BİTLİS EREN ÜNİVERSİTESİ
FEN BİLİMLERİ DERGİSİ

ISSN: 2147-3129 / e-ISSN: 2147-3188

Article Type : Research Article
Received : May 9, 2025
Revised : September 28, 2025
Accepted : September 29, 2025
DOI : 10.17798/bitlisfen.1696097

Year : 2025
Volume : 14
Issue : 3
Pages : 1672-1687

ANTIBIOTIC RESISTANCE, ANTIBACTERIAL ACTIVITY AND EXOPOLYSACCHARIDE DETERMINATION OF *LACTIPLANTIBACILLUS PLANTARUM* OBTAINED FROM TRADITIONAL YOGURTS FROM MUŞ REGION

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ABSTRACT

In this study, 117 natural yoghurt samples were randomly collected from different regions of Muş. After preliminary identification (colony morphology, catalase, gram staining) of suspected *Lactiplantibacillus plantarum* strains isolated from yoghurt samples, molecular identification was performed by PCR method using *recA* gene sequence. Antibacterial activities and antibiotic resistance of 40 identified *L. plantarum* strains were determined. While 42.5% of the isolates were resistant to all antibiotics, three isolates (25, 35 and 36) showed the highest sensitivity. It was determined that strains 5 and 23 showed the best antibacterial activity against seven different pathogenic bacteria. Exopolysaccharides (EPS) synthesized by *L. plantarum* strains were determined qualitatively and quantitatively. It was determined that the isolates produced EPS between 318.30±1.28 mg/L (*L. plantarum* 23) and 205.70±1.44 mg/L (*L. plantarum* 16). Considering the resistance of the isolates in this study to antibiotics, their antibacterial effects on pathogenic microorganisms and EPS synthesis amount data, it was determined that *L. plantarum* isolates 5 and 23 exhibited good characteristics. Therefore, it is believed that isolates that demonstrate good properties (probiotic, anticancer, and antioxidant activity, etc.) as a result of in *vivo* and in *vitro* studies conducted with these isolates will contribute to the food industry and human health.

Keywords: *recA*, Antibiotic Resistance, Antibacterial Activity, Exopolysaccharide.

1 INTRODUCTION

One of the most popular fermented dairy products is yogurt. Pasteurized milk is combined with lactic acid bacteria, specifically *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, under controlled conditions to produce yogurt [1]-[3]. Additionally, other *Lactobacillus* species, including *L. casei*, *L. acidophilus*, *L. plantarum*, and *L. rhamnosus*, may also be incorporated into the yogurt production process [5]. These microorganisms ferment lactose, the primary sugar in milk, resulting in the formation of compounds such as acetaldehyde, diacetyl, carbon dioxide, acetic acid, lactic acid, and various other metabolites that contribute to the distinctive flavor and aroma of yogurt [6]. *L. plantarum* can be isolated from milk and dairy products, meat and meat products, pickles, silage, human lips and digestive system, and fermented plants in nature [7], [8].

Yogurt bacteria produce nisin, known as a natural antibiotic, and lactic acid, which also has antimicrobial activity, which are active against *Escherichia coli*, *Salmonella*, *Shigella*, *Listeria* and *Candida*. These bacteria colonize the intestinal system against harmful microorganisms. Due to the competitive activity of yogurt bacteria and the antimicrobial substances formed, they have a protective effect against infections that cause urogenital and gastrointestinal diseases [1]. Industrially important properties of *L. plantarum* strains such as fermenting various carbohydrates, resistance to antibiotics, ability to grow in acidic environments, production of lactic acid, exopolysaccharide (EPS), bacteriocin, and inhibition of pathogens are also being investigated [9].

Exopolysaccharides (EPS) are high molecular weight carbohydrate polymers synthesized by bacteria and possess nutritional compounds and protection mechanisms against bacteriophages [4]. EPS possess various health beneficial properties such as antioxidant, antibacterial, hypoglycemic, immunomodulatory, and cholesterol-lowering properties [38]. In recent years, many efforts have been made to select LAB and bifidobacteria with high EPS yields for application in yogurt production [39]. Due to differences in LAB qualities, evaluation of strains from various sources is crucial for identifying effective and efficient strains with desired properties for the yogurt industry. Therefore, screening, identification, categorization, and decision-making of strains are important [40]. The EPS-producing ability of LAB and the amount of EPS are largely species- and strain-specific [41].

Therefore, in our study, molecular identification of suspected *L. plantarum* strains isolated from 117 yoghurt samples traditionally produced in Muş region was performed by PCR

method using phenotypic and *recA* gene sequence. The resistance and susceptibility of suspected *L. plantarum* strains to erythromycin, kanamycin, penicillin, chloramphenicol, gentamicin, and trimethoprim were determined, taking into account the antibiotics mechanisms of action. In addition, antibacterial activities of suspected *L. plantarum* strains against pathogenic bacteria *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeroginosa*, *Bacillus megaterium* and *Staphylococcus aureus* were detected. In addition, the presence of EPS synthesized by these strains was reported by qualitative and quantitative methods. It is thought that *L. plantarum* strains showing good characteristics, based on the results obtained and the studies to be carried out, will directly contribute to the food industry by being used in the yoghurt production process and will indirectly make significant contributions to human health.

2 MATERIALS AND METHODS

2.1 Yoghurt Samples and *L. plantarum* Isolation

The 117 yogurt samples used for the isolation of *L. plantarum* were randomly collected from families who traditionally make yogurt in different villages of the Muş region. The collected yogurt samples were delivered to the laboratory in the cold chain as soon as possible under sterile conditions and the isolation process was performed. 5 g of each yogurt sample was weighed and centrifuged in 5 mL of sterile physiological water at 3500 g for 2 min. 1 mL of the resulting supernatant was centrifuged at 10000 g for 30 s, and 1 mL was taken from the resulting supernatant and centrifuged at 10000 g for 15 min. At the end of the period, the sediment remaining in the tube was dissolved with 900 µL of sterile physiological water and serial dilutions up to 10-5 were prepared. For the isolation of LAB from the prepared dilutions, the plates were inoculated on MRS agar and M17 agar petri dishes, respectively, and incubated at 37±2ºC for 24-48 hours. At the end of the incubation, the colonies that were formed and thought to be lactic acid bacteria colonies were taken and activated in the nutrient medium for stocking [10]. In all analyses except for the identification of *L. plantarum*, the *L. plantarum* STD strain supplied by Maysa Gıda-Lab (Tuzla-Istanbul) was used as a standard.

2.2 Phenotypic and *recA* Gene Identification of *L. plantarum* Strains

Preliminary phenotypic identification of the isolates obtained from yogurt was carried out based on colony morphology, Gram staining [11], microscopic characteristics, and the catalase test [12]. A total of 51 presumptive isolates, validated through preliminary assessments,

were cultured in MRS broth at 37 ± 2 °C for 24 to 48 hours. Following this enrichment phase, genomic DNA was extracted using the Genomic DNA Extraction Kit (Vivantis) and the extracted DNA samples were stored at –20 °C for further analysis. For PCR amplification of the recA gene, primers planF (5'-CCGTTTCTGCGGAACACCTA-3') and planR (5'-TCGGGATTACCAAACATCAC-3') were obtained from a commercial supplier [13].

2.3 Determination of Antibiotic Resistance Properties of Isolates

The antibiotic resistance profiles of *L. plantarum* isolates were assessed using the disk diffusion method. To this end, the susceptibility or resistance of the isolates to Penicillin (10 µg), Trimethoprim (25 µg), Erythromycin (10 µg), Gentamicin (10 µg), Chloramphenicol (30 µg) and Kanamycin (30 µg) (Oxoid) was evaluated. After storage at 4 °C, *L. plantarum* strains were reactivated by inoculating them into liquid MRS medium and incubating at 37 °C for 24 hours. Subsequently, 0.1 mL of the activated cultures, containing approximately 10^8 CFU/mL, was added to sterilized MRS agar that had been cooled to 45–50 °C and aliquoted into sterile test tubes. The inoculated medium was thoroughly mixed and poured into sterile 9.0 cm Petri dishes, with 15 mL dispensed per plate to ensure uniform distribution. Once the agar had solidified, antibiotic discs were aseptically positioned on the surface with appropriate spacing. The plates were pre-incubated at 4 °C for 2 hours, followed by incubation at 37 °C for 24 hours. Upon completion of incubation, the diameters of the inhibition zones were measured in millimeters to assess antibiotic susceptibility.

2.4 Antibacterial Activities of Isolates

The antibacterial activity of *L. plantarum* isolates was evaluated using the slurry agar diffusion method. To this end, the resistance and susceptibility profiles of various indicator bacteria *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 25923, *K. pneumoniae*, *B. subtilis* ATCC 6633, *E. aerogenes* ATCC 13048, *B. megaterium* DSM 32 and *E. coli* ATCC 11229 ATCC 13883 were examined. The *L. plantarum* strains were cultured in liquid MRS medium, while the indicator bacteria were cultivated in nutrient broth and incubated at 37°C for 24 hours to ensure activation [14]. Following incubation, bacterial suspensions with a turbidity equivalent to 0.5 McFarland standard were prepared in distilled water from 24-hour cultures of the indicator strains. These suspensions were uniformly spread on appropriate agar media using sterile swabs. Wells were then aseptically created in the agar using a sterile borer, and 100 µL of each *L. plantarum* isolate was dispensed into the wells. The plates were incubated for 24

hours at optimal temperatures for each indicator strain [15]. Finally, the diameters of the inhibition zones were measured in millimeters to assess antibacterial efficacy.

2.5 Qualitative-quantitative Determination of EPS

For exopolysaccharide (EPS) screening, a modified version of the test tube method originally developed by Christensen et al. was employed. *L. plantarum* strains were inoculated into MRS medium at a concentration of 1% and cultured in 6-well polystyrene plates at 37 °C for 24 hours. Following incubation, biofilms formed on the bottom and lateral surfaces of the wells were stained using crystal violet. Excess stain was removed by rinsing the wells with phosphate-buffered saline (PBS), after which residual spots were eliminated. The presence of a visible film on the base and walls of the wells was considered indicative of biofilm formation [16].

For EPS production assessment, the strains were cultured on Congo Red Agar (CRA) medium composed of 37 g/L BHI agar, 0.8 g/L Congo Red, and 36 g/L sucrose [17]. Strains were streaked onto CRA plates and incubated at 37 °C for 24–48 hours to distinguish EPS-producing from non-producing strains. Colonies exhibiting a black, dry, and crystalline texture were classified as EPS-positive, whereas pink, smooth colonies were deemed EPS-negative [18]. Pure cultures of *L. plantarum* were subsequently obtained, and quantitative EPS production was evaluated [19]. For this, activated cultures were incubated in MRS broth at 37 ± 1 °C for 24–48 hours. Cell pellets were then resuspended in 1 mL of sterile distilled water, and EPS content was quantified using the phenol-sulfuric acid method [20]. Absorbance measurements were recorded at 490 nm in triplicate. A standard glucose calibration curve, generated using glucose concentrations ranging from 0–100 mg/L, was employed to calculate EPS concentrations, expressed in mg/L, based on the corresponding absorbance values.

To further assess biofilm formation, bacterial suspensions standardized to approximately 1×10^8 CFU/mL (equivalent to 0.5 McFarland standard) were inoculated into BHI medium supplemented with 1% glucose. These were transferred to 96-well microtiter plates and incubated at 37 °C for 24 hours. After incubation, wells were washed with PBS and dried at 60 °C for one hour. To stain the biofilm layer, 150 µL of crystal violet was added to each well and left for 15 minutes. Excess dye was removed by washing with PBS, followed by drying. Then, 150 µL of methanol was added to solubilize the dye retained in the biofilm layer. Absorbance was measured at 590 nm using a microplate reader. Wells containing only BHI and 1% glucose served as negative controls and were analyzed in triplicate [21].

2.6 Statistical Analysis

The data obtained in this study are presented as mean values accompanied by their standard errors (mean ± SEM), and all graphical representations were prepared accordingly. Each analysis was conducted in a minimum of three replicates. Statistical comparisons were made between each *L. plantarum* strain and the reference *L. plantarum* STD strain. One-way ANOVA was performed followed by Dunnett's multiple comparison test to assess statistical significance. A p-value of less than 0.05 was considered statistically significant. Significance levels were denoted using asterisks as follows: $P < 0.05$ (significant, *), $P < 0.01$ (very significant, **), $P < 0.001$ and $P < 0.0001$ (extremely significant, ***), while $P > 0.05$ was considered not significant (ns).

3 RESULTS AND DISCUSSION

3.1 *L. plantarum* Isolation and Preliminary Identification

Gram staining reaction, colony morphology, and catalase activity of LAB isolates obtained as pure culture were examined. Colony appearances of isolates in petri dishes can be distinguished by white-cream color. Colony images formed after inoculated MRS agar petri dishes and 48 hours of incubation are given in Figure 1a. As a result of Gram staining, 125 isolates were determined to be Gram positive. Microscopic image of suspected *L. plantarum* obtained in Gram staining is given in Figure 1b.

LAB form hydrogen peroxide (H_2O_2) from oxygen derivatives formed as a result of oxidative stress, but due to the lack of catalase enzyme, they cannot break down hydrogen peroxide into oxygen and H_2O. Catalase feature is used as a simple method in LAB determination. After morphological examination, catalase test was performed on the isolates used in the study and it was determined that all 125 isolates were catalase negative. When the microscopic features of the strains were examined, it was determined that 51 bacteria were bacilli and the remaining 74 strains were cocci.

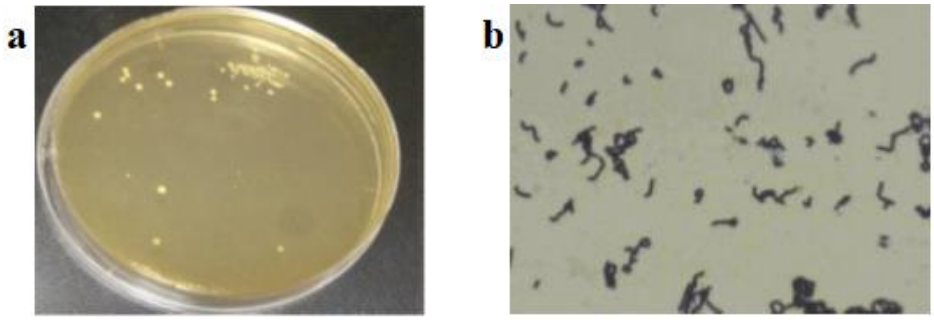

Figure 1. a: Colony image of lactic acid bacteria inoculated on MRS agar petri dishes. b: Gram stain image of lactic acid bacteria strain

The basis of the traditional classification in the identification of lactic acid bacteria is based on phenotypic methods including the investigation of morphological, physiological and metabolic/biochemical properties such as arginine degradation at different temperatures, pH values, development in salt concentrations and carbohydrate catabolism [22]. In our study, 125 LAB were isolated from 117 yogurt samples by considering these phenotypic methods and stored at -20°C to be used in subsequent studies. Table 1 provides information about the places where yoghurt samples were collected and the strains isolated.

3.2 Amplification of *rec A* Gene Regions of Isolates by PCR

The identification and characterization of LAB is gaining importance in scientific and industrial terms. In order to identify *L. plantarum*, in addition to classical methods that include determination of their physiological, morphological and biochemical properties, phage types and antibiotic susceptibilities and serological typing, molecular biology techniques such as PCR, ,DNA sequencing analysis, Restriction Fragment Length Polymorphism, Pulsed Field Gel Electrophoresis, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Restriction Analysis of Amplified rDNA have also been reported to be used [23]. Primer pairs (planF-planR) were synthesized to amplify the *recA* gene region by PCR for *L. plantarum*. The aim of the PCR reaction in *L. plantarum* species was to amplify the 318 bp region. PCR products amplified under PCR operating conditions were visualized on 1% agarose gel (Figure 2).

Table 1. Isolate numbers of lactic acid bacteria obtained from yogurt samples collected from different regions

Place	Number of Yogurts	Number of LAB Isolates	Number of *L. plantarum*
Karaağaçlı Town	25	23	8
Sungu Town	16	13	5
Tandoğan Village	11	11	2
Yarpuzlu Village	10	12*	7
Güzeltepe Village	13	12	5
Suvaran Village	9	14*	2
Sazlıkbaşı Village	33	40*	11
Total	117	125	40

** More than one microorganism was obtained from the same yoghurt sample. Only one colony was taken and stored.*

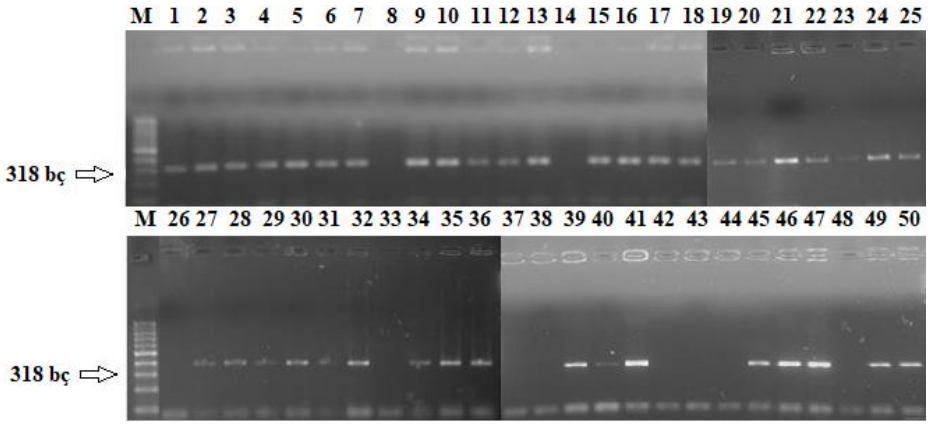

Figure 2. Agarose gel image obtained by PCR amplification of lactic acid bacteria isolated from natural yoghurts. M: 100 bp DNA standard

In the molecular identification of different Lactobacillus species *L. paraplantarum*, *L. plantarum* and *L. pentosus*, *recA* gene region primers were used. According to PCR results, recA gene region primers copied a 318 bp region for *L. plantarum*, while this gene copied a 107 bp region for *L. paraplantarum* and a 218 bp region for *L. pentosus* [24]. The isolated LAB were identified using the *recA* gene region primer by molecular method. As a result of the PCR identification of the obtained isolates, a total of 40 suspicious *L. plantarum* isolates giving a DNA band at 318 bp were obtained. In the study, isolates that could be *L. plantarum* strains were identified and confirmed by biochemical and molecular methods. It was determined that the obtained findings were equivalent to the literature information.

3.3 Antibiotic Resistance Properties of Isolates

In this part of the study, the resistance-sensitivity results of the isolated bacteria to 6 different antibiotics such as Erythromycin (15µg), Kanamycin (30µg), Penicillin (10µg), Chloramphenicol (30µg), Gentamicin (10µg), Trimethoprim (5µg) were determined. The zone image of *L. plantarum* isolates showing resistance and sensitivity to antibiotics is given in Figure 3a and the heat graph created with the obtained data is given in Figure 4.

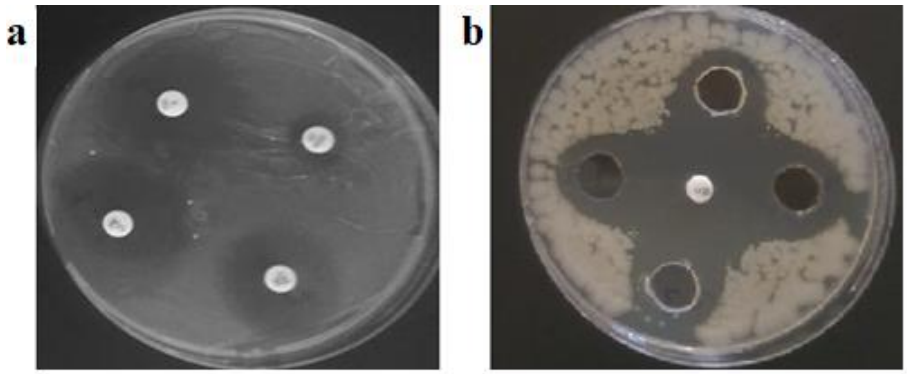

Figure 3. a: Zone images obtained as a result of determining the antibiotic resistance properties of the isolates by the disk diffusion method. b: Zone images obtained from the well agar method of determining the resistance properties of isolates on other bacteria

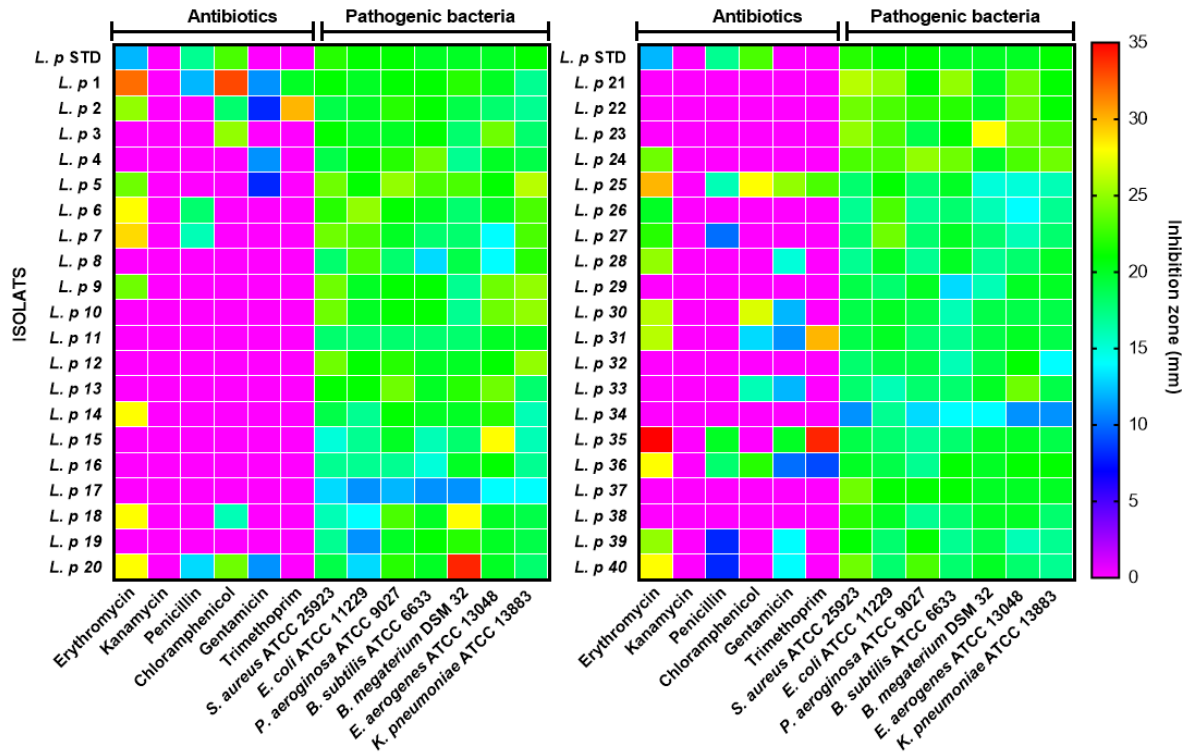

Figure 4. The antibiotic resistance profiles and antibacterial activities of L. plantarum STD and L. plantarum strains were evaluated

L. plantarum strains 8, 10, 11, 12, 13, 15, 16, 17, 19, 21, 22, 23, 29, 32, 34, 37 and 38 were found to be resistant to all antibiotics. It was determined that these strains corresponded to 42.5% of the total isolates. It was determined that *L. plantarum* isolate 35 was most sensitive to erythromycin and trimethoprim and resistant to kanamycin and chloramphenicol. Among the isolates, strains 25, 35 and 36 showed the highest sensitivity to antibiotics. When the resistance-sensitivities to each antibiotic were evaluated separately, *L. plantarum* isolate 36 showed a highly significant difference from *L. plantarum* STD against chloramphenicol. In addition, *L. plantarum* 2, 31 and 35 isolates showed a highly significant difference against trimethoprim ($P<0.0001$).

They stated that the antibiotic resistance profiles of *Lactobacillus*, *Lactococus* and *Bifidobacterium* were quite different [25]. They found that some LAB showed high natural resistance to vancomycin, cefoxitin, kanamycin, ciprofloxacin, fusidic acid, nitrofurantoin gentamicin, streptomycin, metronidazole, norfloxacin and bacitracin. However, they stated that LAB were generally sensitive to penicillin (piperacillin and ampicillin) and ß-lactamase inhibitors, but very resistant to oxacillin and cephalosporins [26]. In studies, Lactobacilli are generally sensitive to antibiotics that inhibit protein synthesis, such as erythromycin, clindamycin, chloramphenicol and tetracycline, and resistant to aminoglycosidases, such as gentamicin, streptomycin, neomycin and kanamycin [27]-[29]. The susceptibility of LAB

isolated from yogurt to chloramphenicol, erythromycin, penicillin G, gentamicin, vancomycin, streptomycin, kanamycin, and tetracycline was determined [42]. Some researchers also found that *Leu. mesenteroides* strains were resistant to tetracycline and streptomycin [43]. All isolates in this study were resistant only to kanamycin, and the resistance and susceptibility of the isolates to antibiotics varied.

3.4 Effects of Isolates on Other Microorganisms

The antibacterial effects of *L. plantarum* isolates isolated from yogurt were determined by the slurry agar diffusion test. The zone image of *L. plantarum* isolates showing resistance and susceptibility properties against the test bacteria is given in Figure 3b and the heat graph created with the obtained data is given in Figure 4. High general inhibitory effects of *L. plantarum* strains on test bacteria were seen on *E. aerogenes*, *S. aureus*, *P. aeruginosa*, *B. megaterium*, *E. coli*, *K. pneumoniae* and *B. subtilis* respectively. When evaluated statistically, *L. plantarum* strains 5, 9, 10, 12, 21, 22, 23 and 24 showed highly significant differences due to their good activities against test bacteria ($P<0.0001$). Statistically, the ranking of these strains is as follows; 5=23>21 >24 >22 >12 >10 >9. The isolate showing the least antibacterial effect on all pathogenic bacteria used in this study was isolate number 34. It was determined that strains 5 and 23 showed the most antibacterial effect among *L. plantarum*.

A very strong antimicrobial effect was observed on *S. aureus* from lactic acid bacteria isolated from fermented food. It was reported that it was not effective on *E. coli* and had a weak antimicrobial effect on *L. monocytogenes* [30]. It was found that *L. curvatus* had a strong antimicrobial effect on *S. aureus* but a weak antimicrobial effect on *E. coli* [31]. In our study, one of the best activities was against *S. aureus*, and one of the weak activities was against *E. coli*, and it can be said that it is partially similar to the literature. It was determined that *Lactobacillus* spp. isolated from dairy products showed high antagonistic activity against pathogenic bacteria [44], [45]. They isolated and identified lactobacilli from natural yogurts and it was determined that the isolates showed antimicrobial activity against *E. coli* and *S. aureus* [46]. They stated that the antimicrobial activities of the lactic acid bacteria they isolated might be due to free or cell-bound metabolites [47], [48]. It is thought that the different results obtained in our study might be due to the metabolites synthesized by the strains.

3.5 Qualitative-quantitative Determination of EPS

The study was carried out with 20 *L. plantarum* isolates (*L. plantarum* isolates 1, 2, 5, 7, 9, 10, 12, 14, 15, 16, 20, 21, 22, 23, 24, 25, 29, 32, 35 and 36) with high and low antibiotic resistance and activities on microorganisms and *L. plantarum* STD. The tube method, a qualitative assay, was employed to evaluate biofilm formation along the bottom and walls of the test tubes. Compared to the control, the wells containing the cultivated strains exhibited more intense crystal violet staining, indicating a higher production of exopolysaccharides (EPS) (Figure 5A). Additionally, following 72 hours of incubation on CRA, both *L. plantarum* and *L. plantarum* STD isolates developed black, dry, crystalline colonies, further confirming their capability to produce EPS (Figure 5B).

The strains and the standard strain (STD) exhibited varying optical density (OD) values in the microtiter plate assay, reflecting differences in their EPS production levels (Figure 6). Among the isolates, *L. plantarum* strain 23 produced the highest amount of EPS (318.30 ± 1.28 mg/L), whereas *L. plantarum* strain 16 yielded the lowest (205.70 ± 1.44 mg/L). It was found that *L. plantarum* strains 2, 5, 7, 9, 10, 12, 14, 21, 22, 23, 24, 32 and 35 showed a highly significant difference by producing more EPS than *L. plantarum* STD ($P<0.0001$) (Figure 6B). Statistically, the ranking of these strains is; It is as follows: 23>21 >5 >12 >10 > 24 >9 >2 >14 >32 >22 >35 >7.

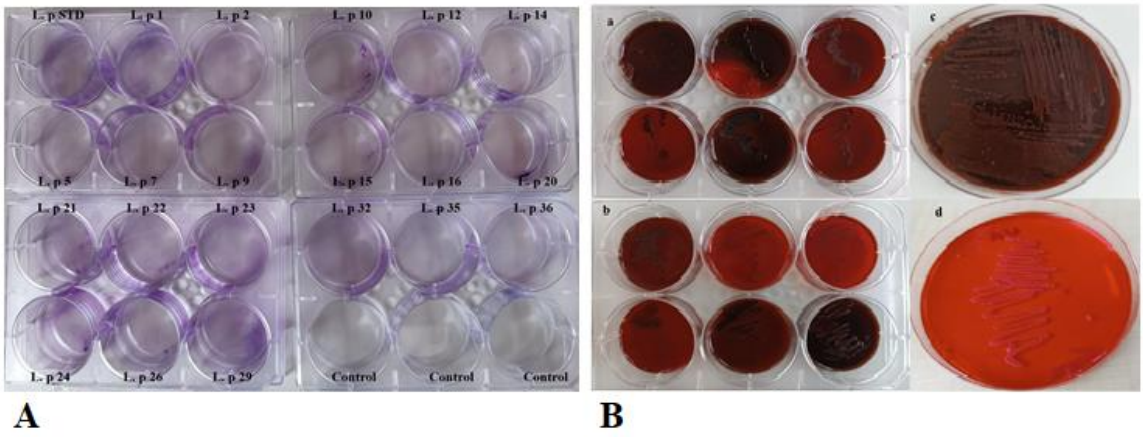

Figure 5. A: Image of a visible film of biofilm-forming bacteria. B: Image of EPSs produced by strains in CRA in six well plate and petri dish. (a, b) Those that form black and dry crystalline colonies in 6 well plate medium are positive, those that do not are negative, (c) Positive, (d) Negative

LABs are widely regarded as safe for consumption, present no health hazards, display substantial genetic and phenotypic diversity, and possess the ability to synthesize exopolysaccharides (EPS) [32].The presence of EPS synthesized by LAB has been verified

through both qualitative and quantitative methods [33]. In the present study, EPS production by the isolated strains was similarly confirmed using established analytical techniques, consistent with findings reported in the literature. For example, Li et al. reported that the maximum EPS yield of *L. plantarum* cultured in MRS medium was below 290.17 mg/L [34]. Other investigations have demonstrated that EPS production among *L. plantarum* strains varies between 75 and 397 mg/L [35], with *L. plantarum* producing up to 280 mg/L [36]. Furthermore, *L. plantarum* strains isolated from human breast milk were shown to produce EPS within a range of 243.30 ± 1.26 to 259.60 ± 1.53 mg/L [37]. Among the isolates analyzed in this study, *L. plantarum* 23 exhibited the highest EPS production at 318.30 ± 1.28 mg/L, while *L. plantarum* 16 produced the lowest amount, measured at 205.70 ± 1.44 mg/L. Overall, the EPS yields of the isolates were relatively consistent. These findings suggest that the EPS production levels of the studied strains fall within the range reported for *L. plantarum* isolates in previous studies.

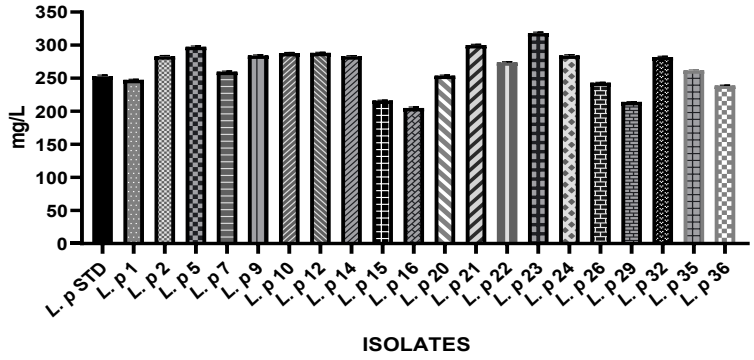

Figure 6. EPS production levels of L. plantarum strains isolated from yoğurt

4 CONCLUSION

Naturally made yoghurt samples were collected from different regions of Muş province and LABs were isolated. Preliminary identification of these isolates (colony morphology, catalase, gram staining) and PCR identification using *recA* gene sequence were performed and as a result, a total of 40 suspected *L. plantarum* strains were detected. 42.5% of our *L. plantarum* strains were found to be resistant to all antibiotics. It was determined that strains 25, 35 and 36 showed the highest sensitivity to antibiotics among the isolates. While isolate 34 showed the least antibacterial effect on pathogenic bacteria, it was determined that strains 5 and 23 showed the highest antibacterial effect. EPSs synthesized by the strains were determined qualitatively and quantitatively. Moreover, *L. plantarum* strain 23 was identified as the highest EPS producer,

yielding 318.30 ± 1.28 mg/L, whereas *L. plantarum* strain 16 exhibited the lowest EPS production at 205.70 ± 1.44 mg/L. In line with these results, it is thought that *L. plantarum* strains 23 and 5 found in yoghurt will contribute to human health by preventing the development of pathogens that harm human flora due to their properties.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Contributions of the Authors

Yusuf ALAN: Conceptualization, Methodology, Validation, Data curation, Visualization, and Investigation Writing- Reviewing and Editing.

Kayhan MANĞ: Conceptualization, Methodology, Formal Analysis, and Investigation Writing Original Draft Preparation.

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