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Research Article

Efficient Screening and Enhanced Exopolysaccharide Production by Functional Lactic Acid Bacteria (LAB) in Lactose Supplemented Media

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Abstract: Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) can be considered as natural biological thickeners that have attracted considerable attention in the food industry. This study aimed to evaluate and select potentially EPS-producing strains LAB and to assess the influence of carbon source and aeration on EPS production. Nine LAB strains were assessed as potential EPS producers, and *Rahnella aquatilis* ATCC 55046 was employed as the positive control strain for EPS. The compaction test and the observation of viscous colonies in a solid medium did not yield sufficient evidence for the presence of EPS. The assessment of capsules through staining provided evidence of EPS presence only for *Rahnella aquatilis* ATCC 55046. The EPS yield was subsequently assessed in De-Man Rogosa and Sharpe (MRS) broth medium supplemented with 2% (w/w) fructose (MRS-f) or lactose (MRS-l), as well as in whey (Whey) and whey supplemented with 2% (w/w) lactose (Whey-l). The EPS production in the various culture media under study ranged from 194 to 1,187 mg of EPS/g of polymer dry mass (PDM). These results suggest that the culture medium and carbon sources had an impact on the EPS production of the different strains. *Bifidobacterium animalis* Bb12 achieved the highest EPS production in MRS-f. In the case of MRS-l, the control strain recorded the highest EPS value, along with *Lactobacillus acidophilus* LAC-1. Regarding Whey, *Lentilactobacillus Kefir* NCFB 2753 exhibited the highest EPS production, while in Whey-l, *Lacticaseibacillus paracasei* LCS-1 emerged as the top performer in terms of EPS production. This suggests that certain strains exhibit potential for use in the production of novel fermented EPS products, whether dairy or non-dairy.

***Keywords*:** Exopolysaccharides, EPS screening, lactic acid bacteria, carbon sources, MRS broth, bovine whey

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1. Introduction

Numerous bacteria are recognized for their ability to produce microbial exopolysaccharides (EPS), which are extracellular macromolecules released into the growth medium, either as a tightly bound capsule or a loosely attached slime layer in microorganisms [1]. A growing interest in EPS produced by lactic acid bacteria (LAB) has been the subject of extensive research work in recent years, spanning physiology, fermentation, chemical and structural characteristics of EPS molecules, biosynthesis, genetic and metabolic engineering, and functional properties of these biomolecules [2].

Bacterial EPS represents potential biopolymers for the food, cosmetic, and pharmaceutical industries, serving as bio-flocculants, bio-absorbents, and drug delivery agents [1]. They have garnered significant interest in pharmacological and nutraceutical applications owing to their biocompatibility [3-4], non-toxicity, and biodegradability. Additionally, they find applications as thickening, stabilizing, emulsifying, or gelling agents in the food industry [2].

These EPS can be classified based on their chemical composition and biosynthesis mechanism into homopolysaccharides, consisting of a single type of monosaccharide (e.g., cellulose and dextran), and heteropolysaccharides containing repeating units of two or more types of monosaccharides (e.g., xanthan), as well as substituted monosaccharides and other units such as phosphate, acetyl, and glycerol [5].

EPS-producing LAB have proven to be significant not only for the properties of the polymers they produce but also because they are generally recognized as safe organisms (GRAS) and play a crucial role in the food sector, particularly in the dairy industry. Some functional strains, specifically LAB, offer various health benefits to consumers when incorporated into food products due to their probiotic properties. One physiological benefit of EPS is their prolonged stay in the gastrointestinal tract, thereby enhancing the colonization by probiotic bacteria [6]. Several health benefits have been attributed to certain EPS produced by LAB, including antitumor effects [7-8], cholesterol-lowering ability [9], and immunostimulatory activity [10]. Additionally, the EPS production capacity of these strains may be an added feature that improves the texture and sensory properties of these food products [11].

The most prominent EPS-producing LAB belong to the bacterial families Lactobacillaceae, Leuconostocaceae, Streptococcaceae, and Bifidobacteriaceae [12]. The yield of EPS is influenced by the bacterial strains, medium composition, and cultural conditions. The type of carbon source has a significant impact on EPS productivity and may also affect the composition of EPS [13]. Various carbon sources, either individually or in combination, are often employed in screening studies to identify the EPS phenotype in LAB and optimize EPS production by the same strains. For instance, van Geel-Schutten et al. [14] screened several Lactobacillus strains from different sources (fermented food, animals' gastrointestinal tract, and human dental plaque) for EPS production in an MRS medium suppleented with high concentrations (100 g/L) of different sugars: glucose, fructose, maltose, raffinose, sucrose, galactose, or lactose.

Many lactobacilli strains have been tested for EPS production using various complex media, including MRS, All Purpose Tween (APT), and synthetic media made from enriched milk ultrafiltrate or cheese whey [4, 15-17]. Gamar et al. [18] found that the yield of EPS was significantly affected by the carbon source and concentration. They observed a substantial increase in EPS production when *L. rhamnosus* was cultivated in a chemically defined medium with the addition of mannose, glucose, and fructose. However, commercial media like MRS are often too expensive for large-scale EPS production. To address this, researchers have explored alternative carbon sources, such as agro-industrial wastes, including dairy, fruit, and vegetable residues, which are still rich in nutrients but can be more cost-effective [19-21].

Different EPS screening methods have already been described for LAB. Smitinont et al. [22] employed visual inspection of bacterial colonies on agar plates, and viscometric analysis of a culture medium during or after fermentation has also been applied to screen for EPS production [23].

The objective of this study was to assess and identify potential EPS-producing LAB, with the intention of using them as functional starter cultures to improve the quality and impart functional attributes to fermented dairy products. To achieve this, EPS production by selected strains of lactobacilli and bifidobacteria was evaluated in MRS medium and whey. Additionally, the impact of carbon source and aeration on EPS production was investigated.

2. Materials and methods

2.1 *Bacterial strains and culture conditions*

Nine probiotic strains (from established culture collections) were tested as potential EPS-producing bacteria (Table 1). The stock cultures of *Bifidobacterium animalis* Bb12, *Bifidobacterium animalis* Bo, *Bifidobacterium animalis* BLC-1, *Lactobacillus acidophilus* LAC-1, *Lactobacillus acidophilus* Ki, and the newly amended genera by Zheng et al. [69], namely, *Lacticaseibacillus paracasei subsp. paracasei* LCS-1, *Lactiplantibacillus pentosus* LMG 10755, *Lentilactobacillus kefir* NCFB 2753, *Levilactobacillus brevis* LMG 6906 (formerly known as *Lactobacillus paracasei, Lactobacillus pentosus*, *Lactobacillus kefir*, *Lactobacillus brevis*, respectively), and *Rahnella aquatilis* ATCC 55046 (used as an EPS-positive control strain), were maintained at -80 °C.

Table 1. Strains tested and their sources

Strain	Source
Bifidobacterium animalis Bb12	Christian Hansen (Hoersholm, Denmark)
Lentilactobacillus kefir NCFB 2753	National Collection of Food Bacteria (UK)
Lactobacillus acidophilus Ki	CSK-frozen concentrates (Leeuwarden, The Netherlands)
Bifidobacterium animalis Bo	
Lactobacillus acidophilus LAC-1	DELVO-PRO® DSM; (Moorebank, Australia)
Lacticaseibacillus paracasei subsp. paracasei LCS-1	
Bifidobacterium animalis BLC-1	
Levilactobacillus brevis LMG 6906	Laboratorium voor Microbiologie en Microbiele Genetica, Rijksuniversiteit (Gent, Belgium)
Lactiplantibacillus pentosus LMG 10755	
Rahnella aquatilis ATCC 55046	American Type Culture Collection (Baltimore, MD)

Working cultures were initiated with a 1% (v/v) inoculum in 10 mL of MRS broth medium [70] (Merck, Darmstadt, Germany) containing peptone from casein 10.0 g/L, meat extract 8.0 g/L, yeast extract 4.0 g/L, D(+)-glucose 20.0 g/L, dipotassium hydrogen phosphate 2.0 g/L, Tween® 80 1.0 g/L, di-ammonium hydrogen citrate 2.0 g/L, sodium acetate 5.0 g/L, magnesium sulfate 0.2 g/L, manganese sulfate 0.04 g/L. In the case of bifidobacteria strains, it was supplemented with cysteine to a final concentration of 0.05% (w/v). *R. aquatilis* ATCC 55046 was grown in Tryptic Soy Broth (TSB) (Difco, Fisher Scientific, Göteborg, Sweden) [71].

Incubation was performed under anaerobic conditions (Gas-Pak Plus system from Becton Dickinson, Maryland MA, USA) for *Bifidobacterium spp*. and *L. acidophilus Ki*, for 24 hours at 30 °C [24-26]. The remaining strains grew under both aerobic and anaerobic conditions (Gas-Pak Plus system, from Becton Dickinson, Maryland MA, USA) for 24 hours at 37 °C, and for *R. aquatilis* ATCC 55046 and *L. pentosus* LMG 10755, at 30 °C [27-28]. The working culture was stored at 4 °C for up to 2 weeks and used as a starter culture for all EPS experiments.

2.2 *Screening of EPS-production*

The screening for EPS production in strains was conducted on MRS agar plates supplemented with 2% (w/w)

of either fructose (MRS-f) or lactose (MRS-l) as a carbon source. The strains were streaked on both types of media and incubated, as previously described in 2.1, for 48-72 hours. At the end of the incubation period, during the initial screening phase, colonies were assessed for compactness or ropiness on solid media by gently touching them with a sterile inoculation loop, following the pick test method [29-30] and mucoid colonies were determined by visual appearance [31]. Additionally, to examine the presence of capsules, all strains underwent analysis using the India ink capsule stain [32] and were observed under optical microscopy *R. aquatilis* ATCC 55046 was used as a positive control.

2.3 *Isolation and quantification of EPS*

The potential EPS-producing strains were cultured in MRS broth medium supplemented with 2% (w/w) of the carbon sources fructose (MRS-f) or lactose (MRS-l), as well as in whey (Whey) and whey supplemented with 2% (w/w) lactose (Whey-l). The whey used in our study was bovine whey obtained as a by-product from the manufacturing of low-fat cheese made from the milk of a native Portuguese bovine breed (Cachena). The whey was promptly refrigerated and stored at 7 °C for up to 2 days until used. Prior to inoculation, the whey was sterilized (110 °C for 20 min).

Following inoculation, cultures were incubated at the appropriate temperatures for 48-72 hours under both aerobic and anaerobic conditions, except for *Bifidobacterium spp*. and *L. acidophilus* Ki, which were subjected only to anaerobic conditions. The insoluble material was subsequently removed by centrifugation at 4,000 rpm for 20 min. For Whey and Whey-l, any residual protein material was precipitated by the addition of one volume of 4% (w/v) trichloroacetic acid (TCA), followed by incubation at 4 °C for 2 hours. The potential EPS material produced in all media was then precipitated by the addition of three volumes of cold ethanol and stored overnight at 4 °C.

The precipitates were subsequently recovered through freeze-drying, and the amounts were determined gravimetrically, expressed as polymer dry mass (mg PDM per liter of culture), and then utilized for the analysis of total carbohydrates. The carbohydrate content of the PDM was assessed using a colorimetric phenol-sulfuric acid assay [72]. A precise amount of PDM was re-dissolved in 2 mL of distilled water, and the EPS (carbohydrate) content was calculated by comparing it with a standard glucose curve of absorption versus glucose concentration ($y = 0.1056x - 0.0022$, $R^2 = 0.9994$), subsequently adjusted with a dilution ratio of 25-fold. The optical density was measured spectrophotometrically at 490 nm, and the EPS quantities of the samples were determined as mg/g of PDM. All analyses were conducted in duplicate, and the results were expressed as mean values with corresponding standard deviations.

2.4 *Statistical analyses*

The SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Data (monitored by the total yield of EPS) of four mediums with different carbon sources, MRS-f, MRS-l, Whey and Whey-l upon the ten different strains were analyzed for normal distribution and by means of independent one-way ANOVA tests and pairwise comparisons of mean values (following Tukey post hoc test) at the 5% significance level were performed to confirm whether the differences occurred between the studied groups.

3. Results

3.1 *Screening of EPS producer strains*

To assess potential EPS producers in the initial screening, we performed the traditional pick test on solid media MRS-f and MRS-l, examining the morphological traits of the colonies growing on the agar surface. These traits, typically associated with EPS synthesis, include slimy or ropiness, and mucoid colonies, identified through visual inspection. The discriminatory value of the methods employed for identifying mucoid and slimy bacterial colonies was limited. The observed differences among the strains did not provide clear evidence of mucoid colonies, indicating that this method alone is not sufficiently discriminatory. The Indian ink staining technique for identifying capsular polysaccharides did not yield enough evidence for the presence of EPS (data not shown) in any strain, except for *R. aquatilis* ATCC 55046 (positive control), which exhibited a polysaccharide capsule. The presence of a transparent halo around the pink cells was observable.

Strains such as *L. paracasei* LCS-1, *L. pentosus* LMG 10755, *L. kefir* NCFB 2753, *L. brevis* LMG 6906, and *R.*

aquatilis ATCC 55046 showed minimal growth in MRS-f under the studied aerobic and anaerobic conditions, hindering the visualization of mucoid colonies and subsequent India ink staining technique in this specific medium. However, they did exhibit growth in the MRS-l.

3.2 *EPS quantification*

After the initial screening phase yielded inconclusive results in the selection of strains as potential EPS producers, LAB strains underwent further evaluation in the second screening phase. Except for strains *L. paracasei* LCS-1, *L. pentosus* LMG 10755, *L. kefir* NCFB 2753, *L. brevis* LMG 6906, and *R. aquatilis* ATCC 55046, which did not exhibit proper growth in MRS-f during the initial phase of the study, these strains were consequently excluded from the second phase of EPS screening in MRS-f. During this subsequent phase, they were cultivated in various liquid media under specified culture conditions, and the resulting EPS-containing material (PDM) was isolated and determined gravimetrically (Table 2).

Table 2. Results of polymer dry mass (PDM) (mg/L) in cultures of EPS-producing LAB in MRS broth supplemented with fructose (MRS-f) and lactose (MRS-l) and in whey (Whey) and whey supplemented with lactose (Whey-l)

Strain, aeration	Medium			
	MRS-f	MRS-l	Whey	Whey-l
	(mg/L)	(mg/L)	(mg/L)	(mg/L)
B. animalis Bo, *ana*	4,336 ± 181	4,576 ± 11	1,480 ± 91	3,132 ± 796
B. animalis BLC-1, *ana*	3,712 ± 701	3,384 ± 170	1,588 ± 458	2,520 ± 249
B. animalis Bb12, *ana*	3,428 ± 730	4,736 ± 554	1,972 ± 107	2,560 ± 147
L. acidophilus Ki, *ana*	3,688 ± 136	3,596 ± 639	1,556 ± 775	2,712 ± 238
L. acidophilus LAC-1, *ana*	2,404 ± 198	3,604 ± 96	2,172 ± 280	2,168 ± 147
L. acidophilus LAC-1, *a*	2,144 ± 611	2,904 ± 385	1,592 ± 215	2,228 ± 356
L. paracasei ssp. *paracasei* LCS-1, *ana*	-	2,740 ± 6	1,452 ± 175	1,708 ± 311
L. paracasei ssp. *paracasei* LCS-1, *a*	-	3,128 ± 305	1,092 ± 6	1,212 ± 96
L. pentosus LMG 10755, *ana*	-	2,956 ± 243	1,260 ± 164	1,452 ± 209
L. pentosus LMG 10755, *a*	-	2,708 ± 164	1,260 ± 107	1,396 ± 28
L. kefir NCFB 2753, *ana*	-	4,624 ± 1,720	1,068 ± 175	1,192 ± 532
L. kefir NCFB 2753, *a*	-	3,260 ± 74	1,236 ± 164	1,368 ± 158
L. brevis LMG 6906, *ana*	-	3,064 ± 170	1,376 ± 419	1,632 ± 34
L. brevis LMG 6906, *a*	-	3,216 ± 373	1,364 ± 255	1,104 ± 339
R. aquatilis ATCC 55046, *ana*	-	4,248 ± 339	1,596 ± 153	2,984 ± 57
R. aquatilis ATCC 55046, *a*	-	4,280 ± 1,018	1,648 ± 407	2,704 ± 1,143

ana = anaerobic conditions, *a* = aerobic conditions

The observed PDM values (mg/L) were consistently higher in MRS-f and MRS-l culture media compared to Whey and Whey-l media for all tested strains. However, in Whey-l, PDM values were generally higher than those in Whey for most strains. The highest PDM values were recorded for *B. animalis* Bb12, *B. animalis* Bo, and *L. kefir* NCFB 2753 under anaerobic conditions, and *R. aquatilis* ATCC 55046 under both aeration conditions in MRS-l medium (Table 2).

The carbohydrate content of the PDM was determined using a colorimetric phenol-sulfuric acid method with glucose as the standard. All strains demonstrated the ability to produce EPS, with yields ranging from 194 to 1,187 mg of EPS/g of PDM (Table 3). However, as observed in Tables 2 and 3 and previously mentioned in 3.1, it is important to note that several strains were incapable of producing EPS in MRS-f.

The strains *B. animalis* Bb12 and *L. acidophilus* Ki produced 528 and 516 mg/g of EPS, respectively, in liquid MRS-f medium-these values represented the highest recorded EPS production in this medium. In contrast, the *B. animalis* Bo strain exhibited the lowest production at 290 mg/g.

Table 3. Results of EPS concentration (mg EPS/g of PDM) in cultures of EPS-producing LAB in MRS broth supplemented with fructose (MRS-f) and lactose (MRS-l) and in whey (Whey) and whey supplemented with lactose (Whey-l)

Strain, aeration	Medium			
	MRS-f	MRS-l	Whey	Whey-l
	(mg/g)	(mg/g)	(mg/g)	(mg/g)
B. animalis Bo, *ana*	290 ± 3 ^{a}	252 ± 33 ^{a}	490 ± 195 ^{ab}	741 ± 47 ^{b}
B. animalis BLC-1, *ana*	397 ± 37 ^{a}	389 ± 75 ^{a}	584 ± 66 ^{a}	522 ± 199 ^{a}
B. animalis Bb12, *ana*	528 ± 77 ^{ab}	326 ± 140 ^{a}	917 ± 119 ^{b}	851 ± 62 ^{b}
L. acidophilus Ki, *ana*	516 ± 24 ^{a}	359 ± 6 ^{a}	905 ± 37 ^{a}	903 ± 266 ^{a}
L. acidophilus LAC-1, *ana*	444 ± 23 ^{ab}	342 ± 9 ^{a}	558 ± 21 ^{bc}	635 ± 75 ^{c}
L. acidophilus LAC-1, *a*	464 ± 171 ^{a}	430 ± 60 ^{a}	415 ± 152 ^{a}	719 ± 13 ^{a}
L. paracasei ssp. *paracasei* LCS-1, *ana*	-	392 ± 8 ^{a}	638 ± 15 ^{b}	625 ± 34 ^{b}
L. paracasei ssp. *paracasei* LCS-1, *a*	-	377 ± 65 ^{a}	831 ± 38 ^{ab}	1,187 ± 260 ^{b}
L. pentosus LMG 10755, *ana*	-	323 ± 41 ^{a}	600 ± 51 ^{b}	704 ± 57 ^{b}
L. pentosus LMG 10755, *a*	-	381 ± 47 ^{a}	729 ± 35 ^{b}	800 ± 17 ^{b}
L. kefir NCFB 2753, *ana*	-	304 ± 0 ^{a}	923 ± 35 ^{b}	1,014 ± 104 ^{b}
L. kefir NCFB 2753, *a*	-	399 ± 25 ^{a}	733 ± 84 ^{b}	959 ± 10 ^{c}
L. brevis LMG 6906, *ana*	-	194 ± 15 ^{a}	907 ± 173 ^{b}	1,178 ± 94 ^{b}
L. brevis LMG 6906, *a*	-	285 ± 59 ^{a}	721 ± 17 ^{b}	905 ± 84 ^{b}
R. aquatilis ATCC 55046, *ana*	-	420 ± 35 ^{a}	574 ± 46 ^{ab}	771 ± 81 ^{b}
R. aquatilis ATCC 55046, *a*	-	433 ± 18 ^{a}	912 ± 311 ^{a}	660 ± 261 ^{a}

$^{a-c}$Means in rows without common letters are significantly different ($P < 0.05$; $n = 8$)
ana = anaerobic conditions, *a* = aerobic conditions

In the MRS-l medium, under aerobic conditions, strains *L. acidophilus* LAC-1 and *R. aquatilis* ATCC 55046 exhibited the highest EPS concentrations. Specifically, these strains demonstrated EPS concentrations more than twice that of *L. brevis* LMG 6906, which displayed poor EPS production (194 mg/g).

In Whey medium, *L. kefir* NCFB 2753, under anaerobic conditions, achieved the maximum EPS concentration (923 mg/g), approximately double that of *L. acidophilus* LAC-1 under anaerobic conditions, which showed the lowest production (415 mg/g). Additionally, in Whey-l, *L. paracasei* LCS-1, under aerobic conditions, displayed the highest EPS concentration (1,187 mg/g), while *B. animalis* BLC-1 had the lowest EPS yield (522 mg/g).

Most strains exhibited statistically significant differences ($p < 0.05$) in EPS production among the different media studied, with the exceptions of *B. animalis* BLC-1 ana, *L. acidophilus* Ki *ana*, *L. acidophilus* LAC-1 a, and *R. aquatilis* ATCC 55046 a. When comparing EPS production between MRS-f and MRS-l media, no statistical differences ($p > 0.05$) were observed for any of the strains under analysis.

Nevertheless, the results indicated significantly higher EPS yields for all strains in Whey and Whey-l compared to MRS media supplemented with two carbon sources. Particularly noteworthy were the differences observed for *L. brevis* LMG 6906 *ana*, which produced approximately six times more EPS in Whey-l than in liquid MRS-l as well as *L. paracasei* LCS-1 *a*, and *L. kefir* NCFB 2753 *ana*, displayed nearly three times more EPS production in Whey-l than MRS-l. In contrast, *B. animalis* BLC-1 exhibited the lowest EPS yield (522 mg/g) in Whey-l.

When comparing Whey and Whey-l, the strains that exhibited the highest EPS yields in Whey-l were *L. acidophilus* LAC-1 *a*, *L. paracasei* LCS-1 *a*, *L. brevis* LMG 6906 *ana*, *R. aquatilis* ATCC 55046 *a*, and *L. kefir* NCFB 2753 *a*. Conversely, strains that displayed lower EPS production in Whey-l were *L. acidophilus* Ki *ana* and *L. paracasei* LCS-1 *ana*.

Aeration conditions significantly influenced the EPS production of *L. kefir* NCFB 2753, *L. acidophilus* LAC-1, and *L. brevis* LMG 6906 in MRS-l, with increases of 1.3 to 1.5-fold observed under aerobic conditions.

Under aerobic conditions, *R. aquatilis* ATCC 55046 exhibited higher EPS yield in Whey compared to Whey-l. In contrast, *L. paracasei* LCS-1 showed greater EPS production in the Whey-l medium under aerobic conditions.

4. Discussion

The visual inspection of bacterial colonies on agar plates represents the simplest method for EPS screening [2, 33-37]. LAB may produce EPS in the form of capsules tightly associated with the cell wall or secrete them into the environment in the form of slime. Bacteria can produce one or both forms of EPS [38]. Colonies of encapsulated bacteria typically appear smooth and often iridescent, while those of non-encapsulated bacteria tend to be rough (dry to the touch) and smaller [39]. The presence of a translucent or creamy material surrounding a mucoid colony indicates EPS production. However, in the present study, the visualization of mucoid colonies was inconclusive for discriminating between different strains as potential EPS producers and non-producers. The strains' capacity to produce capsular polysaccharides or EPS, along with their sticky nature, is genetically unstable. These capabilities can be lost by the strains after several passages of the organisms in the laboratory [2, 40]. Occasionally, detecting mucoid colonies can be challenging, hindering the acquisition of discriminatory information, particularly for low EPS-producing LAB strains [41-43]. Capsule formation can be observed in both non-ropy and ropy strains. Light microscopy, combined with staining bacterial cells, offers an inexpensive, simple, and fast method applicable at the industry level for detecting EPS-producing bacteria. However, in our study, we did not find evidence of capsules for the different strains included, except for the *R. aquatilis* ATCC 55046 used as an EPS-positive control strain, which presented a capsule. This uncertainty arises because the dye application step is a critical aspect of staining methods. An appropriate amount of dye must be used to achieve films of adequate thickness. If the film is too thick, the bacteria may spread, becoming obscured by the overlying ink and barely visible against the dark background of the slides. Additionally, the regular background can interfere with the image of the capsules. If the film is pressed too thin, the capsules may become flattened, distorted, and possibly disintegrated [44].

The careful selection of strains and the optimization of culture conditions can significantly enhance the production of EPS by LAB. Several studies have highlighted the dependence of LAB's EPS production on various culture conditions, including the composition of the medium and physicochemical parameters, as well as the physiological state

of the cells [18, 45-53]. MRS, a complex and nutrient-rich medium composed of yeast extract and peptones, is widely employed for the laboratory cultivation of LAB. It is commonly used for the enrichment, cultivation, and isolation of EPS-producing LAB. Our study aimed to assess strains with the potential for EPS production using two approaches: MRS medium supplemented with two different carbon sources, namely lactose (MRS-l) and fructose (MRS-f). These variations were tested to cultivate the microorganisms and increase the yield of EPS. In the second phase of the study, the strains that did not exhibit proper growth in MRS-f during the initial phase of the study (*L. paracasei* LCS-1, *L. pentosus* LMG 10755, *L. kefir* NCFB 2753, *L. brevis* LMG 6906, and *R. aquatilis* ATCC 55046) were not tested in this medium. The strains that thrived in both MRS-l and MRS-f media exhibited similar EPS production yields, suggesting that lactose and fructose promoted EPS production equally. In the case of bifidobacteria, EPS yield production was slightly higher in MRS-f than in MRS-l. Audy et al. [54] observed that *B. longum subsp. longum* CRC 002 exhibited the highest EPS production capacity (1,080 ± 120 mg/L) when cultivated in an MRS broth medium with lactose without pH adjustment. Conversely, in the MRS medium with fructose, galactose, and glucose, EPS production was lower, at 512 ± 63, 564 ± 165, and 616 ± 93 mg/L, respectively.

In our study, in the whey with and without supplemented with lactose media, all tested strains exhibited EPS production. The basic fermenting sugar for LAB in the growth medium is lactose, a naturally occurring component in milk. The addition of lactose to milk media has been employed in certain studies to stimulate EPS production [55-56]. Whey is recognized as a nutrient-rich medium, providing not only lactose but also serving as a source of amino acids, which can significantly enhance EPS production. Rabha et al. [57] demonstrated the ability of *S. thermophilus* BN1 to produce EPS using whey as a sole substrate. Our results showed that disparities in EPS production between the two media were not statistically significant ($p > 0.05$) for most strains, however, it was observed that the addition of lactose positively influenced EPS production. The Bifidobacterium strain with the lowest EPS production capacity in MRS was *B. animalis*. However, in Whey, and especially in Whey-l, it exhibited higher EPS production, supporting the idea that lactose also positively influenced its EPS production capacity. It is noteworthy to observe the strain-dependent nature of EPS production. Several other studies have demonstrated similar trends, albeit at lower concentrations than those reported in the present study. Abbad Andaloussi et al. [58] observed that the EPS production capacity of *B. longum* varied within the range of 115-145 mg/L in peptone/yeast medium and 105-132 mg/L in skim milk. Prasanna et al. [59] noted that, for both *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486, the maximum growth and EPS production levels were achieved in skim milk supplemented with casein hydrolysate, reaching around 241 and 366 mg/L of EPS, respectively.

Regarding the *Lactobacillus* strains, the anaerobic strain *L. acidophilus* KI exhibited similar results to *B. animalis* Bb12 across various media studied. Notably, *L. acidophilus* LAC-1 demonstrated the highest levels of EPS production in MRS-l comparable to the control strain *R. aquatilis* ATCC 55046. However, in the case of this facultative anaerobic strain, slightly higher EPS levels were observed under aerobic conditions in all media, except for Whey, where the EPS production was the lowest among all strains. Our results in MRS-l for this strain align positively with those reported by Deepak et al. [60], who indicated maximum EPS levels of 597 mg/L for *L. acidophilus* 10307 under optimal growth conditions. Contrastingly, Amiri et al. [16] reported lower values for *L. acidophilus* compared to our findings, with the maximum observed EPS production in this study in cheese whey being 349.82 ± 5.39 mg/L.

The maximum EPS production yield in the Whey-l medium was observed in *L. paracasei* LCS-1 under aerobic conditions. The impact of aeration on EPS production for this strain was evident in both Whey and Whey-l. However, in the MRS medium, this difference was not noticeable. Zhang et al. [61] reported higher EPS production values than ours in MRS-l for *L. paracasei* TD 062, with EPS levels reaching 0.609 g/L. On the other hand, Dupont et al. [62] investigated *L. paracasei* Type V in a chemically defined medium with the addition of glucose or lactose, as well as in milk, and observed lower EPS values, with maximum production levels of 93 mg/L, 85 mg/L, and 79 mg/L, respectively.

For *L. pentosus* LMG 10755, a higher EPS production was observed under aerobic conditions in all media, specifically in Whey and Whey-l. Sánchez et al. [53] observed EPS production values close to ours for *L. pentosus* LPS26 in a chemically semi-defined medium with various carbon sources. Under optimal production conditions, the values obtained (514 mg/L) closely resembled ours, leading the authors to conclude that glucose and lactose were the most favorable sugars for EPS production.

L. kefir NCFB 2753 exhibited slightly higher EPS production under aerobic conditions in MRS-1. However, in

Whey and Whey-l, the trend was reversed, with higher EPS production observed under anaerobic conditions. While slightly lower, our results align with those obtained by Wang et al. [63], who investigated *L. kefiranofaciens* ZW3, an EPS-producing strain isolated from Tibet kefir grain. This strain demonstrated significant EPS production, reaching up to 1,215 mg/L in supplemented whey media. Moreover, it achieved even higher levels, up to 1,675 mg/L, when the incubated broth was subjected to heating at 100 °C for 30 minutes.

L. brevis LMG 6906 exhibited the lowest EPS yield in the MRS-l medium, particularly under anaerobic conditions. Although the production slightly increased under aerobic conditions this strain demonstrated the lowest EPS production after *B. animalis* Bb12. However, in Whey and Whey-l, the EPS yields were significantly higher than in MRS-1, confirming that lactose had a positive influence on EPS production. While studies on EPS production with this strain are limited, Ermiş et al. [64] observed much higher production values for *L. brevis* E25, which produced from 10 to 35 g/L in MRS broth.

The *R. aquatilis* ATCC 55046 strain was selected as the positive control in our study due to its recognition in previous research for its high EPS production capacity. This facultative anaerobic bacterium, associated with *Erwinia* spp., can generate a polysaccharide gum from lactose and other sugars. Specifically, it produces lactan, a polysaccharide composed of mannose, galactose, and galacturonic acid (at the molar ratios 5:3:2). This occurs in a semi-defined lactose-rich medium through fermentation, as detailed by Flatt et al. [65] and Flatt et al. [66]. Our results indicate that *R. aquatilis* ATCC 55046 produced EPS in MRS media and whey, with the highest quantity observed in Whey under aerobic conditions (912 mg/g). Although these results were lower than those previously reported by [27], who compared the fermentative production of lactan from sweet cheese whey and synthetic medium, noting polysaccharide production reaching 22 g/L and 26 g/L, respectively. Matsuyama et al. [67] reported EPS concentrations of approximately 1.63 g/L for insoluble EPS and 2.58 g/L for soluble EPS in a synthetic medium produced by *R. aquatilis*.

The bacterial polymers obtained in this study were characterized as polymers with a low degree of purity, or crude EPS extracts. Since this study aimed to compare the EPS production capacity among LAB, there was no immediate requirement for high EPS purity. The polymeric dry mass (PDM) results indicated significantly higher values than the EPS amounts estimated through the phenol-sulfuric acid method across all media and for all strains. These discrepancies can likely be attributed to the presence of interfering compounds in the medium, which are carried along during the EPS extraction process. Previous studies, such as Kimmel et al. [52], have confirmed that the MRS medium contains carbon, nitrogen sources, and other nutrients and supplements aimed at enhancing bacterial growth and EPS production. These elements can potentially interfere with the purity of the extracts, introducing components that are not efficiently eliminated in the isolation process. This interference may affect the accuracy of detection and quantification methods, leading to an overestimation of the EPS levels. The MRS medium is widely utilized in EPS production, particularly in screening studies, special attention should be given to EPS extraction and purification methods when conducting production and characterization studies. The MRS medium is a significant source of interfering compounds in EPS quantification, predominantly glucose-rich material, and to a lesser extent, mannoproteins from yeast extract. This could explain the higher polymeric dry mass (PDM) obtained, especially in MRS-f and MRS-l, as a result of the precipitation of components from the MRS growth medium. During EPS isolation with solvent precipitation, these components may co-precipitate with EPS, contributing to the elevated polymeric mass obtained [2, 68].

This difference may arise from an additional treatment step in the EPS precipitation method for Whey and Whey-l, where residual protein material was pre-precipitated with the addition of TCA before solvent precipitation. This likely contributed to the EPS extracts from these media containing fewer impurities and may explain the lower PDM results observed in these media.

5. Conclusions

All strains exhibited the capacity to produce EPS in both culture media (MRS medium and whey). However, whey and lactose-supplemented whey emerged as the preferred media, demonstrating the highest EPS production for all strains. This highlights the significant impact that carbohydrates in the culture medium can have on EPS production yield. Among the strictly anaerobic bacteria, the one with the highest EPS production capacity in the MRS-f medium was *B. animalis* Bb12 whereas in the MRS-l medium, it was *B. animalis* BLC-1. The *L. acidophilus* LAC-1 was the

strain that showed the highest value of EPS production in MRS-l and *L. paracasei* LCS-1 produced the highest EPS in Whey-l under aerobic conditions. *L. brevis* LMG 6906 showed the greatest influence in terms of EPS productivity which produced in Whey-l ca. six times more EPS than in the MRS-1 medium in anaerobic conditions as well as *L. paracasei* LCS-1, and *L. kefir* NCFB 2753, displayed nearly three times more EPS production in Whey-l. Our results demonstrated the potential of EPS production for all examined strains. Notably, specific strains exhibited higher production levels under both aerobic and anaerobic conditions, positioning them as promising candidates to function as starter cultures in the production of EPS for novel fermented dairy or non-dairy products. This encourages further investigation through continued characterization and optimization studies for EPS production with these particular strains.

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Data Availability

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

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Conflict of interest

The authors declare no competing financial interest.

References

[1] Angelin J, Kavitha M. Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules*. 2020; 162: 853-865.

[2] Ruas-Madiedo P, de los Reyes-Gavilán CG. Invited review: Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *Journal of Dairy Science*. 2005; 88(3): 843-586.

[3] Gassem M, Schmidt K, Frank J. Exopolysaccharide production from whey lactose by fermentation with Lactobacillus delbrueckii ssp. bulgaricus. *Journal of Food Science*. 1997; 62(1): 171-173.

[4] Prete R, Alam MK, Perpetuini G, Perla C, Pittia P, Corsetti A. Lactic acid bacteria exopolysaccharides producers: A sustainable tool for functional foods. *Foods*. 2021; 10(7): 1653.

[5] Florou-Paneri P, Christaki E, Bonos E. Lactic acid bacteria as source of functional ingredients. In: Kongo JM. (ed.) *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes*. IntechOpen; 2013. p.589-614. Available

from: doi:10.5772/47766.
[6] German B, Schiffrin EJ, Reniero R, Mollet B, Pfeifer A, Neeser J-R. The development of functional foods: Lessons from the gut. *Trends in Biotechnology*. 1999; 17(12): 492-529.
[7] Laiño J, Villena J, Kanmani P, Kitazawa H. Immunoregulatory effects triggered by lactic acid bacteria exopolysaccharides: New insights into molecular interactions with host cells. *Microorganisms*. 2016; 4(3): 27.
[8] Sun N, Liu H, Liu S, Zhang X, Chen P, Li W, et al. Purification, preliminary structure and antitumor activity of exopolysaccharide produced by streptococcus thermophilus CH_9. *Molecules*. 2018; 23(11): 2898. Available from: doi:10.3390/molecules23112898.
[9] Korcz E, Kerényi Z, Varga L. Dietary fibers, prebiotics, and exopolysaccharides produced by lactic acid bacteria: potential health benefits with special regard to cholesterol-lowering effects. *Food & Function*. 2018; 9(6): 3057-3068.
[10] Xiu L, Zhang H, Hu Z, Liang Y, Guo S, Yang M, et al. Immunostimulatory activity of exopolysaccharides from probiotic Lactobacillus casei WXD030 strain as a novel adjuvant in vitro and in vivo. *Food and Agricultural Immunology*. 2018; 29(1): 1086-1105.
[11] Silva LA, Lopes Neto JHP, Cardarelli HR. Exopolysaccharides produced by Lactobacillus plantarum: technological properties, biological activity, and potential application in the food industry. *Annals of Microbiology*. 2019; 69(4): 321-328.
[12] Guerin M, Robert-Da Silva C, Garcia C, Remize F. Lactic acid bacterial production of exopolysaccharides from fruit and vegetables and associated benefits. *Fermentation-Basel*. 2020; 6(4): 115. Available from: doi:10.3390/fermentation6040115.
[13] Looijesteijn PJ, Boels IC, Kleerebezem M, Hugenholtz J. Regulation of exopolysaccharide production by lactococcus lactis subsp. cremoris by the Sugar Source. *Applied and Environmental Microbiology.* 1999; 65(11): 5003-5008. Available from: doi:10.1128/aem.65.11.5003-5008.1999.
[14] van Geel-Schutten G, Flesch F, Ten Brink B, Smith M, Dijkhuizen L. Screening and characterization of Lactobacillus strains producing large amounts of exopolysaccharides. *Applied Microbiology and Biotechnology*. 1998; 50(6): 697-703.
[15] Salazar N, Prieto A, Leal JA, Mayo B, Bada-Gancedo JC, de los Reyes-Gavilán CG, et al. Production of exopolysaccharides by Lactobacillus and Bifidobacterium strains of human origin, and metabolic activity of the producing bacteria in milk. *Journal of Dairy Science*. 2009; 92(9): 4158-4168.
[16] Amiri S, Mokarram RR, Khiabani MS, Bari MR, Khaledabad MA. Exopolysaccharides production by Lactobacillus acidophilus LA5 and Bifidobacterium animalis subsp. lactis BB12: Optimization of fermentation variables and characterization of structure and bioactivities. *International Journal of Biological Macromolecules*. 2019; 123: 752-765.
[17] Zhou K, Zeng Y, Yang M, Chen S, He L, Ao X, et al. Production, purification and structural study of an exopolysaccharide from Lactobacillus plantarum BC-25. *Carbohydrate Polymers*. 2016; 144: 205-214.
[18] Gamar L, Blondeau K, Simonet JM. Physiological approach to extracellular polysaccharide production by Lactobacillus rhamnosus strain C83. *Journal of Applied Microbiology*. 1997; 83(3): 281-287.
[19] Briczinski EP, Roberts R. Production of an exopolysaccharide-containing whey protein concentrate by fermentation of whey. *Journal of Dairy Science*. 2003; 85: 3189-3197.
[20] Vaishnav A, Upadhyay K, Tipre D, Dave S. Characterization of potent exopolysaccharide producing bacteria isolated from fruit pulp and potato peels and enhancement in their exopolysaccharide production potential. *Journal of Microbiology, Biotechnology and Food Sciences*. 2021; 2021: 874-877.
[21] Vaishnav A, Upadhay K, Tipre D, Dave S. Utilization of mixed fruit waste for exopolysaccharide production by Bacillus species SRA4: medium formulation and its optimization. *3 Biotech*. 2020; 10(12): 550.
[22] Smitinont T, Tansakul C, Tanasupawat S, Keeratipibul S, Navarini L, Bosco M, et al. Exopolysaccharide-producing lactic acid bacteria strains from traditional Thai fermented foods: Isolation, identification and exopolysaccharide characterization. *International Journal of Food Microbiology*. 1999; 51(2-3): 105-111. Available from: doi:10.1016/S0168-1605(99)00094-X.
[23] Sanni AI, Onilude AA, Ogunbanwo ST, Fadahunsi IF, Afolabi RO. Production of exopolysaccharides by lactic acid bacteria isolated from traditional fermented foods in Nigeria. *European Food Research and Technology*. 2002;

214(5): 405-407.
[24] Hoover DG. Bifidobacterium. In: Batt CA, Tortorello ML. (eds.) *Encyclopedia of Food Microbiology* (2nd ed.). Oxford: Academic Press; 2014. p.216-222.
[25] Ozogul F, Yazgan H, Ozogul Y. Lactic acid bacteria: Lactobacillus acidophilus. In: McSweeney PLH, McNamara JP. (eds.) *Encyclopedia of Dairy Sciences* (3rd ed.). Oxford: Academic Press; 2022. p.187-197.
[26] Gomes AMP, Malcata FX. Bifidobacterium spp. and Lactobacillus acidophilus: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science & Technology*. 1999; 10(4): 139-157.
[27] Pintado M, Pintado A, Malcata F. Production of polysaccharide by Rahnella aquatilis with whey feedstock. *Journal of Food Science*. 1999; 64(2): 348-352.
[28] Imbert M, Blondeau R. On the iron requirement of lactobacilli grown in chemically defined medium. *Current Microbiology*. 1998; 37: 64-66.
[29] Dierksen KP, Sandine WE, Trempy JE. Expression of ropy and mucoid phenotypes in Lactococcus lactis. *Journal of Dairy Science.* 1997; 80(8): 1528-1536. Available from: doi:10.3168/jds.S0022-0302(97)76082-X.
[30] Vescovo M, Scolari GL, Bottazzi V. Plasmid-encoded ropiness production lactobacillus casei SSP.casei. *Biotechnol Lett*. 1989; 11(10): 709-712.
[31] Welman AD, Maddox IS, Archer RH. Screening and selection of exopolysaccharide-producing strains of Lactobacillus delbrueckii subsp. bulgaricus. *Journal of Applied Microbiology*. 2003; 95(6): 1200-1206. Available from: doi:10.1046/j.1365-2672.2003.02122.x.
[32] Breakwell DP, Moyes RB, Reynolds J. Differential staining of bacteria: Capsule stain. *Current Protocols in Microbiology*. 2009; 15(1): A.3I.1-A.3I.4. Available from: doi:10.1002/9780471729259.mca03is15.
[33] Fusconi R, Godinho M. Screening for exopolysaccharide-producing bacteria from sub-tropical polluted groundwater. *Brazilian Journal of Biology*. 2002; 62(2): 363-369.
[34] Ruas-Madiedo P. Detection, isolation, and purification of bifidobacterial exopolysaccharides. *Methods in Molecular Biology*. 2021; 2278: 101-115. Available from: doi:10.1007/978-1-0716-1274-3_9.
[35] Emnace IC, Dizon E. Selection of lactic acid bacteria for exopolysaccharide production. *Annals of Tropical Research*. 2018; 40(2): 61-68. Available from: doi:10.32945/atr4025.2018.
[36] Abdellah M, Hakem A, Yabrir B, Saad B, Abdelmalek B. Screening for exopolysaccharide-producing strains of thermophilic lactic acid bacteria isolated from Algerian raw camel milk. *African Journal of Microbiology Research*. 2014; 8: 2208-2214.
[37] Welman A, Maddox I, Archer R. Screening and selection of exopolysaccharide-producing strains of Lactobacillus delbrueckii subsp. bulgaricus. *Journal of Applied Microbiology*. 2003; 95(6): 1200-1206.
[38] Mozzi F, Torino MI, de Valdez GF. Identification of exopolysaccharide-producing lactic acid bacteria. In: Spencer JFT, de Ragout Spencer AL. (eds.) *Food Microbiology Protocols*. Totowa, NJ: Humana Press; 2001. p.183-190.
[39] Wen Z, Zhang J. *Bacterial Capsules: Molecular Medical Microbiology*. London: Academic Press; 2015.
[40] Yamanaka T, Yamane K, Furukawa T, Matsumoto-Mashimo C, Sugimori C, Nambu T, et al. Comparison of the virulence of exopolysaccharide-producing Prevotella intermedia to exopolysaccharide non-producing periodontopathic organisms. *BMC Infectious Diseases*. 2011; 11(1): 228.
[41] Xu R, Ma S, Wang Y, Liu L, Li P. Screening, identification and statistic optimization of a novel exopolysaccharide producing Lactobacillus paracasei HCT. *African Journal of Microbiology Research*. 2010; 4(9): 783-795.
[42] Abdellah M, Ahcene H, Benalia Y, Saad B, Abdelmalek B. Evaluation of biofilm formation by exopolysaccharide-producer strains of thermophilic lactic acid bacteria isolated from Algerian camel milk. *Emirates Journal of Food and Agriculture*. 2015; 27(6): 513-521.
[43] Abdalrahim S, Zohri A-N, Khider M, El-Dean A, Abulreesh H, Ahmad I, et al. Phenotypic and genotypic characterization of exopolysaccharide producing bacteria isolated from fermented fruits, vegetables and dairy products. *Journal of Pure and Applied Microbiology*. 2019; 13: 1349-1362.
[44] Oleksy M, Klewicka E. Capsular polysaccharides of lactobacillus spp.: Theoretical and practical aspects of simple visualization methods. *Probiotics Antimicrob Proteins*. 2017; 9(4): 425-434.
[45] Oleksy-Sobczak M, Klewicka E. Optimization of media composition to maximize the yield of exopolysaccharides production by lactobacillus rhamnosus strains. *Probiotics Antimicrob Proteins*. 2020; 12(2): 774-783.

[46] Malaka R, Maruddin F, Dwyana Z, Vargas MV. Assessment of exopolysaccharide production by Lactobacillus delbrueckii subsp. bulgaricus ropy strain in different substrate media. *Food Science & Nutrition*. 2020; 8(3): 1657-1664.
[47] Fraunhofer ME, Jakob F, Vogel RF. Influence of different sugars and Initial pH on β-Glucan formation by lactobacillus brevis TMW 1.2112. *Current Microbiology*. 2018; 75(7): 794-802.
[48] Bengoa AA, Llamas MG, Iraporda C, Dueñas MT, Abraham AG, Garrote GL. Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains. *Food Microbiology*. 2018; 69: 212-218.
[49] Zamfir M, Stefan IR, Grosu-Tudor SS. Influence of growth medium composition on the bacteriocin activity of some lactic acid bacteria. *Romanian Biotechnological Letters*. 2016; 21(6): 12126-12135.
[50] Alhudhud M, Humphreys P, Laws A. Development of a growth medium suitable for exopolysaccharide production and structural characterization by Bifidobacterium animalis ssp lactis AD011. *Journal of Microbiological Methods*. 2014; 100: 93-98.
[51] Adebayo-Tayo B, Onilude A. Comparative influence of medium composition on biomass growth, lactic acid and Exopolysaccharides Production by some Strains of Lactic Acid Bacteria. *Internet Journal of Microbiology*. 2009; 7(2): 1-8.
[52] Kimmel SA, Roberts RF. Development of a growth medium suitable for exopolysaccharide production by Lactobacillus delbrueckii ssp. bulgaricus RR. *International Journal of Food Microbiology*. 1998; 40(1-2): 87-92.
[53] Sánchez J-I, Martínez B, Guillén R, Jiménez-Díaz R, Rodríguez A. Culture conditions determine the balance between two different exopolysaccharides produced by Lactobacillus pentosus LPS26. *Applied and Environmental Microbiology*. 2006; 72(12): 7495-7502.
[54] Audy J, Labrie S, Roy D, LaPointe G. Sugar source modulates exopolysaccharide biosynthesis in Bifidobacterium longum subsp. longum CRC 002. *Microbiology*. 2010; 156(3): 653-664.
[55] Zhang Y, Li S, Zhang C, Luo Y, Zhang H, Yang Z. Growth and exopolysaccharide production by Lactobacillus fermentum F6 in skim milk. *African Journal of Biotechnology*. 2011; 10(11): 2080-2091.
[56] Zajšek K, Goršek A, Kolar M. Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains. *Food Chemistry*. 2013; 139(1): 970-977.
[57] Rabha B, Ladero V, Alvarez M, María F, Bensoltane A. Effect of fermentation conditions (culture media and incubation temperature) on exopolysaccharide production by Streptococcus thermophilus BN12011. *International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE)*. 2011; 24: 433-437. Available from: http://hdl.handle.net/10261/50278.
[58] Andaloussi SA, Talbaoui H, Marczak R, Bonaly R. Isolation and characterization of exocellular polysaccharides produced by Bifidobacterium longum. *Applied Microbiology and Biotechnology*. 1995; 43(6): 995-1000.
[59] Prasanna PHP, Grandison AS, Charalampopoulos D. Effect of dairy-based protein sources and temperature on growth, acidification and exopolysaccharide production of Bifidobacterium strains in skim milk. *Food Research International*. 2012; 47(1): 6-12.
[60] Deepak V, Pandian SRK, Sivasubramaniam SD, Nellaiah H, Sundar K. Optimization of anticancer exopolysaccharide production from probiotic Lactobacillus acidophilus by response surface methodology. *Preparative Biochemistry and Biotechnology*. 2016; 46(3): 288-297.
[61] Zhang Y, Dai X, Jin H, Man C, Jiang Y. The effect of optimized carbon source on the synthesis and composition of exopolysaccharides produced by Lactobacillus paracasei. *Journal of Dairy Science*. 2021; 104(4): 4023-4032.
[62] Dupont I, Roy D, Lapointe G. Comparison of exopolysaccharide production by strains of Lactobacillus rhamnosus and Lactobacillus paracasei grown in chemically defined medium and milk. *Journal of Industrial Microbiology and Biotechnology*. 2000; 24(4): 251-255.
[63] Wang Y, Ahmed Z, Feng W, Li C, Song S. Physicochemical properties of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir. *International Journal of Biological Macromolecules*. 2008; 43(3): 283-288.
[64] Ermiş E, Poyraz E, Dertli E, Yılmaz MT. Optimization of exopolysaccharide production of Lactobacillus brevis E25 using RSM and characterization. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi*. 2020; 24(1): 151-160.
[65] Flatt JH, Hardin RS, Gonzalez JM, Dogger DE, Lightfoot EN, Cameron DC. An anionic galactomannan

polysaccharide gum from a newly-isolated lactose-utilizing bacterium. I. Strain description and gum characterization. *Biotechnology Progress*. 1992; 8(4): 327-334.

[66] Flatt JH, Cooper TA, Gonzalez JM, Dogger DE, Lightfoot EN, Cameron DC. An anionic galactomannan polysaccharide gum from a newly-isolated lactose-utilizing bacterium. II. Fermentation kinetics and lactose transport. *Biotechnology Progress*. 1992; 8(4): 335-339.

[67] Matsuyama H, Sasaki R, Kawasaki K, Yumoto I. Production of a novel exopolysaccharide by Rahnella aquatilis. *Journal of Bioscience and Bioengineering*. 1999; 87(2): 180-183.

[68] Leroy F, De Vuyst L. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health. *Journal of Dairy Science*. 2016; 99(4): 3229-3238.

[69] Zheng J, Wittouck S, Salvetti E, Franz CM, Harris H, Mattarelli P, et al. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *International Journal Ofsystematic And Evolutionary Microbiology*. 2020; 70(4): 2782-2858. Available from: doi:10.1099/ijsem.0.004107.

[70] De Man J, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli. *Journal of applied Bacteriology*. 1960; 23(1): 130-135.

[71] Pintado ME, Pintado AIE, Malcata FX. Production of polysaccharide by rahnella aquatilis with whey feedstock. *Journal of Food Science*. 1999; 64(2): 348-352.

[72] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method determination of sugars and related substances. *Analytical Chemistry*. 1956; 28(3): 350-356.