °C. For growth test at different pH values, the selected strains were incubated for 7 days at 30 °C in MRS broth medium adjusted to four pH values (pH 2.0, 3.0, 3.5, and 4.0) (Plessas et al., 2017). For all tests, an increase in OD of 0.3 units in the MC-Farland device during the incubation process was considered as positive.

Resistance test of selected LAB strains to simulated gastric-intestinal systems

Phosphate Buffered Saline (PBS) solution containing 0.3 % (w/v) pepsin and 0.5 % (w/v) NaCl was prepared for resistance to the gastric environment and the prepared gastric solution was adjusted to a pH of 2.0. Active cultures of the tested strains (18-hours) were centrifuged (3500 g 15 min) to obtain pellets and the pellets were washed with physiological salt water (0.85 % NaCl; pH 7.0). Subsequently, prepared gastric solution pellets were added (10^9 log CFU/mL simulated gastric solution), and the mixture was incubated at 37 °C under 5 % CO_2 for 120 min. At the 0, 60, and 120 minutes of the incubation, the number of surviving strains was determined by the plate count method in MRS agar (Reuben et al., 2020).

A PBS containing 0.5 % bile salt, 0.1 % trypsin and 0.2 % pancreatin was prepared, and adjusted to pH 8.0 for the intestinal medium. The LAB strains to be tested

were inoculated into the solution prepared similarly to the method used in the resistance analysis to the gastric condition. Incubation was carried out at 37 °C under 5 % CO_2 for 240 min. At the 0, 120, and 240 minutes of the incubation, the number of surviving strains was determined by the plate count method in MRS agar (Tavakoli et al., 2017).

Statistical analysis

All tests were performed in triplicate (n=3). The results were statistically evaluated using SPSS software (SPSS Inc., Chicago, IL) at a 5 % significance level. Means were compared using Duncan's test with significance level $p<0.05$.

Results and discussion

EPS producing capable and antimicrobial activity of LAB isolates

In the study, 30 LAB isolates were tested for EPS production and antimicrobial activity. It was determined that 4 of these LAB isolates had ropy or mucoid characteristics and were selected as EPS-producing strains. All 5 of these isolates were isolated from tarhana. In addition, 5 of these 30 isolates were screened for antimicrobial activity against selected pathogenic microorganisms, and it was determined that all of them, 4 of which were previously selected as EPS producing isolates, had antimicrobial activity. Therefore, 5 samples were selected for the continuation of the study. These isolates were identified by 16S rDNA sequence and pheS gene sequence in a previous study by Özel (2012).

Four isolates, identified as *Lactiplantibacillus plantarum* CL6, *Lp. plantarum* KL2, *Levilactobacillus brevis* EL1 and *Lactococcus lactis* CL3 presented a stable mucoid/ropy character. These four ropy strains were selected as EPS-producing. When the antimicrobial activities of five isolated species were tested against six foodborne pathogen indicator strains, *Lc. lactis* CL3, *Lp. plantarum* KL2 and BL4, showed especially strong antibacterial activity against all pathogenic strains. Overall, the antimicrobial activity of five isolates against at least four of the test microorganisms was observed. All tested isolates showed activity against both bacteria and yeast. The five isolates with antimicrobial activity and/or the ropy EPS producing strains were suitably chosen for further experiments. Antimicrobial and EPS-producing strains are summarized in Table 1B.

Quantification of EPS

EPS amounts of the selected strains are shown in Fig. 1A. The CL6 strain had the highest amount of EPS with a value of 0.96 g L^{-1}, followed by the CL3 strain at 0.64 g L^{-1} ($p<0.05$). Among the EPS contents of other strains, that of the *Lp. plantarum* BL4 strain was quite low.

Zehir-Şentürk et al. (2020) reported; similarly, the EPS production amounts of *Lp. plantarum* strains isolated from tarhana samples were determined in the range of 0.12-0.40 g L^{-1}. In the present study, it was shown that the results were considerably higher than these values. This situation showed that even the same species of microorganism strains which obtained from the same type

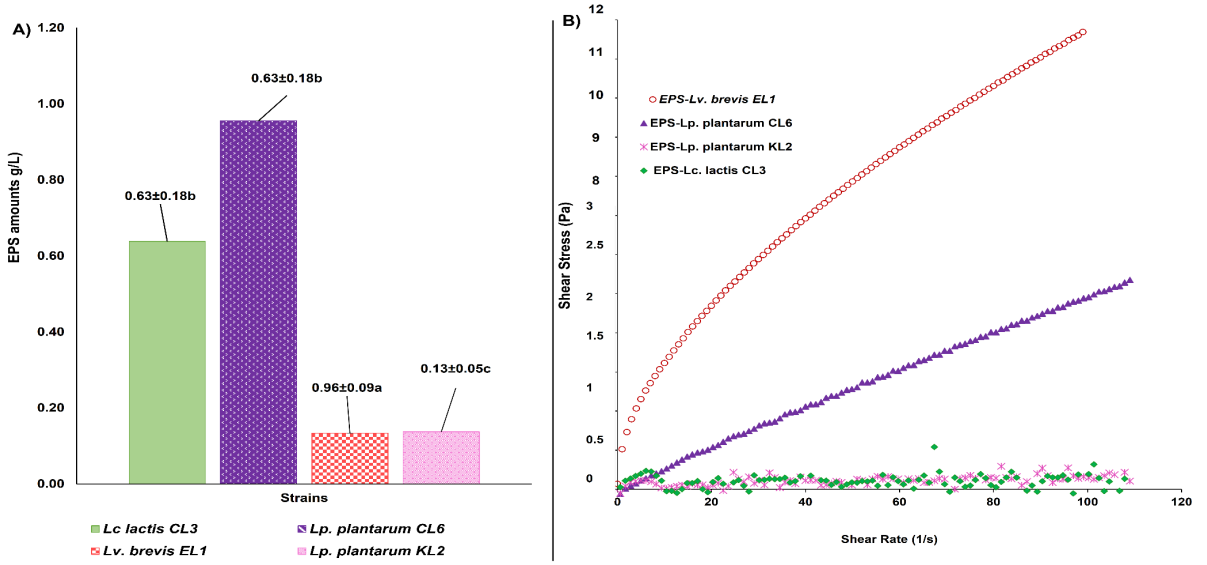

Figure 1. The amount of exopolysaccharides produced by the selected LAB-strains (A) and Apparent viscosity; shear stress change rheogram depending on the shear rate of the EPSs produced by the isolates (B)

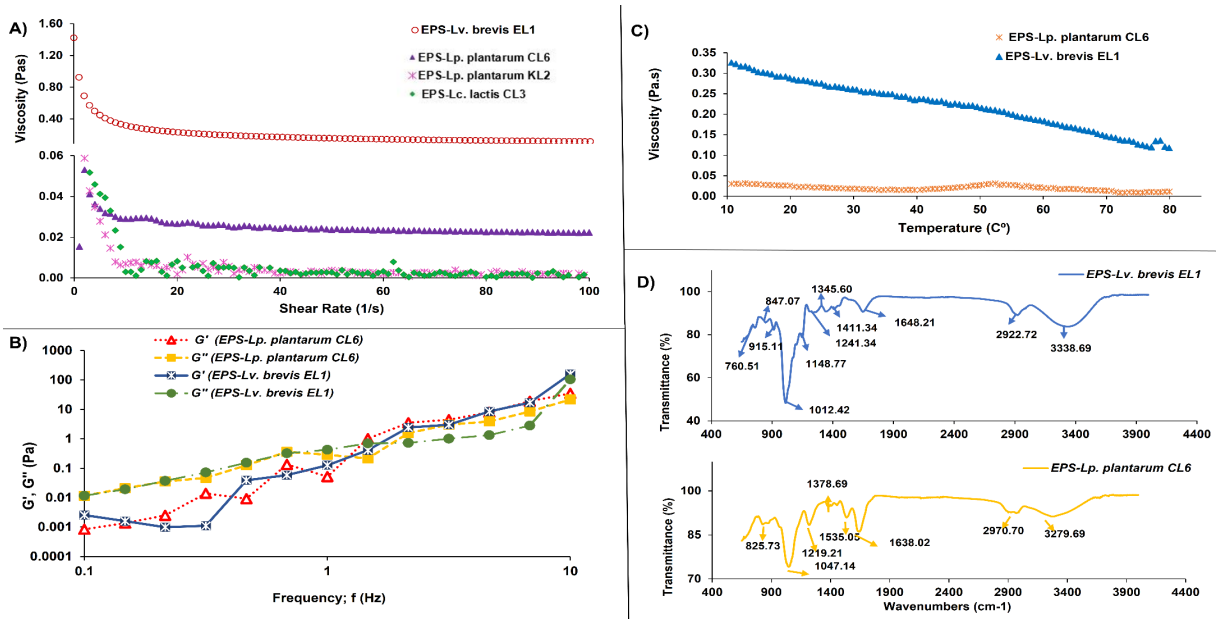

Figure 2. Viscosity change rheogram depending on the shear rate of the EPSs produced by the isolates(A); storage G' and loss G"(B); temperature-dependent behavior of the EPS-Lp. plantarum CL6 and EPS-Lv. brevis EL1 (C); Fourier-transform infrared (FT-IR) spectra of the EPSs produced by the isolates (D)

of fermented foods can have different EPS production levels. In a study conducted by Tok and Aslim, (2010) the EPS produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* strains was examined. In this study, the amount of EPS was determined to be between 0.027-0.211 g L^{-1}. And, in other a study conducted by Looijesteijn et al. (2001) a *Lc. lactis* strain was found to produce 0.40 g L^{-1} EPS.

It has been determined in the literature that many strains belonging to *Lc. lactis*, *Lv. brevis* and especially *Lp. plantarum* species have the ability to produce EPS (Looijesteijn et al., 2001; Fukao et al. 2019; Zehir-Şentürk et al. 2020; Hao et al., 2025). In addition, many of these strains were isolated from fermented products such as tarhana cheese, as in this study. This indicates that fermented products are a rich source of EPS producing LAB strains.

All five isolates were screened for their antagonistic activity against *E. coli* ATCC 25922, *B. cereus* NRRL B209, *S. aureus* ATCC 33862, *L. monocytogenes* ATCC 7644, *C. albicans* ATCC 10131 and *S. cerevisiae* ATCC 9763. All of them showed positive activity against one or more tested strains. However, the EL1 and CL3 strains showed the highest zone of inhibition against all the yeasts targeted in this study. This strain and the BL4 strain showed activity against all pathogenic indicators. CL6 and EL1 strains did not show any inhibitory activity against *L. monocytogenes* ATCC 7644.

In the literature, LAB strains with antimicrobial activity are frequently isolated from fermented products. Since the basis of the fermentation process is to transform the food into another structure that can be stored for a long time without spoiling, LAB strains with antimicrobial activity are highly likely to be found in fermented products (Purutoğlu et al., 2020).

Rheological properties of the produced-EPS

Since it is important to foresee the areas where EPS can be used in the food industry, the rheological properties of the isolated EPS were determined. The rheograms of the shear stress variations of the EPS samples depending on the shear rate are shown in Fig. 1B. The shear stress of EPSs produced by strains EL1 and CL6 behaves inconsistently with the shear rate, as it initially increases but eventually decreases with further increase in shear rate. The solution of the EPS-EL1 showed shear thinning behavior, which is characteristic of pseudoplastic fluids. Mayonnaise is the best-known example of a shear thinning system. The solution of the EPS-CL6 in the concentration showed shear thinning behavior with a very slight slope. At the same time, the solutions of the tested EPS-KL2 and the EPS-CL3 also indicated shear thinning behavior with a quite slight slope. However, the flow behaviors of the EPS-KL2 and the EPS-CL3 are insignificant as seen in Fig. 1B.

In Fig. 2A, the viscosity values of the EPS samples change as the shear rate increases. The increase in viscosity is related to the proportion of monosaccharides, due to their higher water-binding capacity. These EPS producing strains with the EPS flows properties are important for industrial applications. Inclusion such as EPS producing strains will have a positive impact on certain desirable properties.

Wang et al. (2015) have reported that the reduction in viscosity of EPS concurs with loosened polymer structure which related to the decreased interactions between the EPS molecules as the temperature increases. It is known that hydrodynamic forces generated during the shear can led to the breakdown of the structural units and the physical networks EPS structures (Zarour et al. 2017; Zhang et al., 2025). Therefore, the shear-thinning data presented suggest that the EPS studied would be very suitable to improve the texture and palatability of food products. Therefore, in the current study, the EPSs produced by the EL1 and CL6 strains attracted attention. The apparent viscosity (η) of the EPS sample was determined as 23.68 mPas and 157 mPas for the EPS-CL6 and the EPS-EL1 at a 50 s^{-1} (shear rate) (Fig. 2A), and the 50 s^{-1} is an appropriate estimation of oral shear rate for food samples.

As for viscoelastic properties of the EPS-EL1 and EPS-CL6 (Fig. 2B and 2C), the variations of elastic (storage module; G') and viscous (loss modulus; G") moduli of these EPS samples with frequency are shown in Fig. 2A. The storage modulus (G') quantifies the energy retained within the material's structure, reflecting its elastic nature. In contrast, the loss modulus (G") represents the material's viscous characteristics, where energy dissipation occurs due to deformation and internal friction. Since these rheological evaluations are conducted under the linear conditions, it was necessary to perform a preliminary linear viscoelastic region (LVR) scan to ensure the integrity of the sample was not compromised. For these EPS samples, the LVR was identified within a strain range of less than 1%.

Both G' and G" exhibited a continuous increase with frequency. This variation indicates a shift in the viscoelastic behavior of the material: at lower frequencies, the material's behavior is predominantly viscous (G">G'), while at higher frequencies, it becomes predominantly elastic (G'>G"). The point at which the G' and G" curves intersect, known as the cross-over frequency, marks the transition from liquid-like to solid-like behavior. Consequently, polymers can be characterized by the frequency at which G' equals G", referred to as the cross-over frequency and by the plateau value of G' observed at high frequencies, where the tan delta (δ) is less than 1.0.

In this study, G' was not observed to be lower than G" at frequencies below 9.03 Hz for these aqueous EPS solutions, indicating a predominance of viscous behavior over elastic behavior. This does not suggest that the sample exhibits liquid-like properties at low frequencies, with tan delta (δ) greater than 1.0. Overall, both modules of each ESP increased with frequency; G' exceeds G", indicating a shift towards elastic properties at higher frequencies.

Another factor affecting the EPS structure is temperature. In this study, the apparent viscosity of the EPS-EL1 solution decreased significantly with increasing temperature (Fig. 2C). This showed that the thinning properties of the EPS-EL1 solution decreased with increasing temperature. This was mainly due to decreased molecular flexibility at low temperatures. This result means that the thermal energy is sufficient to break down the polymer structure. The findings indicate that the EPS-EL1 is not suitable for high thermal processes. On the other hand, it was observed that the apparent viscosity of the EPS-CL6 solution, although low, did not change significantly with increasing temperature. This showed that the thinning properties of the EPS-EL1 solution did not change with temperature.

FTIR analysis of produced EPS

FTIR spectra of EPS produced by the bacterial strains were presented in Fig. 2D. The FTIR spectra showed characteristic peaks ranging from 760.51 to 3338.69 cm^{-1} which are regarded as the fingerprint regions of EPS. Peaks in the spectral range 813.92-973.26 cm^{-1} indicate α-D-glucosidic linkages. These spectral features revealed the presence of monosaccharides and polysaccharides (Sasikumar et al., 2017). Peaks near 1012.42 cm^{-1} and 1047.14 cm^{-1} were assigned to the polymer (Abinaya et al., 2018); a stretched peak at 1345.60 to 1535.05 cm^{-1} corresponds to the C-O, R-COO-R group; the absorption peaks ranging from 1148.77 cm^{-1} to 1241.34 cm^{-1} revealed the carbohydrates composition. At the peaks near 1012.42 cm^{-1} and 1047.14 cm^{-1}, the FTIR spectrum of EPS-L1 showed sharper specific absorbances compared to the FTIR spectrum of EPS-CL6. This means that the EPS-EL1 showed higher polysaccharides content than the EPS-CL6. These results agree with the earlier reports of Xu et al. (2019). A strong absorption at 1638.02 cm^{-1} and 1652.16 cm^{-1} showed the presence of carboxyl groups (Wang et al., 2014). Another peak at 2922.12 or 2970.70 cm^{-1} denoted the C=H, C=O, O=H stretching vibration. The peak corresponding to hydroxyl groups appeared in the range of 3279.69 - 3338.69 cm^{-1} (Dertli et al., 2016; Saravanan et al., 2016; Abinaya et al., 2018; Wang et al., 2014). These findings confirmed that the samples to be EPS.

Monosaccharide composition of EPS

EPSs of the productive strains revealed a variation in their sugar composition. It consists of more than one sugar such as maltose, glucose, galactose, and fructose. The sugar compositions and the bacteria that produce them are given in Table 1C. All strains in this case had glucose and galactose in their sugar composition. Glucose was the most dominant sugar in the EPS produced by EL1and KL2 with 15.87 and 19.71 mg mL^{-1}, respectively. The gluco-galactose ratio demonstrates an inverse relationship in all the cultured EPSs ($p<0.05$). Maltose and fructose were not present in EPS produced by CL3 and CL6. However, these strains produced EPS with relatively high percentages of galactose. Maltose is a disaccharide and ranged from 0.58 to 0.61 mg mL^{-1} of EPSs produced by both EL1 and KL2. It is clearly suggested that these two strains share the same EPSs. Variations in the ratio of sugar among strains ($p<0.05$)

reflect changes in the compositional diversity; and different molecules contribute to the variation among isolates variation in their ability to produce specific EPSs. The amount of EPS produced by LABs was similar to literature (Ispirli et al., 2019; Li et al., 2020; Zehir-Şentürk et al., 2020; Wang et al., 2025). The presence of different monomers detected in the EPSs of all LABs revealed that these EPSs are heteropolysaccharides. This characteristic is mostly found in other lactic acid bacteria (Sungur et al., 2017).

The antibiotic susceptibilities of the selected LAB strains

In the study, seven different antibiotic discs were used for the LAB strains. The antibiotic-susceptibility pattern of the LABs to different antibiotics is shown in Table 2A. According to the results, the BL4, the CL3 and the KL2 strains showed the highest sensitivity against amoxicillin, followed by ampicillin. These strains showed the highest sensitivity to metronidazole and vancomycin, followed by clindamycin except for CL6. The CL6 also shows high resistance to clindamycin. Antibiotic resistance should be low to use microorganisms in starter cultures or foods for different purposes. *Lactobacilli* can function as reservoir for antibiotic resistance genes and may be susceptible to gene transfer for other bacteria. Tetracycline resistance of *L. lactis* strains was due to *tet*(S) and *tet*(M) (Walther et al., 2008). Egervärn (2009) revealed that acquired tetracycline resistance of *Lp. plantarum* strains was related to plasmid-bound *tet*(M). *Lc. lactis* acquired the *tet*(M) gene due to gene transfer from *Lp. plantarum* at a pork abattoir (Toomey et al., 2010). *Lp. plantarum* was intrinsically resistant to vancomycin and streptomycin (Walther et al., 2008; Toomey et al., 2010). The erythromycin -resistant Lc. lactis and *Lp. plantarum* harbored *erm*(B) gene (Walther et al., 2008; Shao et al., 2015). In a study conducted by Wakil and Olorode (2018), three strains of *Lp. plantarum* created 28, 36 and 49 mm zones against the amoxicillin. However, a strain of the same species did not form an inhibition zone. In this study, tested strains showed greatest sensitivity against amoxicillin and ampicillin.

Generally, most lactobacilli are intrinsically resistant to aminoglycosides (streptomycin and gentamycin), glycopeptides (vancomycin), inhibitors of nucleic acid synthesis (ciprofloxacin) and inhibitors of folic acid synthesis (trimethoprim). However, they are susceptible to penicillins, chloramphenicol, streptomycin, tetracycline and erythromycin (Abriouel et al., 2015).

Effects of different conditions on the growth of the selected LAB strains

The effect of different temperatures, pH and NaCl concentration on the growth of LAB strains was represented in Table 2B. All the strains had wider adaptability and were able to grow at 15-45 °C, 2-4 pH, and at 3-9 % salt conc. For the five isolates tested, the best growth rate was achieved at 37 °C, pH 4, and 5 % salt concentration under optimum conditions. Similarly, growth at 30 °C and 3% NaCl concentration was not significantly different from that at

Table 2. *Antibiotic susceptibility of LAB isolates (A), Growth characterizations of tested isolates at different salt (NaCl), temperature, and pH values (B)*

A. Diameter of inhibition zone(mm)*							
Strains	**TE**	**MET**	**DA**	**AM**	**E15**	**VA**	**AMC**
Lp. plantarum BL4	22.7±0.11^{c}(S)	ND	18.2±0.23^{b}(M)	41.2±0.13^{b}(S)	33.5±0.40^{b}(S)	ND	47.2±1.10^{ab}(S)
Lp. plantarum CL6	29.6±0.85^{a}(S)	ND	45.1±1.14^{a}(S)	45.8±0.21^{a}(S)	31.2±1.14^{c}(S)	ND	40.5±0.76^{b}(S)
Lp. plantarum KL2	25.3±1.07^{b}(S)	ND	16.4±0.69^{c}(M)	40.7±0.71^{b}(S)	31.7±0.27^{c}(S)	ND	46.4±1.24^{ab}(S)
Lv. brevis EL1	23.2±0.54^{c}(S)	ND	18.3±0.35^{b}(M)	39.4±0.18^{b}(S)	38.3±2.03^{a}(S)	ND	39.2±0.62^{b}(S)
Lc. lactis CL3	30.2±1.23^{a}(S)	ND	17.8±0.65^{bc}(M)	45.3±0.67^{a}(S)	34.8±0.85^{b}(S)	ND	50.9±0.32^{a}(S)

**TE: Tetracycline (30 µg), MET: Metronidazole (5 µg), DA: Clindamycin (2 µg), AM: Ampicillin (10 µg), E_{15}: Erythromycin (30 µg), VA: Vancomycin (30 µg), AMC: Amoxicillin (20/10 µg). ^{a,b} Means at each column with different superscripts lowercase are significantly different (p<0.05). Results provided as average (n=3) ± SD (standard deviation). Also; Low;11-15 mm, Moderate(m); 15–20 mm, Sensitive(S); >21 mm.*

B. Growth characterizations of tested isolates at different salt (NaCl), temp. and pH values**												
Salt (NaCl)				**pH**				**Temperature (°C)**				
3 %	**5 %**	**7 %**	**9 %**	**2.0**	**3.0**	**3.5**	**4.0**	**15 °C**	**30 °C**	**37 °C**	**40 °C**	**45 °C**
1	1	4	5	5	5	3	1	2	1	1	2	3
1	1	3	3	5	4	1	1	3	1	1	2	3
1	1	3	6	4	2	2	1	3	1	1	2	3
1	1	3	3	3	2	1	1	4	1	1	1	1
1	1	2	3	5	5	2	1	3	1	1	2	2

**The difference between samples marked with different lowercase letters (a,b,c,d,) in the same column is significant (p<0.05). ND not determined. **: The days of incubation on which the growth of the strains was observed, is given in parentheses. Early development shows high resistance and adaptation to the environment.*

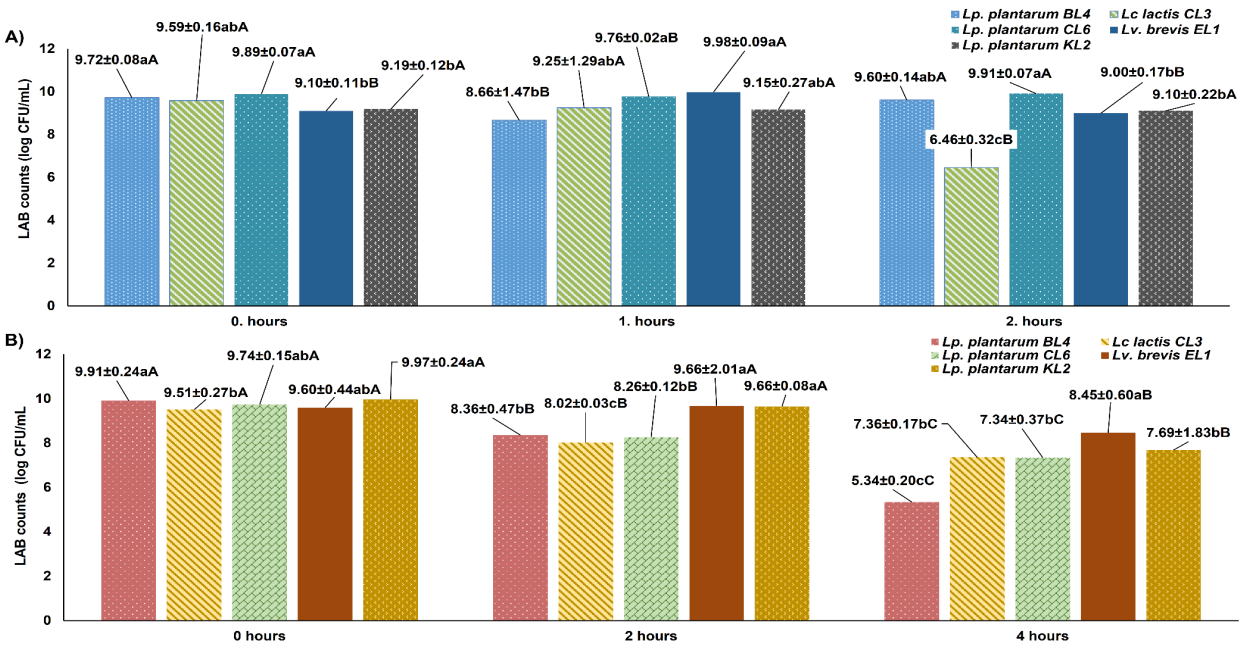

Figure 3. *The resistances of the selected LAB strains to the simulated gastric condition (A) to the simulated intestinal condition (B)*

37 °C and 3 % NaCl concentration. The growth rate of all isolates greatly reduced at 15 °C, 3.5 pH and 7 % salt concentration invariably to 75 % or less of that achieved at 30 °C, 4.0 pH and 5 % salt concentration respectively. EL1 showed growth at 40-45 °C and 3.5 pH and CL6 showed growth at 3.5 pH during one day of cultivation. The growth of the others was preceded by a lag period of 2-3 days. Only two of the five isolates (KL2 and EL1) were able to grow at pH 3 over the 2 days of observation. For the other three isolates, was detected in a period of 4-5 days. These results suggested that EL1 and KL2 could be used as starter and protective culture, as they exhibited heat stably, a wide pH range, antibacterial activity and EPS production.

In a study conducted by Ertekin and Çon (2014), the growth of 14 *Lp. plantarum* and one *Lv. brevis* strain was tested at different temperatures, pH and salt concentration values. All of these strains grew at 15 °C, however; one strain growth only at 45 °C and 11 strains grew at 6 °C. One strain of *Lv. brevis* species tested did not grow at only 45 °C. In the same study, only one of the *Lp. plantarum* strains grew at 8 % and 9 % salt concentrations. All strains grew at all salt concentrations below these values. These results are similar to those of the present study. The strains grown in the same study showed growth at pH 3.5 and above; however, only one strain of *Lp. plantarum* species grew at pH 3. However, in this study, incubation was applied at 24-48 h. On the other hand, in a study conducted by Sagdic et al. (2014) All 20 *Lp. plantarum* strains and 2 of 3 *Lv. brevis* strains grew at pH 2.5.

The survival of the selected LAB strains in the simulated gastric-intestinal conditions

The survival of these five strains to simulated gastrointestinal environment and conditions was analyzed. This test (*in vitro*) is one of the most important tests required to identify a potential probiotic isolate. The results of survival under simulated gastric and intestinal fluid are summarized in Figs. 3A and B. After 120 min of incubation at pH 2.0, the BL4, CL6, EL1 and KL2 were able to survive above 90 %. Less than 3 log reduction of cell viability occurred for the CL3. However, survival rates remained above 67 % after 2 h of simulated gastric juice treatment. Compared to the survival of the *Lactococcus* strain, the other strains exhibited high tolerance to artificial gastric conditions (Varsha et al., 2014; Zeghad et al., 2025; Iliev et al., 2025). After 4 h of exposure to bile salts, the isolates exhibited different levels of survival. Compared with the initial number of cells the reduction varied significantly among all strains. The EL1 strain had slightly less than a 2 log reduction. Just one strain BL4, possessed lower tolerance to bile salt ($<10^6$ log at 4 h). Similarly, in one study (Kiliç-Kanak and Yilmaz, 2023), *L. plantarum* SM27, *L. plantarum* S74 and *L. paracasei* RU39-7 strains were found to have the best probiotic properties. Hence, survival of the tested isolates at gastrointestinal simulation environment conditions could be considered potential probiotic candidates for application.

Conclusion

This study investigated the valuable functional properties of LAB isolates from tarhana for their potential use in the dairy industry. Among these isolates, the *Lp. plantarum* BL4, CL6 and KL2, *Lv. brevis* EL1 and *Lc. Lactis* CL3 were selected as strongs EPS producers. Especially EPS production ability is a very important feature for strains recommended for use in the dairy industry. When the EPS structures produced by the strains were examined, the EPS-EL1 obtained from the *Lv. brevis* EL1 showed shear-thinning behavior. It was determined that EPS-EL1 had a significant rheological contribution to the dairy industry applications. When the resistance of the tested strains to the stimulating gastrointestinal simulation system was evaluated together, the strains, especially *Lp. plantarum* CL6 and *Lv. brevis* EL1 stood out. However, except for *Lp. plantarum* BL4 and *Lc. Lactis* CL3, their viability rates were quite good. This situation shows that they can be preferred as potential probiotic candidate isolates for new functional products to be produced in the dairy industry. Moreover, *Lv. brevis* EL1 showed high antimicrobial activity against the bacterial pathogen *E. coli* ATCC 25922, while *Lp. plantarum* BL4 and *Lc. lactis* CL3 showed high antimicrobial activity against the yeast pathogen the *C. albicans* ATCC 10131. This shows that the *Lv. brevis* EL1 isolate is highly suitable for different functions, especially for co-culture, as an adjuvant culture for the dairy industry.

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Potencijal sojeva *Lactiplantibacillus plantarum*, *Levilactobacillus brevis* i *Lactococcus lactis* kao funkcionalnih kultura za mljekarsku industriju

Sažetak

Funkcionalna svojstva bakterija mliječne kiseline (BMK) koje se prenose hranom povezana su s njihovim metabolitima. U ovom radu istražena su najvažnija funkcionalna svojstva izolata BMK izoliranih iz različitih fermentiranih namirnica. To uključuje prvenstveno sposobnost proizvodnje egzopolisaharida (EPS-a) i svojstva njihovih struktura, antimikrobnu aktivnost i otpornost na simulirani gastrointestinalni sustav, što je jedan od važnih testova za koncept potencijalnih probiotičkih izolata. Među izolatima, utvrđeno je da su BL4, CL6 i KL2 koji pripadaju vrsti *Lactiplantibacillus plantarum*, EL1 koji pripada vrsti *Levilactobacillus brevis* i CL3 koji pripada vrsti *Lactococcus lactis* dobri proizvođači EPS-a. Kada su ispitane EPS strukture koje proizvode istraživani sojevi, posebno EPS-EL1, vidljiv je potencijal pseudoplastičnih svojstava tekućina koji bi mogao pružiti značajan reološki doprinos za primjenu u mljekarskoj industriji. Štoviše, ovaj soj, kao i soj CL6, pokazao je visoku antimikrobnu aktivnost prema patogenoj bakteriji *E. coli* ATCC 25922 i visoku otpornost na gastrointestinalni simulacijski medij. Osim toga, utvrđena je visoka antimikrobna aktivnost sojeva *Lp. plantarum* BL4 *i Lc. lactis* CL3 protiv patogenih kvasaca *C. albicans* ATCC 10131. Rezultati upućuju na mogućnost korištenja soja *Lv. brevis* EL1 za različite fermentacije u obliku čiste ili mješovite kulture u mljekarskoj industriji. Ovih 5 istraživanih sojeva imaju različita svojstva za različite procese fermentacije te se mogu koristiti u mljekarskoj industriji.

Ključne riječi: egzopolisaharid; antimikrobno sredstvo; *Levilactobacillus brevis*; *Lactiplantibacillus plantarum*; *Lactococcus lactis*; potencijalni probiotički kandidat

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