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A New Approach to Exopolysaccharides of Post Probiotic *Lactobacillus paracasei* L1 Strain: Anti-quarum Sensing Activity

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Background: Multi-antibiotic resistance, which has increased in recent years, poses a serious societal threat as it makes the fight against deadly infection-causing pathogens even more complex and difficult. As such, the search for naturally resistant probiotic microorganisms and metabolic products obtained from these organisms to prevent infections, as an alternative to antibiotics, is crucial. In this context, preventing the quorum sensing (QS) mechanism that provides communication among bacteria is considered a mechanism that can prevent the colonization and progression of deadly infections.

Aims: To determine the QS mechanism and the immunological effects and various biological and biochemical characterizations of exopolysaccharide (EPS) obtained from the *Lactobacillus paracasei* L1 strain isolated from the vaginal microflora of healthy women.

Study Design: Experimental laboratory study.

Methods: The antibacterial ability, the antibiofilm and QS forming activities, and interferon (IFN)-γ and interleukin (IL)-10 production capacities of EPS were determined. The total antioxidant capacity (TAC), the surface morphology of EPS by scanning electron microscopy (SEM), the presence of functional groups, and the monosaccharide composition were determined by gas chromatography-mass spectrometry (GC-MS).

Results: *L. paracasei* L1-EPS demonstrated a strong antibiofilm activity on *Escherichia coli* (65.14%), *Staphylococcus aureus* (63.27%), and *Pseudomonas aeruginosa* (54.21%) at a concentration of 5.0 mg/ml. The anti-QS activity of EPS was found to be quite high at 10 mg/ml EPS concentration. In the study performed with human peripheral blood mononuclear cells (hPBMC), the immunostimulatory IFN-γ value was higher (45 ± 0.0.3) than that in the experimental group, while the IL-10 value was lower than that in the control group (36 ± 0.05). The TAC value of *L. piracies* L1- EPS was found to be 76 µg/ml at 1,000 µg concentration. According to the GC-MS analysis results, glucose constituted 13.80% of the monosaccharide composition of EPS, while alpha-D-galactose constituted 13.89%.

Conclusion: Interestingly, EPSs of *L. paracasei* L1 strain, which have not been reported previously, demonstrated high anti-QS and antibiofilm properties, making EPSs a prospective compound for application in the pharmaceutical and food industries owing to their strong antimicrobial and antioxidant capacities.

INTRODUCTION

Exopolysaccharides (EPSs) are microbial polysaccharides produced by various bacteria in response to biotic and abiotic stress factors or to adapt to harsh environmental conditions. EPSs have the capacity to form a capsule that can be released into the external environment as slime EPS by the enzymes associated with the cell wall or to adhere to a bacteria's outer cell wall.[1-3] EPS production during or at the end of the logarithmic developmental stage[4] facilitates physiological tasks such as phagocytosis, phage attacks, antibiotics, toxic metal ions, and protection against osmotic stress.[5]

EPSs have attracted the significant interest of researchers at the food institute due to their various industrial properties and physiological characteristics.[6] In addition, EPS offers various physiological functions such as antioxidant, anti-cancer, immunomodulatory, antibacterial, low glycemic index, anti-hypertensive, anticholesterol effects, and biofilm formation.[7,8] Therefore, the structure-function relationship and the various biological properties of EPS continue to be an important research topic.[1]

Quorum sensing (QS) is a system that enables the communication between bacteria through extracellular signaling molecules after

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the bacterial population density reaches a certain critical point.[9,10] Through QS, bacteria modulate the virulence gene expression and biofilm formation and interact with other microbes. In addition, bacteria affect cellular processes such as spore formation, toxin production, and disinfectant resistance. The inhibition of the QS mechanism appears to be a good mediator in preventing the formation of a biofilm layer by pathogens, thereby controlling the bacterial infection.[11]

Studies that determined the effectiveness of EPS obtained from lactic acid bacteria on the QS mechanism are limited in the literature[3] and there is no study published on the biological characterization of EPS obtained from *L. paracasei* L1 strain, which has a particularly strong probiotic characteristic. Therefore, the results of this study can be considered preliminary for future applications.

This study aimed to determine the anti-microbial, anti-QS, and antibiofilm activity of EPSs obtained from probiotic *Lactobacillus paracasei* L1 species isolated from the vaginal flora as well as to determine the IFN-γ- and IL-10-production capacities and the various biochemical characterizations of EPS.

MATERIALS AND METHODS

Obtaining and Culture Conditions of Bacteria

L. paracasei L1 strain was isolated from the vaginal flora of healthy women. Wholesome women who applied during 2016-2017 were selected for this study. The subjects were of age 18-45 years, non-menopausal, used contraception for protection, and had not used antibiotics in the last three months.[12] *L. paracasei* L1 was developed and activated in solid media and Man Rogosa Sharpe (MRS), Merck broth. The cultures were incubated in an anaerobic jar at 37 °C.[12]

The strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 14579, *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Enterobacter aerogenes* ATCC 1304 ve *Shigella dysenteriae* ATCC 11456) used in this study were sourced from the biotechnology laboratory's stock culture. Clinical samples (*P. aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* ve methicillin-resistant *S. aureus* [MRSA]) were obtained from the patients hospitalized in the intensive care unit. The clinical and type strains were grown in tryptone soy broth (TSB) medium at 37 ºC for 24 h.

Purification of Exopolysaccharides

To obtain pure EPS from the tested bacteria, some changes were applied to the methods described by Onbas et al.[13] before use. In this study, the test bacteria were activated at the appropriate temperature the night before. After being heated to 100 °C for 15 min, the activated cultures were centrifuged for 15 min at 13,000 rpm. The next stages of the study were conducted as stated by Onbas et al.[13] Pure EPS products were stored at 4 °C before being used in subsequent analyzes.

Antimicrobial Activity of *L. paracasei* L1-EPS

The antimicrobial property of EPS produced from the *L. paracasei* L1 strain was investigated by using the agar well-diffusion method. The cultures of the pathogen test bacteria were homogenized into Mueller Hinton Agar (Merck) media spread after activation in TSB medium at 37 °C for 18 h in the appropriate media. The antimicrobial activity study was conducted as indicated by Kiray, 2021.[14] The study was evaluated by taking an average of three tests.

Antibiofilm Activity of *L. paracasei* L1-EPS

Various biofilm-producing pathogenic microorganisms were used to determine the antibiofilm activity of pure EPS extract from *L. paracasei* L1. At 37 °C, the pathogenic bacteria were grown overnight in the TSB medium. After serial dilutions, 100 ml of pure EPS extracts (0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml) and 100 ml of bacterial cultures (OD_{600} 0.132) were transferred into a 96-well polystyrene microtiter plate following serial dilutions and heating to 37 °C. The plate was incubated for 24 h at 37 °C. The antibiofilm study described by Theodora et al.[15] was performed as stated. The measurement of the wells was performed at 595 nm with the ELISA reader (Epoch2 BioTek, USA). Bacterial cultures without EPS were used as positive control and the TSB medium as blank. The formula given below was used to calculate the percentage of biofilm inhibitory potential. The experiment was repeated thrice.[15]

Antibiofilm activity (%) = (1 - OD sample/OD control) x100

Antiquorum Sensing Activity of *L. paracasei* L1-EPS

In our study, *C. violaceum* ATCC 12472 was used as a monitor strain to determine the anti-QS activity of EPS extracts obtained from *L. paracasei* L1 strain, which we used as the test bacteria *C. violaceum* was cultivated in 50 ml of the TSB culture and incubated for 48 h at 28 °C. A sterilized Drigalski spatula was used to spread 100 ml of *C. violaceum* (OD_{600} 0.132) on TSA. Wells with a diameter of 6 mm were opened on the cultivated culture plates and EPS extracts (100 ml) were applied to the wells at the concentrations of 10 mg/ml and 5 mg/ml. Through the zone generated in the violacein pigment background, the anti-QS activity was measured.[16]

The Immunomodulatory Effect of L. paracasei L1-EPS

According to Vissers' instructions, healthy volunteer hPBMCs were isolated, and LAB treatment was performed as previously explained. Human PBMCs (2 x 10^5 cells/well) were seeded in a 96-well tissue culture plate for 48 h at 37 °C and 5% CO_2 with 2 x 10^6 colony-forming unit LAB PBMCs (LAB ratio 1:10). *E. coli* lipopolysaccharide (1 µg/ml) was used as a control in the study. IFN-γ and IL-10 concentrations were determined in accordance with the manufacturer's instructions depending on the enzyme.[17]

Determination of the Total Antioxidant Capacity of *L. paracasei* L1-EPS

The test solution was prepared by dissolving 1,235 g of 4 mM ammonium molybdate [0.9942 g of sodium sulfate (28 mM) and 45 ml of sulfuric acid (0.6 M) in 250 ml of distilled water] and then

tested to assess its overall antioxidant capability. EPS of various concentrations (50-1,000 µg) was dissolved in 1 ml of antioxidant and then incubated for 15 min. Post-incubation absorbance values were measured at 695 nm [UV-VIS spectrophotometer (Shimadzu 1601)]. Ascorbic acid was used as the standard.[18] The test was conducted thrice.

Electron Microscope Analysis of *L. paracasei* L1-EPS

EPSs obtained from the *L. paracasei* L1 strain were glued to aluminum studs and sprayed with gold. The surface morphologies of the EPSs were then visualized with field emission SEM (Quanta FEG-250 SEM) at 2-KV acceleration voltage.[19]

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra were determined by using the Thermo Scientific (Nicolet 6700 FTIR) spectrometer according to the method reported by Shang et al.[20] so as to detect the presence of various functional groups in EPS.

Monosaccharide Composition of *L. paracasei* L1-EPS

The monosaccharide content of EPS was ascertained by using gas chromatography-mass spectrometry (GC-MS). In the study, 2 ml of purified EPS and 2 ml of 2 M trifluoroacetic acid were hydrolyzed at 120 °C for 2 h. After reduction with potassium borohydride dissolved in the hydrolysates, it was methylated with ammonium hydroxide (NH_4OH) and acetic anhydride $(CH_3CO)_2O$. The procedure for determining the monosaccharide composition was performed as specified by Kanamarlapudi and Muddada.[18]

Statistical analysis

Unless stated otherwise, all assays were repeated at least thrice on separate occasions, and the mean and standard deviations were calculated. The SPSS (Ver23, Chicago, IL, USA) package program was used to perform the statistical analysis for the study. All findings are expressed as the mean standard deviation. Student's t-test was used to compare the reference and test groups to determine the antimicrobial, immunomodulatory, and antioxidant capacities of EPS. EPS's antibiofilm test data were analyzed by two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. $p = 0.05$ was considered to indicate the significant difference between the groups.

RESULTS

Antimicrobial Activity of *L. paracasei* L1-EPS

The antimicrobiological action of EPS obtained from the *L. paracasei* L1 strain, which possesses strong probiotic properties, was determined. EPS, which exhibits an inhibitory effect on both gram-negative and gram-positive bacteria, is found on *P. aeruginosa* ATCC 27853 and *E. aerogenes* ATCC 1304 strains, which are especially of high clinical importance from gram-negative bacteria, and *S. aureus* ATCC 25923 and *B. cereus* ATCC 14579 strains were determined to have a strong antimicrobial effect. The antimicrobial zone diameters of EPS are listed in Table 1.

Antibiophilim Activity of *L. paracasei* L1-EPS

According to Figure 1, the antibiofilm activity of EPS obtained from *L. paracasei* L1 on three pathogens (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923) was investigated and their inhibitory activities on biofilm formation were determined by increasing the concentration accordingly. In parallel with the increase in the EPS concentration (0.2-5.0 mg/ml), an increase in biofilm inhibition was also observed. At a concentration of 5.0 mg/ml, EPS showed the highest biofilm inhibitions for *E. coli* ATCC 25922 (84.17%), *S. aureus* ATCC 25,923 (88.15%) and *P. aeruginosa* ATCC 278,533 (68.31%). EPS products demonstrated strong inhibitory effects on *E. coli* and *S. aureus* biofilm, but lower activity on *P. aeruginosa*. These results indicate that EPS from *L. paracasei* L1 can be used in the food industry against various biofilm-forming microorganisms to control microbial biofilm formation.

Antiquorum Sensing Activity of *L. paracasei* L1-EPS

C. violaceum ATCC 12472 was used as an indicator strain to determine the anti-QS activity of EPS obtained from *L. paracasei* L1. In the study performed at different concentrations, the anti-QS activity of EPS was found to be quite high at 10 mg/ml and 5 mg/ml concentrations. These results were expressed in centimeters of the blurred zone created against the background of the violacein pigment. As shown in Figure 2, EPS formed a zone diameter of 24 cm at the concentration of 20 mg/ml, with a zone diameter of 18 cm at the concentration of 10 mg/ml.

The Immunomodulatory Effect of L. paracasei L1-EPS

PBMCs obtained from healthy participants were cultured in tissue culture plates for 48 h with pure EPS obtained from the *L. paracasei* L1 strain. In the study, the levels of immunostimulatory IFN-γ and

TABLE 1. The Antimicrobial Activity of EPSs (5.0 mg/ml) Obtained from *L. paracasei* L1 Strain on Pathogenic Microorganisms.

	E. coli 25,922	*E. coli*	PA 27,853	PA	K.P	E A 1,304	SD 11,456b	*LM* 19,115	*S. aureus* 25,923	*S. aureus* (MRSA)	*B. cereus* 14,579	*E. faecalis*
EPS	16 ± 0.26*	14 ± 0.7	18 ± 2.13	15 ± 0.37	14 ± 0.75	18 ± 0.43	17 ± 0.17	n.d[a]	17 ± 1.26	16 ± 0.71	17 ± 0.84	14 ± 0.52
GN	19 ± 0.11	18 ± 0.5	15 ± 0.28	16 ± 0.32	18 ± 0.58	21 ± 0.56	19 ± 0.32	n. d	18 ± 0.52	19 ± 0.47	23 ± 0.14	n. d

Antimicrobial activities were determined as the lowest concentration of peptide that inhibited bacterial growth, as determined from three independent experiments, each performed in triplicate.
PA: *Pseudomonas aeruginosa*, KP: *Klebsiella pneumoniae*, EA*: Enterobacter aerogenes*, SD: *Shigella dysenteriae*, LM*: Listeria monocytogenes*, GN: Gentamicin, [a]n.d: Not done. The data are presented as the average ± SD of three replicates. *$p < 0.05$ with respect to the control.

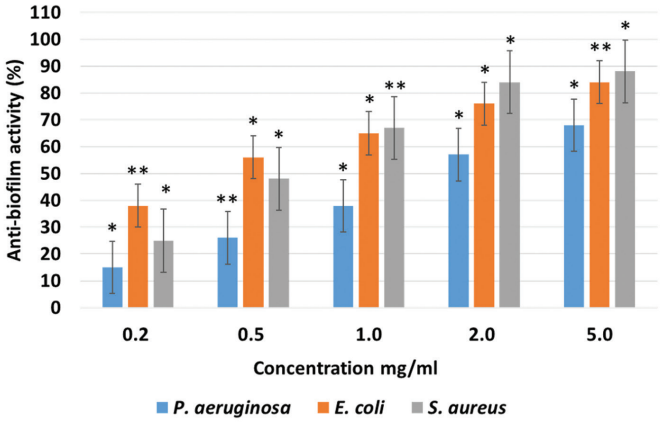

FIG. 1. The concentration-dependent % antibiofilm activity of EPS from *L. paracasei* L1 on *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. Data were analyzed by 2-way analysis of variance and Tukey's multiple comparison post-hoc tests. The data are presented as the average ± SD of three replicates. Asterisks indicate significant differences compared to the untreated control (*$p < 0.05$; **$p < 0.01$). *SD: Standard deviation.*

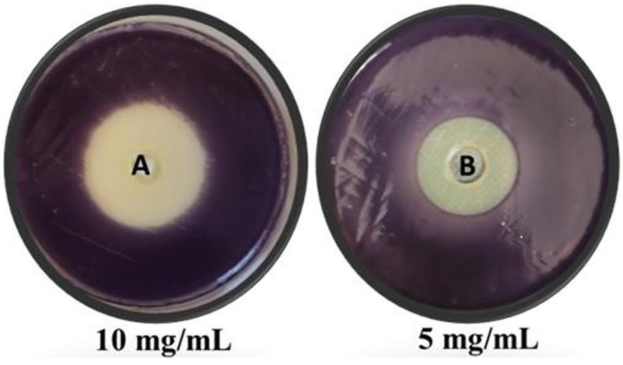

FIG. 2. The inhibition zone activity of EPS from *L. paracasei* L1 strain on *C. violaceum* ATCC 12472 A: 10 mg/ml and B: 5 mg/ml.

immunomodulatory cytokine production were measured by ELISA As seen in Table 2, pure EPS produced higher IFN-γ than controls and the IL-10 concentrations were lower than the corresponding control values. The anti-inflammatory response of EPS from *L. paracasei* appeared to be strong. The test was conducted thrice.

Determination of Total Antioxidant Capacity of *L. paracasei* L1-EPS

In this study, the total antioxidant capacity (TAC) of EPS obtained from *L. paracasei* L1 strain demonstrated dose-dependent activity in the concentration range of 36-76 µg/ml. As seen in Figure 3, the antioxidant capacity increased with an increase in the EPS concentration.

SEM Analysis of *L. paracasei* L1-EPS

It is a common practice to apply SEM to examine the morphology of macromolecules. EPS images of the *L. paracasei L1* strain are

TABLE 2. Effect of EPS from *L. paracasei* on Interferon-y (IFN-y) and Interleukin-10 (IL-10) Production.

Cytokine concentration (pg/ml)			
Sample (n)	IFN-y mean ± SD[a]	IL-10 mean ± SD	*P* value*
Control	17 ± 1.0	81 ± 1.0	0.024
EPS	45 ± 0.3	36 ± 0.5	0.010

*Student's t-test was applied to compare the reference and test groups. All results showed significant differences from the control ($p < 0.05$).
[a]SD: Standard deviation

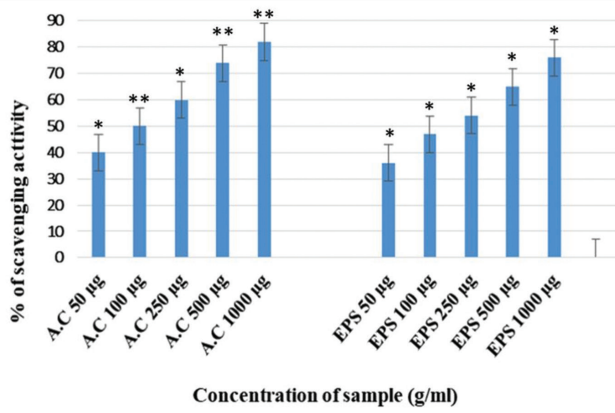

FIG. 3. The total antioxidant capacity of EPS-*L. paracasei* L1. (All data are representative of three independent experiments, each performed in triplicate and expressed as the mean ± SD values in each bar. *$p < 0.05$; **$p < 0.01$.)

given in Figure 4. Large numbers of free hydroxyl groups were exposed by the very porous structures, as shown by SEM, in the polysaccharides, which improved hydration and water-retention capacities.[19]

FTIR Analysis of *L. paracasei* L1-EPS

FTIR spectra were determined in the absorbance mode of 4,000 to 400 cm to further investigate the functional groups of purified EPS. In the FTIR analysis of EPSs of *L. paracasei* L1 strain, a dense and wide band of approximately 3342.67 cm^{-1} was obtained. These bands belong to the OH group. The band at around 1636.37 cm^{-1} appeared as bands of C = O bonds in a monosaccharide structure. Bands near 1,100 cm^{1} are associated with the C-O tensions of carbohydrates and aromatic groups (Figure 5). This observation was made in accordance with the EPS spectra provided in the literature.[23]

Monosaccharide Composition of *L. paracasei* L1-EPS

The composition of EPS monosaccharides was established by using the GC-MS data. According to Figure 6, GCMS analysis of bacterial EPS peaks revealed a matching glucose concentration of 13.80% and an alphaD-galactose concentration of 13.89%.

DISCUSSION

In various studies, EPSs obtained from *Lactobacillus* strains have demonstrated antimicrobial activities. The antimicrobial activity

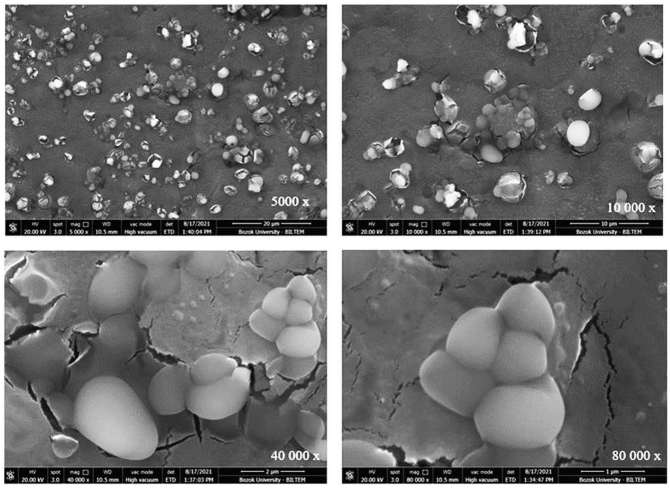

FIG. 4. Electron microscope images of EPS of *L. paracasei* L1 strain (5,000x-10,000x-40,000x-80,000x).

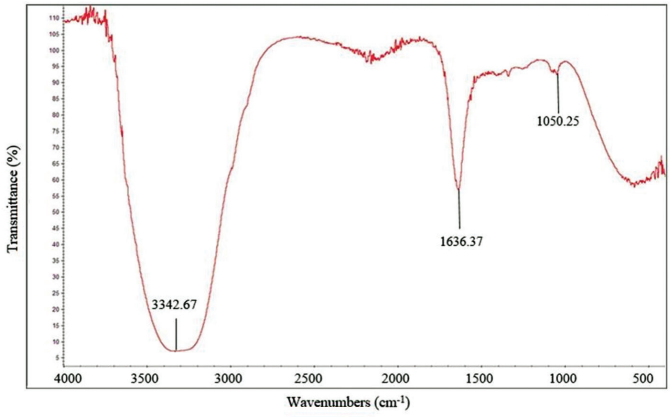

FIG. 5. FTIR analysis of EPS-*L. paracasei* L1 strain.

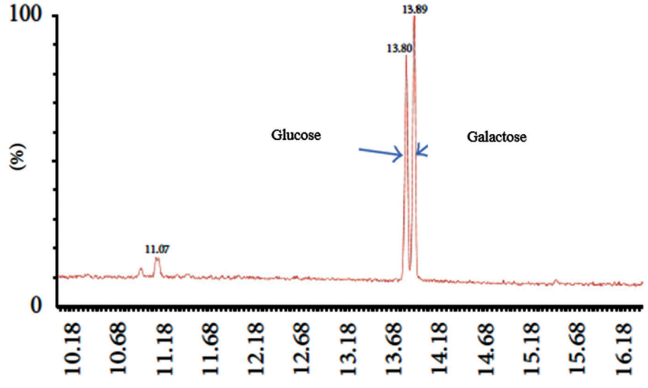

FIG. 6. The GC-MS spectrum of the EPS extract-*L. paracasei* L1 strain. GC-MS.

of EPS obtained from the *L. plantarum* S123 strain was performed on *E. coli* ATCC25922 and *S. aureus* ATCC29213 strains showed a higher effect on *E. coli*.[21] In another study, EPS obtained from *L. rhamnosus* strain was found in *E. coli*, *Salmonella typhimurium*, and *Staphylococcus petrasii* subsp. *pragensis* KY196531, with different degrees of antimicrobial activities in each.[22] In an additional study, EPS belonging to the *Lactobacillus brevis* strain demonstrated a strong antimicrobial effect on *E. coli* and *S. aureus* strains. These results obtained were similar to our study data.[23]

The biofilm-inhibiting ability of EPS obtained from *L. fermentum* S1 strain (0.25-2 mg/ml) on *E. coli* and *S. aureus* was investigated, and the highest inhibition rate on *E. coli* was found to be 32.25%, while the highest inhibition rate on *S. aureus* was found to be 42.77%.[19] In our study, EPS obtained from *L. paracasei* L1 strain at a concentration of 5.0 mg/ml revealed the highest biofilm inhibitions in *E. coli* ATCC 25922 (84.17%), *S. aureus* ATCC 25923 (88.17%), and *P. aeruginosa* ATCC 278533 (68.31%). EPS products demonstrated a strong inhibitory effect on *E. coli* and *S. aureus* biofilm, albeit with lower activity on *P. aeruginosa*. These results suggested that EPS obtained from *L. paracasei* L1 can be used in the food industry as a food-grade biofilm inhibitor against various biofilm-forming microorganisms to control microbial biofilm formation.

The physiological formations applied by probiotic microorganisms to prevent the biofilm formation process remain to be fully clarified. In some in vitro studies, the autoaggregation and coaggregation abilities of probiotic microorganisms prevented the biofilm formation as well as QS formation, and the various virulence effects were affected by probiotic microorganisms.[24,25] In addition, some probiotic microorganisms possessed properties that prevented the physiological results of bacteria because of QS. Gómez et al.[26] demonstrated comparable inhibiting effects on *E. coli* O157:H7, *Salmonella typhimurium,* and *L. monocytogenes*. In our previous study, various probiotic *Lactobacillus* spp. (*L. paracasei* and *L. rhamnosus*, *L. gasseri*, *L. crispatus*, *L. acidophilus,* and *L. acidophilus*) the strain was determined to have anti-QS characterization, and the *L. paracasei* L1 strain used in our study demonstrated strong anti-QS activity.[14]

In this study, EPS obtained from *L. paracasei* L1 strain demonstrated strong anti-QS activity at the concentrations of 10 mg/ml and 5 mg/ml, which is consistent with our previous findings. According to the results obtained, a feature that enabled the probiotic *Lactobacillus* strains to display anti-QS activities is the production of EPS. There are limitations studies in this area. In the literature, no similar study has been reported on EPS obtained from the vaginal microflora-derived *L. paracasei* strain. We believe that the present results will facilitate future work in this direction.

In this research, we compared the cytokine profiles, IL-10-production capabilities, and antibiofilm and anti-QS properties of strains with known probiotic relevance. Comparisons between the immunomodulatory impact of *L. paracasei* EPS on hPBMC cytokine profiles and proliferative response revealed that *L. paracasei* L1 EPS produced higher IFN-γ than the controls, and the IL-10 concentrations were lower than the control concentrations. The production of inflammatory cells by *Lactobacillus* spp. is a pathway that demonstrates the helpful influence of healthy bacteria on immune function.[27]

One of the most significant macrophages with cytokine-stimulating abilities is IFN-. It is crucial for both acquired immunity and innate immune response. Notably, in several studies, lactic acid secreted by vaginal *Lactobacillus* species was found to inhibit the production of IFN- in T- and natural killer cells. This production is a factor that protects against bacterial vaginosis.[10,28] The immunomodulatory effect of EPSs obtained from *L. paracasei* has not been investigated so far. In this regard, our study is expected to set the standard for other researches.

Functional units of EPS obtained from the *L. paracasei* L1 strain were detected by FTIR spectroscopy. This analysis identified a dense and wide band around 3342.67 cm^{-1}. These bands indicate a significant number of hydroxyl groups, representing the characteristic absorption band of carbohydrates. The present data conform to those reported previosuly.[18]

Owing to their capacity to scavenge free radicals, bind metal ion catalysts, and engage in reduction activities, natural antioxidants can perform preventive roles against various illnesses.[29] The antioxidant activity of EPS obtained from *L. paracasei* L1 under in vitro conditions is illustrated in Figure 3. The antioxidant capacity of EPS suggested varying activity depending on the dose increase in the concentration range of 36-76 µg/ml. The 76.25% capacity determined in the present study was obtained from EPS belonging to the

L. paracasei strain isolated from TAC sauerkraut with a similar rate of 76.34%.[30] Past studies in this area have reported that EPSs obtained from various lactic acid bacteria demonstrate a similarly strong antioxidant activity.[31]

In this study, glucose, which has been accepted as a valuable product for the food industry, was found at 13.80%, while alpha-D-galactose was found at the rate of 13.89%. The information gleaned from the study's GC-MS analysis is compatible with those of previous reports on EPS. In previous studies, EPSs obtained from *Lactobacillus* strains contained both glucose and galactose, generally with the same 1:1 molar ratios.[30]

In this study, EPS derived from *L. paracasei* L1 strain, which was previously proven to possess a strong probiotic characteristic,[12] demonstrated strong properties in terms of various biological activities. It has been determined that EPS, which exhibits strong antimicrobial, antibiofilm, and anti-QS activities, exhibits high antioxidant activity and strong anti-inflammatory response to the immune system. Considering the outcomes of this study, we concluded that *L. paracasei* L1-EPS can disperse *E. coli* and *S. aureus* biofilms and inhibit biofilm formation by inhibiting the QS mechanism involved in biofilm formation. In this study, the heteropolysaccharide structure of EPS was determined by FTIR and GC-MS analysis, and its porous structure was identified by SEM images. Owing to its potent antibacterial antibiofilm, anti-QS, and antioxidant activities, it could optimize the production and composition of antioxidant and antibiofilm agents in food and pharmaceutical industries for potential applications.

Ethics Committee Approval: The Kırıkkale University Faculty of Medicine Ethics Committee gave its approval to the protocol. Testing was performed three times.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- E.K., N.M.R.; Design- E.K., N.M.R.; Data Collection or Processing- E.K., N.M.R.; Analysis or Interpretation- E.K., N.M.R.; Literature Search- E.K., N.M.R.; Writing- E.K., N.M.R.

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REFERENCES

1. Xu Y, Cui Y, Wang X, et al. Purification, characterization and bioactivity of exopolysaccharides produced by Lactobacillus plantarum KX041. *Int J Biol Macromol.* 2019;128:480-492. [CrossRef]
2. Donot F, Fontana A, Baccou JC, Schorr-Galindo S. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohyd Polym.* 2012;87:951-962. [CrossRef]
3. Genc Karadeniz D, Kaskatepe B, Eylul Kiymaci M, Tok KC, Gumustas M, Karaaslan C. Microbial exopolysaccharide production of Streptococcus thermophilus and its antiquorum sensing activity. *Arch Microbiol.* 2021;203:3331-3339. [CrossRef]
4. Ergene E, Avcı A. Microbial Exopolysaccharides. SAÜ Fen Bil Der. 2016;20:193-202. [CrossRef]
5. Fukuda K, Shi T, Nagami K, et al. Effects of carbohydrate source on physicochemical properties of the exopolysaccharide produced by Lactobacillus fermentum TDS030603 in a chemically defined medium. *Carbohyd Polym.* 2010;79:1040-1045. [CrossRef]
6. Caggianiello G, Kleerebezem M, Spano G. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. *Appl Microbiol Biotechnol.* 2016;100:3877-3886. [CrossRef]
7. El-Deeb NM, Yassin AM, Al-Madboly LA, El-Hawiet A. A novel purified Lactobacillus acidophilus 20079 exopolysaccharide, LA-EPS-20079, molecularly regulates both apoptotic and NF-kappa B inflammatory pathways in human colon cance. *Microb Cell Fact.* 2018;17:29. [CrossRef]
8. Zhu Y, Wang C, Jia S, et al. Purification, characterization and antioxidant activity of the exopolysaccharide from Weissella cibaria SJ14 isolated from Sichuan paocai. *Int J Biol Macromol.* 2018;115:820-828. [CrossRef]
9. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol.* 2016;14:576-588. [CrossRef]
10. Zhao X, Yu Z, Ding T. Quorum-Sensing Regulation of Antimicrobial Resistance in Bacteria. *Microorganisms.* 2020;17:425. [CrossRef]
11. Barzegari A, Kheyrolahzadeh K, Hosseiniyan Khatibi SM, Sharifi S, Memar MY, Zununi Vahed S. The Battle of Probiotics and Their Derivatives Against Biofilms. *Infect Drug Resist.* 2020;26:659-672. [CrossRef]
12. Kiray E, Kariptas E, Dağlı S. Characterisation Of Probiotic Properties Of Lactic Acid Bacteria Isolated From Vaginal Microflora Of Healthy Turkish Women. *Fresenius Environ Bull.* 2020;29:4240-4252. [CrossRef]
13. Onbas T, Osmanagaoglu Ö, Kiran F. Potential Properties of Lactobacillus plantarum F-10 as a Bio control strategy for Wound Infection. *Probiotics Antimicrob Proteins.* 2019;11:1110-1123. [CrossRef]
14. Kiray E. Antibiofilm and Anti-Quorum Sensing Activities of Vaginal Origin Probiotics. *Eur J Biol.* 2021;80:82-90. [CrossRef]
15. Theodora NA, Dominika V, Waturangi DE. Screening and quantification of anti-quorum sensing and antibiofilm activities of phyllosphere bacteria against biofilm forming bacteria. *BMC Res Notes.* 2019;12:732. [CrossRef]
16. Abudoleh SM, Mahasneh AM. Anti-Quorum Sensing Activity of Substances Isolated from Wild Berry Associated Bacteria. *Avicenna J Med Biotechnol.* 2017;9:23-30. [CrossRef]

17. Vissers YM, Snel J, Zuurendonk PF, Smit BA, Wichers HJ, Savelkoul HFJ. Differential effects of Lactobacillus acidophilus and Lactobacillus plantarum strains on cytokine induction in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol.* 2010;59:60-70. **[CrossRef]**
18. Kanamarlapudi SLRK, Muddada S. Characterization of Exopolysaccharide Produced by Streptococcus thermophilus CC30. *Biomed Res Int.* 2017;2017:4201809. **[CrossRef]**
19. Wang K, Niu M, Song D, et al. Preparation, partial characterization and biological activity of exopolysaccharides produced from Lactobacillus fermentum S1. *J Biosci Bioeng.* 2020;129:206-214. **[CrossRef]**
20. Shang N, Xu RH, Li PL. Structure characterization of an exopolysaccharide produced by Bifidobacterium animalis RH. *Carbohydr Polym.* 2013;91:128-134. **[CrossRef]**
21. Wang K, Li W, Rui X, et al. Characterization of a novel exopolysaccharide with antitumor activity from Lactobacillus plantarum 70810. *Int J Biol Macromol.* 2014;63:133-139. **[CrossRef]**
22. Riaz Rajoka MS, Mehwish HM, Fang H, et al. Characterization and anti-tumor activity of exopolysaccharide produced by Lactobacillus kefiri isolated from Chinese kefir grains. *J Funct Foods.* 2019;63:103588. **[CrossRef]**
23. Riaz Rajoka MS, Mehwish HM, Zhang H, et al. Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle synthesized by Lactobacillus brevis isolated from Chinese koumiss. *Colloids Surf B Biointerfaces.* 2020;186:110734. **[CrossRef]**
24. Wasfi R, Abd El-Rahman OA, Zafer MM, Ashour HM. Probiotic Lactobacillus sp. inhibit growth, biofilm formation and gene expression of caries-inducing Streptococcus mutans. *J Cell Mol Med.* 2018;22:1972-1983. **[CrossRef]**
25. James KM, MacDonald KW, Chanyi RM, Cadieux PA, Burton JP. Inhibition of Candida albicans biofilm formation and modulation of gene expression by probiotic cells and supernatant. *J Med Microbiol.* 2016;65:328-336. **[CrossRef]**
26. Gómez NC, Ramiro JM, Quecan BX, de Melo Franco BD Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of Listeria monocytogenes , Salmonella Typhimurium, and Escherichia coli O157: H7 biofilms formation. *Front Microbiol.* 2016;7:863. **[CrossRef]**
27. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-Inflammatory and Immunomodulatory Effects of Probiotics in Gut Inflammation: A Door to the Body. *Front Immunol.* 2021;12:578386. **[CrossRef]**
28. Sturme MHJ, Francke C, Siezen RJ, de Vos WM, Kleerebezem M. Making sense of quorum sensing in lactobacilli: a special focus on Lactobacillus plantarum WCFS1. *Microbiology.* 2007;153:3939-3947. **[CrossRef]**
29. Wang J, Zhao X, Yang Y, Zhao A, Yang Z. Characterization and bioactivities of an exopolysaccharide produced by Lactobacillus plantarum YW32. *Int J Biol Macromol.* 2015;74:119-126. **[CrossRef]**
30. Shankar T, Palpperumal S, Kathiresan D, et al. Biomedical and therapeutic potential of exopolysaccharides by Lactobacillus paracasei isolated from sauerkraut: Screening and characterization. *Saudi J Biol Sci.* 2021;28:2943-2950. **[CrossRef]**
31. Xu R, Shang N, Li P. In vitro and in vivo antioxidant activity of exopolysaccharide fractions from Bifidobacterium animalis RH. Anaerobe. 2011;17:226-231. **[CrossRef]**