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Assessment of anti-biofilm activity and bifidogenic growth stimulator (BGS) effect of lyophilized exopolysaccharides (I-EPs) from *Lactobacilli* strains

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ABSTRACT

In the present study, a total of used six lactic acid bacterial strains that isolated from children's feces and milk products. This study investigated the exopolysaccharide production of these strains. In the culture broth, highest exopolysaccharide production capacity was detected in GD-11, LB-69, and B-3 strains. Monosaccharide compositions of the exopolysaccharides that were purified and lyophilized in the culture medium were investigated and dominant monomer was found to be mannose in all strains. The biofilm-forming capacity of 13 pathogen bacteria was determined and four strains with the highest biofilm-forming capacity were selected as test bacteria for anti-biofilm studies. Different concentrations of I-exopolysaccharides of three lactic acid bacteria were tested for anti-biofilm effect. Highest anti-biofilm effects were exhibited by the *L. fermentum* LB-69 (90%) against *B. cereus* RSKK 863. In the statistical analyses performed using the available data, a strong correlation was found between the amount of mannose present in I-exopolysaccharides and the anti-biofilm activity exhibited by I-exopolysaccharides ($p < 0.01$). In this study, it was concluded that the I-exopolysaccharides of the *L. fermentum* LB-69 strain could be used as efficient agents with both bifidogenic growth stimulator effect and anti-biofilm effect. If these agents are taken via oral route, it would be possible to provide beneficial effects for the host by increasing the development of bifidobacteria present in the gastrointestinal tract as well as to prevent and treat the diseases by hindering the biofilm formation by harmful bacteria.

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Introduction

Bacteria from the lactic acid bacteria (LAB) group have mostly been used in the production and protection of foods. Some species are acknowledged as probiotics, which according to the definition recommended by the World Health Organization (WHO) meet a health benefit on the host when led in satisfactory amounts.^[1,2] In addition LAB are identified as generally recognized as safe (GRAS) foods by the Food Drug Administration (FDA). As the resident microbiota of the intestinal track the microorganisms that are closely related to human beings.^[3]

Exopolysaccharides (EPSs) are long-chain high-molecular-mass polymers that are primarily produced by the metabolic pathways of LAB.^[4] In LAB, many species have been indicated to produce EPS molecules. The diversity of the produced molecules is huge in terms of monomer composition, molecular mass, degree of branching, and structure.^[5] The EPS can also consist in different forms. Many probiotic bacteria (FAO/WHO, 2002) with diverse informed health effects have been indicated to produce EPS, including the well-known *Lactobacillus rhamnosus* strain GG,^[6] which produces two types of molecules: one galactose-rich (the most abundant)^[7] and the

other glucose-rich.^[8] The defined roles of EPS in probiotic actions are numerous,^[9] including prebiotic potential, possible involvement in the adhesion to the intestinal epithelium, and modulation of host immunity.^[10–12]

EPS-producing LAB are generally regarded as safe due to their long history of safe use in substances for human consumption.^[13] In recent years, an enhancing body of evidence has displayed that EPSs produced by different LAB strains have potential biological activities such as antioxidant,^[14] immunomodulating,^[15] anti-tumor, anti-biofilm,^[16] and antibacterial properties.^[15] EPSs are also involved in the formation of biofilms,^[17,18] which may develop colonization and assist survival.^[19,20]

Biofilm is the predominant mode of growth for bacteria in clinical, industrial, and most natural environments.^[16,21] Biofilm formation by bacterial pathogens has been indicated to enhance their tolerance to exogenous stress and their ability to escape from the host immune system, causing many serious problems such as food spoilage, biofouling, and ineffective treatment for chronic and repetitive infections.^[22] Biofilms are sustainable sources of contaminations that are accountable of food-related illness and substantial economic losses. This lifestyle negotiates protection to bacterial cells and reduces the efficiency of cleaning and disinfection procedures.^[23] As a consequence, the need for the progress of new strategies and anti-biofilms agents allowing the suppression and control of biofilm-formation by various pathogens is of major importance in order to diminish the risk of contamination in food sector and human health.

Prebiotics such as fructooligosaccharides (FOS) and inulin, are designated as “a dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health” by the *International Association of Probiotics and Prebiotics*.^[24] Prebiotic oligosaccharides and polysaccharides are employed during different stages of gut transit, generally the proximal and distal colon, respectively. This is thought to be due to the fact that long chain carbohydrates are metabolized more slowly than oligosaccharides.^[25] As inulin is recently the only commercially present prebiotic polysaccharide, it may be of interest to find new polymeric substances which could be delivered in dissimilar food vectors, such as dairy or bakery products with EPS producing adjuncts, to improve the distal colonic microbiome. It is only in the last few years that LAB EPS have taken attention with regard to prebiotic potential.^[26] Due to the lack of wide information on EPSs from the food-associated and human originated LABs to determine this research investigated the use of lyophilized EPS (l-EPS) synthesized by LAB as potential prebiotics. The aim of the present work was determined the EPS production of six lactobacilli strains, monosaccharide composition of the l-EPSs, regulatory effects of the l-EPSs on bifidobacteria development and their anti-biofilm abilities against some pathogens.

Materials and methods

Bacterial strains media and culture conditions

This research comprised *Bifidobacterium breve* BASO-1 and six strains of lactobacilli, of which these strains were conducted at Gazi University, Biotechnology Laboratory Collection for Type Cultures. The six lactobacilli species utilized in the research were isolated from children's feces and milk products. The lactobacilli were classified by their morphological and cultural properties, catalase test, and the API 50 CHL kit (BioMerieux, France) analyzed by API LAB plus software version 4.0 databases (data not show). In addition, the strains were approved using 16S rDNA sequence analysis. Uni27F (20 bp) 5' AGAGTTTGATCCTGGCTCAG 3' and Uni1492R (19 bp) 5' GGTACCTTGTTACGACTT 3' were used in PCR amplification of 16S rDNA gene regions and sequencing (Table 1). The sequences were compared with the sequences deposited in the GenBank database using the BLAST algorithm. Lactobacilli were inoculated in de Man Rogosa Sharpe (MRS) broth (Oxoid, Istanbul, Turkey). All of the lactobacilli strains were stored at −40°C in MRS broth with 10% glycerol, and regenerated twice before the use in the manipulations.

To detect the effect of l-EPs on bifidobacteria, *Bifidobacterium breve* BASO-1 strain were utilized. The strain were cultured under anaerobic conditions for 24–48 h at 37°C in modify MRS (Merck) broth supplemented with 0.05% w/v L-cysteine-HCl (Merck; MRS-C).^[27]

Thirteen pathogen bacteria (*Bacillus cereus* RSKK 863, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 25175, *Staphylococcus aureus* EB-1, *S. aureus* EB-12, *S. aureus* ATCC 25923, *S. aureus* ATCC 2392, *S. aureus* EB-36, *S. aureus* EB-18, *Escherichia coli* 0157:H7, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 72853, *Salmonella typhimurium* BAST-01) were used to research biofilm production. The strains were stored at –40°C in Nutrient broth plus 10% (v/v) glycerol. Before experimental use the cultures were propagated twice in nutrient broth at 37°C; the transfer inoculum was 2% (v/v) of a 24 h culture grown in fresh medium.

Isolation and quantification of EPS

EPS was isolated with a method of Frengova et al.^[28] Cultures were boiled at 100°C for 15 min. After cooling, they were treated with 17% (v/v) of 85% trichloroacetic acid solution and centrifuged at 13,000 rpm for 20 min. Removal of cells and protein was done by centrifugation. The EPS was precipitated with 1 volume of cold absolute ethanol and then centrifuged at 13,000 rpm for 15 min. The pellet containing EPS was dissolved in deionized water. EPS was precipitated with ethanol again, and the EPS that dissolved in deionized water was lyophilized (Christ alpha 2- 4 LD Plus freeze Dryer, UK). The total amount of carbohydrate in the l-EPS was described using the phenol-sulfuric acid method^[29] with glucose as standard.

Monomer analysis of l-EPS

The l-EPS was analyzed for carbohydrate content. l-EPS was dissolved with ultra-pure water. Hydrolysis of the EPS was performed with 4 M HCl at 120°C for 15 min. Determination of monosaccharides was made by HPLC (Varian ProStar) with Carbohydrates Ca (300 × 6.5 mm) CP28351 column using water as mobile phase at a flow rate of 0.6 mL/min and the eluted were conformed with a detector (VARIAN 350 RI).

l-EPS fermentation by *Bifidobacterium breve* BASO-1

For this study, l-EPS belonging GD-11 strain was selected among the six strains according to their capacity of EPS production. Ten milliliters of modified carbohydrate-free basal medium with 1% (wt/vol) l-EPS was inoculated (2% [vol/vol]) with culture of *Bifidobacterium breve* BASO-1 and incubated for 48 h at 37°C under anaerobic conditions. The number of live (cfu/mL) bacteria was designated by total plate count after 48 h of cultivation.

Effect of l-EPs on *Bifidobacterium breve* BASO-1 development

l-EPs added to the fresh MRS-C broth at final concentrations of 0.5 and 1 mg/mL. A negative control (without l-EPS) and a positive control (0.5 and 1 mg/mL inulin) were also used simultaneously. *Bifidobacterium breve* BASO-1 strain was inoculated with an initial turbidity at 10⁷–10⁸ CFU/mL. After 48 h incubation at 37°C under anaerobic conditions, the number of viable bacteria was calculated as follows:

$$\text{The number of viable bacteria} = (A \times B)/C$$

where A is the number of colonies, B is dilution factor = 1 /ratio of dilution, C is volume of planted.

Microtiter plate biofilm production assay

The method used was a modified version of that defined by Djordjevic et al.^[30] Overnight cultures of pathogen bacteria strains grown at 37°C in nutrient broth were refreshed in nutrient broth. Two hundred microliters of inoculum were transferred in the 96-well polystyrene microtiter plates with an initial turbidity at 600 nm of 0.05 OD. The microtiter plate was then left at 37°C for 24 h in static condition. To analysis the biofilm formation, the remaining medium in the incubated microtiter plate was taken out and the wells were washed five times with sterile distilled water to remove loosely associated bacteria. Plates were air dried for 45 min and each well was stained with 200 µL of 1% crystal violet solution for 45 min. After staining, plates were washed with sterile distilled water five times. The quantitative analysis of biofilm production was made by adding 200 µL of ethanol-acetone solution (4:1) to de-stain the wells. The level (OD) of the crystal violet present in the de-staining solution was measured at OD₅₇₀ and the cell turbidity was monitored using a microtiter plate reader (Multiscan Spectrum, Thermo Electron Corporation). The rate of biofilm formation was designated by the value of OD. Five replicate wells were performed for each experimental parameter and each data point was averaged from these five. Strains were divided into three; weak biofilm formation, middle biofilm formation and strong biofilm formation. Four strains that were the highest biofilm forming were utilized for test anti-biofilm experiment.

In vitro anti-biofilm effect of l-EPS on pathogen bacteria

The highest biofilm forming pathogen bacteria (*L. monocytogenes* ATCC-7644, *B. cereus* RSKK-863, *E. fecalis* ATCC-25175, and *P. aeruginosa* ATCC-72853) were grown at 37°C in nutrient broth. The culture, grown overnight, was diluted to 0.1 OD with nutrient broth at 620 nm and then added to a 96-well polystyrene microtiter plate together with different concentrations of l-EPS (0.1 and 1.0 mg/mL). The plate was incubated for 24 h (100 rpm, 37°C) and a biofilm developed by strain in the absence of l-EPS was used as the control. Wells were washed, dried and then, plates were stained for 5 min with 0.2 mL of 2% crystal violet. To correlate biofilm formation with planktonic growth in each well, the planktonic cell fraction was transferred to a new microtiter plate and the OD₅₇₀ was monitored using a microtiter plate reader (Multiscan Spectrum, Thermo Electron Corporation). The level (OD) of the crystal violet present in the de-staining solution was measured at 570 nm.^[31,32] Percent of biofilm inhibition was calculated according to the following formula:

$$\text{Anti - Biofilm \%} = [a / (a + b)] \times 100$$

where a is biofilm OD 570 nm, b is planktonic OD 570 nm.

Statistics

All experiments were realized in triplicate, and mean values are presented. The results were stated as means ± standard deviations (SD). Statistical analyses were made using SPSS version 16.0 (SPSS, Chicago, 182 IL, USA). Pearson's correlation analysis was used to designate the statistical significance of differences between the values.

Results and Discussion

In the present study, six *Lactobacillus* spp. were isolated from human feces and milk products. The isolates were identified by sequencing of their 16S rRNA gene region which amplified a 1492 bp fragment. They identified as lactobacilli and displayed a similarity of ≥99% to type strains deposited in the GenBank database. As results of sequence analysis; isolates identified as *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Lactobacillus plantarum* (Table 1).

Table 1. Origin and molecular identification results of lactobacilli in this study by sequencing the 16S rRNA gene region of lactobacilli in this study.

Strain	Origin	Identity%	GenBank accession number
<i>Lactobacillus fermentum</i> LB-69	Feces	99	JQ013298.1
<i>Lactobacillus rhamnosus</i> GD-11	Feces	99	AB008211.1
<i>Lactobacillus casei</i> LB-8	Feces	100	JQ0466411.1
<i>Lactobacillus plantarum</i> GD-2	Feces	99	AB112083.1
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> A-12	Milk product	100	AY735407.1
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> B-3	Milk product	99	FJ915697.1

The rheological properties of EPS produced by LAB have determined a major application in the manufacture of fermented dairy products such as yogurt, cheese, or fermented milks, although they also play a role in the elaboration of fermented meats and vegetables.^[33–35] The EPSs will remain for longer in the gastrointestinal tract, thus increasing colonization by probiotic bacteria^[36] and the prebiotic effect will be in direct or indirect role.

The l-EPSs of *Lactobacillus* spp. content ranged between 255–320 mg/L (Table 2). The l-EPS of *L. rhamnosus* GD-11 strain produced the highest amount 320 mg/L. A considerable variation can be observed in EPS quantifications. In previous studies, the amount of EPS informed varies from 25 to 132 mg/L for *L. lactis* ssp. *cremoris*^[37] and *L. rhamnosus* C83,^[38] and from 130–250 mg/L for *Lactobacillus casei* CG11 and *L. delbrueckii* ssp. *bulgaricus* NCFB 2772,^[39] respectively. When we compared our results with these findings; *Lactobacillus* spp. produced comparatively higher quantities of EPS.

The monomer compositions of EPSs that lyophilized and produced by the strains displaying the EPS production were analyzed by high-performance liquid chromatography (HPLC). The l-EPSs have the same monomers in their composition, however, the ratio among the two polymers were different. The l-EPSs defined in samples consist of mannose and glucose monosaccharides. Mannose was the major component of l-EPS from all strains (Table 2). The l-EPS produced by these closely related strains also differed in their monosaccharide ratio composition. Other studies declare on the presence of mannose in exocellular polymers in lactobacilli, which was identified as a main monomer in the polysaccharides analyzed in our study.^[40–42]

The EPS fermentation capability of the *B. breve* BASO-1 was evaluated by using the l-EPS of the *L. rhamnosus* GD-11 strain which is the highest EPS producer. *B. breve* BASO-1 was able to grow in both MRS-C medium and carbohydrate-free basal medium with 1% (wt/vol) l-EPS (Fig. 1). Figure 1. *B. breve* BASO-1 was tested *in vitro* for its ability to use all the l-EPSs studied. The l-EPSs were added to the medium to a final concentration of 0.5 and 1 µg/mL. Bifidogenic growth stimulator (BGS) activities of l-EPSs were compared with inulin. Inulin enhanced the number of bifidobacteria cell viability, and this stimulation was not higher than BGS activities of the l-EPSs ($p < 0.05$; Table 3). After 2 days of incubation, all l-EPSs tested, as well as inulin, promoted higher raises in levels of bifidobacteria populations than those occurring in control cultures without carbohydrates addition. Even though an interindividual variability in BGS activities was found, the highest stimulation of bifidogenic growth was promoted by l-EPS from *L. fermentum* LB-69 (10.70 log cfu/mL at 1 mg/mL; Table 3). Our results exhibited that the l-EPSs produced by these lactobacilli had the ability to

Table 2. The amount and carbohydrate composition of l-EPS isolated from lactobacilli and carbohydrate composition of l-EPSs.

Strains	Quantity of l-EPS (mg/L)	Monosaccharides (%)	
		Mannose	Glucose
<i>L. fermentum</i> LB-69	310 ± 2	96.00	4.00
<i>L. rhamnosus</i> GD-11	320 ± 4	95.96	4.04
<i>L. paracasei</i> ssp. <i>paracasei</i> LB-8	255 ± 5	93.20	6.80
<i>L. plantarum</i> GD-2	270 ± 5	88.20	11.80
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> A-12	286 ± 6	82.80	17.20
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> B-3	286 ± 3	91.20	8.80

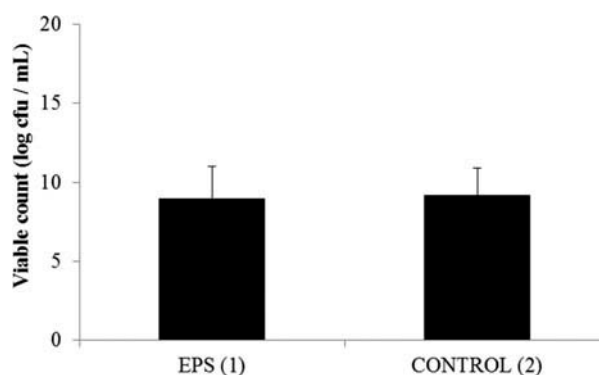


Figure 1. EPS fermentation by *Bifidobacterium breve* BASO-1. (1) The viability in MRS-C medium. (2) The viability in carbohydrate-free basal medium with 1% (wt/vol) I-EPS.

Table 3. Effect of I-EPS on *Bifidobacterium breve* BASO-1 development.

Strain	<i>Bifidobacterium breve</i> BASO-1 Viable count (log cfu/mL)	
	0.5 mg/mL	1 mg/mL
<i>L. fermentum</i> LB-69	9.91 ± 1.20	10.70 ± 1.02
<i>L. rhamnosus</i> GD-11	10.10 ± 1.05	10.59 ± 1.89
<i>L. paracasei</i> ssp. <i>paracasei</i> LB-8	9.79 ± 1.89	10.50 ± 1.16
<i>L. plantarum</i> GD-2	9.69 ± 1.20	10.35 ± 1.84
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> A-12	9.60 ± 1.84	10.00 ± 1.10
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> B-3	9.60 ± 1.50	10.10 ± 1.78
Inulin (positive control)	9.49 ± 1.55	9.98 ± 1.55
Negative control (without I-EPS)	9.31 ± 1.35	9.31 ± 1.35

modulate the intestinal microbiota, promoting a bifidogenic effect comparable to that of the prebiotic inulin. Also, our results defined that EPS which has different types of monosaccharide composition stimulated the growth of bifidobacteria at different rates.

Ruas-Madiedo et al.^[43] reported that the susceptibility of the LAB to biological degradation is directly related to the EPSs. The *Bifidobacterium* strains characterized in their study possess degradative extracellular enzymes belonging to glycoside hydrolase families 2, 13, 36, and 42, such as α -galactosidases, β -galactosidases, and an enzyme active toward glucooligosaccharides. These results exhibited that; EPS synthesized by *Lactobacillus* spp. could act as fermentable substrates as BGS.^[44]

We initially tested the ability of 13 pathogen bacteria to form biofilms *in vitro* in a classic micro well assay. The highest biofilm forming pathogen bacteria (*B. cereus* RSKK 863, *L. monocytogenes* ATCC 7644, *E. fecalis* ATCC 25175, and *P. aeruginosa* ATCC 72853) were selected to determine the anti-biofilm effect of I-EPSs (Table 4). EPSs, as one of the primary metabolic products of LAB, were strongly associated with functional properties such as antioxidant, antimicrobial and cholesterol-lowering effects due to their unique characteristics and strong biological activities.^[45] However, so far few studies have been reported on the characterization and biological activities (e.g., antioxidant, anti-biofilm, and anti-tumor activities) of the I-EPS from lactobacilli.

A biofilm formation assay with varying concentration of I-EPSs (0.1 and 1 mg/mL) was made in order to study the anti-biofilm potential of the I-EPSs against four pathogen bacteria. As portray in Table 5, the inhibitory activities of the I-EPS from three lactobacilli strains on biofilm formation by the four pathogens *B. cereus* RSKK 863, *L. monocytogenes* ATCC 7644, *E. fecalis* ATCC 25175, and *P. aeruginosa* ATCC 72853 were concentration dependent. The I-EPSs of three lactobacilli indicated potent anti-

Table 4. Biofilm formation of some pathogenic bacteria.

Strains	Biofilm
<i>B. cereus</i> RSKK 863	+++
<i>L. monocytogenes</i> ATCC 7644	+++
<i>E. fecalis</i> ATCC 25175	+++
<i>P. aeruginosa</i> ATCC 72853	+++
<i>E. coli</i> 0157:H7	++
<i>E. coli</i> ATCC 35218	++
<i>S. aureus</i> EB-1	+
<i>S. aureus</i> ATCC 25923	+
<i>S. aureus</i> EB-12	+
<i>S. aureus</i> ATCC 2392	+
<i>S. aureus</i> EB-36	+
<i>S. aureus</i> EB-18	+
<i>S. typhimurium</i> BAST-01	+

+++ : Strong biofilm formation.
++ : Middle biofilm formation.
+ : Weak biofilm formation.

Table 5. Anti-biofilm activity of lyophilized EPSs from the exopolysaccharide (EPS)-produced lactobacilli strains.

Strains	Inhibition of biofilm (%)					
	LB-69		GD-11		B-3	
	0.1 mg/mL I-EPS	1 mg/mL I-EPS	0.1 mg/mL I-EPS	1 mg/mL I-EPS	0.1mg/mL I-EPS	1 mg/mL I-EPS
<i>L. monocytogenes</i> ATCC 7644	50	88	36	83	21	76
<i>E. fecalis</i> ATCC 25175	47	85	30	87	15	75
<i>B. cereus</i> RSKK 863	55	90	38	83	20	78
<i>P. aeruginosa</i> ATCC 72853	52	88	36	85	18	74

biofilm activities by inhibiting the four biofilm forming pathogen bacteria such as the abilities of BGS. The inhibition abilities of I-EPSs on biofilms forming pathogen bacteria were in a concentration dependent way. Biofilm formation in *B. cereus* RSKK 863, *L. monocytogenes* ATCC 7644, *E. fecalis* ATCC 25175, and *P. aeruginosa* ATCC 72853 diminished significantly ($p < 0.01$) with enhancing concentrations of I-EPSs (Table 5). The I-EPSs of *L. fermentum* LB-69 exhibited significant biofilm inhibition, especially with four pathogen bacteria. At a concentration of 1 mg/mL, *L. fermentum* LB-69 I-EPS significantly inhibited 90% of biofilm formation by *B. cereus* RSKK 863 followed by *L. monocytogenes* ATCC 7644 (88%) and *P. aeruginosa* ATCC 72853 (88%) among these pathogen bacteria. These results are similar to those reported previously for the lactobacilli in the studies of Kim et al.^[31] and Li et al.^[16] Kim et al.^[31] declared that enterohemorrhagic *E. coli* biofilms were dramatically diminished by 87% in plastic 96-well microplates that contained 1 mg/mL of released EPS (r-EPS) by the *Lactobacillus acidophilus* A4 strain. Li et al.^[16] indicated that, the EPS from *Lactobacillus helveticus* MB2-1 had anti-biofilm activity against several biofilm-forming bacteria.

In the present study, it was also found that the level of biological activity was depended on the type of EPS ($p < 0.01$). It was found remarkable that the I-EPSs of *L. fermentum* LB-69 had high level of mannose (96%). Bifidobacteria are very important microorganisms for taking part in the health of the human gastrointestinal tract. In order to depict these health-beneficial effects, bifidobacteria should raise their population at a definite number. So, the growth stimulator factors of bifidobacteria are very important for stabilizing the microflora in the gut.^[26] Prebiotics stimulate the growth of probiotic bacteria in the colon; especially inulin is used for bifidobacteria. This prebiotic carbohydrate is obtained from chicory plants. However, it is very difficult to get this plant. So, BGS molecules should be utilized as a probable prebiotic in food, and microorganisms are easier than plants. Since this activity is not influenced by heat, a wide pH range, and storage conditions, BGS would not lose influence in the gastrointestinal conditions and can be used as a prebiotic alternative to inulin in food products.^[44] Microbial EPSs that

produced by the probiotic bacteria very important for human health. As well as EPS has immunomodulating, antiulcer, antitumor, cholesterol-lowering effects; prebiotic activity of the EPS is a new found and important feature.^[46]

Conclusion

This is the first article giving a detailed anti-biofilm activity and potential prebiotic application of l-EPSs from the lactobacilli. The l-EPS produced by *L. fermentum* LB-69, which may make it potential in the design of new therapeutic strategies for bacterial biofilm-associated infections and limiting the biofilm formation on medical indwelling devices. The investigation displayed that the l-EPSs produced by lactobacilli had potentials as a multi-functional natural ingredient for therapeutic and health foods. These results may also promote a new recognition about the functions of bacterial EPSs. The prebiotic effect and anti-biofilm activity of the EPSs may contribute to its possible pharmaceutical applications. Also, this is due to their properties, EPSs may be a new potential in the development of functional foods.

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