

# Sustainable Food Technology

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Md. S. Tahmid, Md. Abunaser, A. K. M. Masum, M. A. Islam and Md. Harun-ur-Rashid, *Sustainable Food Technol.*, 2025, DOI: 10.1039/D5FB00943J.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

### Sustainability Spotlight Statement

This work promotes sustainable food technology through the use of lactic acid bacteria (LAB) to produce bioactive exopolysaccharides (EPS) and conjugated linoleic acid (CLA) via natural fermentation. These microbial-derived compounds offer eco-friendly alternatives to synthetic additives, enhancing food functionality, safety, and sustainability.



1 **Exopolysaccharides and conjugated linoleic acid production by selected lactic acid**  
2 **bacteria: physicochemical attributes, antimicrobial activity, and techno-functional**  
3 **properties of exopolysaccharides**

4 **Md. Sadman Tahmid, Md. Abunaser, A. K. M. Masum, Mohammad Ashiqul Islam, and**  
5 **Md. Harun-ur-Rashid\***

6 Department of Dairy Science, Bangladesh Agricultural University, Mymensingh- 2202,  
7 Bangladesh

8 \*Corresponding Author.

9 *E-mail address:* harunds@bau.edu.bd (MH. Rashid)

10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31



## ABSTRACT

View Article Online  
DOI: 10.1039/D5FB00943J

32  
33 Lactic acid bacteria (LAB) exopolysaccharides (EPS) are high-value biopolymers that have  
34 predominant applications in the food sector due to their thickening, gelling, stabilizing,  
35 texturizing, emulsifying, and flocculating properties, while conjugated linoleic acid (CLA) has  
36 potential health benefits for humans. This study aims to screen and isolate bacterial strains  
37 based on their EPS and CLA production, as well as to characterize the carbohydrate, protein  
38 content, water and oil-holding capacity, emulsification, antimicrobial, and hydroxyl radical  
39 scavenging activity of EPS. In this study, 50 out of 300 bacterial strains stocked in the  
40 laboratory were screened by agar media, and 18 strains were selected based on their EPS  
41 production. Afterward, 10 highest EPS producing bacterial strains (*Lactobacillus acidophilus*,  
42 *Enterococcus faecium*, *Lactiplantibacillus plantarum*, *Streptococcus thermophilus*, *Lactococcus*  
43 *lactis*, *Lacticaseibacillus casei*, *Lactobacillus delbruki* spp. *bulgaricus*, *Pediococcus*  
44 *pentosaceus*, *Lactobacillus paraplantarum*, and *Lactobacillus delbruki* spp. *lactis*) were  
45 evaluated for their CLA production, followed by EPS techno-functional properties. The EPS  
46 production varied significantly, from 39.02 mg/L in *Lactococcus raffinolactis* B425 to 2210.18  
47 mg/L in *Lactobacillus acidophilus* LDMB01. Considering CLA production, *Lactobacillus del.*  
48 spp. *bulgaricus* M240 exhibited the highest yield (30.60 µg/mL). In terms of techno-functional  
49 properties, *Enterococcus faecium* D325 showed the highest carbohydrate content (749.89  
50 µg/mL), while *Lactobacillus del.* spp. *bulgaricus* M240 exhibited the highest protein content  
51 (194.87 µg/mL). *Lacticaseibacillus casei* LDMB03 demonstrated the highest water-holding  
52 (204.28%) and oil-holding (309.59%) capacity. The 10% EPS exhibited the highest  
53 antimicrobial activity against the tested pathogenic bacteria, even at relatively low  
54 concentrations. *Pediococcus pentosaceus* B225 showed the strongest emulsification index,  
55 whereas the highest hydroxyl radical scavenging activity was recorded in *Lactococcus lactis*  
56 LDMB10 (94.40%). These findings highlight the diverse functional properties of LAB-derived  
57 EPS, underscoring their potential in developing functional foods, pharmaceuticals, and  
58 cosmetic products.

59

60 **Key words:** Exopolysaccharides, conjugated linoleic acid, Emulsification activity, Water  
61 holding capacity, Antimicrobial activity, Hydroxyl radical scavenging activity.

62

63

64



## 65 1. Introduction

66 Lactic acid bacteria (LAB) are Generally Recognized as Safe (GRAS), facultatively anaerobic  
67 or microaerophilic, non-motile, gram-positive, catalase-negative, and non-spore-forming  
68 bacterial strains those generate organic acids, bacteriocins, and exopolysaccharides in their  
69 metabolic activities.<sup>1</sup> LAB metabolic activities vary significantly across different genera and  
70 even strain to strain within the same species due to their genetic makeups and enzyme  
71 repertoires.<sup>2</sup> LABs are capable of producing microbial exopolysaccharides (EPS), which are  
72 non-toxic antioxidants, and high molecular weight biodegradable natural carbohydrate  
73 polymers. In addition to bacteria, yeasts, fungi, algae, and both eukaryotic and prokaryotic  
74 organisms with diverse biological activities have the potential to secrete EPS.<sup>3-5</sup> On the basis  
75 of EPS monosaccharide compositions, it is classified into homopolysaccharides and  
76 heteropolysaccharides.<sup>6</sup> According to Dueholm *et al.*,<sup>7</sup> the EPS is mostly produced  
77 intracellularly, and different pathways are involved in its secretion. It is synthesized by any of  
78 the four biosynthesis pathways -1. the ATP-binding cassette (ABC) transporter-dependent, 2.  
79 the Wzx/Wzy-dependent, 3. the extracellular, and 4. the synthase-dependent.<sup>8-10</sup> The  
80 biosynthetic pathway employed for the EPS production in LAB significantly influences EPS's  
81 functional and structural properties. The structural modifications (chemical and biomolecular  
82 modifications) of EPS can improve the yield of EPS and also enhance their desired  
83 physicochemical and biological activities.<sup>6,10,11</sup>

84 The molecules of *Leuconostoc mesenteroides*, *Limosilactobacillus fermentum*,  
85 *Limosilactobacillus reuteri*, *Limosilactobacillus reuteri*, and *Streptococcus salivarius*  
86 followed the extracellular biosynthesis pathways and produced alternan, dextran, reuteran, and  
87 mutan types of EPS but their properties are significantly different. In a similar way, the  
88 properties of the commercial EPS, including *Xanthomonas campestris*, *Lactobacillus del.*  
89 subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* were distinct,  
90 though they use Wzx/Wzy-dependent pathway. Stingle *et al.*,<sup>12</sup> mentioned that the ST11 and  
91 YS4 strain of *Str. thermophilus* as non-ropy, while *Str. thermophilus* Sfi6 strain is a ropy one.  
92 Even differences exist between *Str. thermophilus* Sfi6 and *Str. thermophilus* Sfi39 and the  
93 variation in their gene level has been evident from Stingle *et al.*<sup>12</sup> and Germond *et al.*<sup>13</sup>. In  
94 addition to the bacterial strain, exopolysaccharides production, structural characterization,  
95 biosynthesis, biological properties, physicochemical properties, and effective applications  
96 depend on the composition of the fermentation medium, including carbon, nitrogen, salt, and  
97 the condition of the culture, such as pH, time, and temperature.<sup>14,15</sup> The *Lactobacillus*



98 *helveticus* MB2-1 strain in their optimum growing condition, produced 340 mg/L of EPS when  
99 an optimal temperature of 37°C was used.<sup>16</sup> In contrast, under similar conditions (25% (w/w)  
100 dipotassium hydrogen phosphate, 30% (w/w) ethanol, 2% (w/w) 1-butyl-3-methylimidazolium  
101 octyl sulfate, and a pH of 10, the EPS yields of *Lactobacillus acidophilus* and  
102 *Limosilactobacillus reuteri* were found to be 63.30 µg/mL and 146.48 µg/mL, respectively.<sup>17</sup>

103 The industrial production of EPS is costly, with 40% of expenses for microbial growth  
104 substrates.<sup>18</sup> The extraction of EPS from LAB with distinctive bioactivities is becoming  
105 progressively attractive, especially for industrial applications. They are increasingly used in  
106 agriculture, medicine, cosmetics, and food due to their beneficial properties, such as stability,  
107 solubility, viscosity, elasticity, water-holding capacity, oil-holding capacity, emulsification  
108 ability, biocompatibility, gelation, and thickening agent.<sup>15,19</sup> During fermentation, the produced  
109 EPS enhances texture and flavor and provides health benefits.<sup>14</sup> *Streptococcus thermophilus*  
110 EPS is valuable in dairy, improving texture stability, handling tolerance, and meltability while  
111 reducing chewiness and hardness in low-fat Mozzarella cheese.<sup>20</sup> EPS-producing probiotics  
112 significantly enhance food safety, quality, shelf life, and functionality. Because EPS enhance  
113 the rheological properties and water-holding capacity of food matrices, improve emulsion and  
114 foam stability, facilitate the formation of protective biofilms that suppress spoilage and  
115 pathogenic microorganisms, and function as prebiotic and immunomodulatory compounds that  
116 confer additional health benefits.<sup>19,21</sup> LAB EPS regulate gut health, influencing microbiome  
117 composition and short-chain fatty acid production, which impacts intestinal function and  
118 energy metabolism.<sup>22</sup> They also offer biological effects, including anti-cholesterol,  
119 antibacterial, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antiviral, antioxidant,  
120 hypoglycemic, immunomodulatory, and removing *Helicobacter pylori* activities.<sup>23–25</sup>  
121 *Lactobacillus plantarum* KX041 EPS favors the molecular mechanisms against inflammation  
122 and obesity<sup>26</sup> and *Lactiplantibacillus plantarum* LCC-605 increased the stability of the  
123 fermented yoghurt, and antioxidant and cholesterol-reducing abilities.<sup>27</sup>

124 Like EPS, conjugated linoleic acid (CLA) received significant attention as a health-  
125 promoting compound that prevents atherosclerosis, regulates gut microbiota and body  
126 immunity, reduces fat or lipid deposition, promotes bone formation, and exerts anti-  
127 cardiovascular, antioxidant, anti-obesity, anti-diabetic, and anti-carcinogenic effects.<sup>28–31</sup>  
128 According to Wu *et al.*,<sup>32</sup> CLA can enhance insulin signalling, fatty acid oxidation, and glucose  
129 transport in adipose tissue or the liver. Moreover, the EPS-producing LAB have the ability to  
130 convert free linoleic acid (LA) to conjugated linoleic acid.<sup>33,34</sup> Among the LAB, it has been



131 found that many food-grade LAB strains, including *Lactobacillus lactis*, *Lactobacillus casei*,  
132 *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* can produce CLA in yogurt,  
133 cheese, and milk products.<sup>34</sup>

134 Given the variability among the LAB strains and their diverse metabolic end products, it  
135 was hypothesized that the LAB selected for this study would exhibit different EPS production  
136 potentials with distinct physicochemical and techno-functional properties of the produced EPS.  
137 Similarly, they were also expected to differ in their CLA production capabilities, herein, which  
138 was considered as an additional health and wellbeing promoting features of the EPS producing  
139 LAB. Therefore, the aim of the study was to screen the EPS-producing LAB strains from the  
140 selected LAB lot including the evaluation of the produced EPS and an assessment of the CLA  
141 production potentials of the selected EPS-LAB. The set objectives were the quantification of  
142 the EPS and CLA produced by screened LAB strains, followed by monitoring, recording, and  
143 assessing the physicochemical and techno-functional properties of the produced EPS *viz.* total  
144 carbohydrate and protein content, hydroxyl radical scavenging activity, antimicrobial activity,  
145 emulsification index, oil holding capacity, water holding capacity, and solidification ability of  
146 EPS. It is anticipated that the outcome of this study will provide valuable insights into the  
147 techno-functional properties of EPS produced by bacterial strains and their potential  
148 applications in the food industry, especially with regard to product development and well-being  
149 of consumers.

View Article Online  
DOI: 10.1039/D3FB00943J



## 150 2. Materials and methods

### 151 2.1. Selection of bacteria and processing

152 The bacterial strains were obtained from the Laboratory of Dairy Microbiology and  
153 Biotechnology (LDMB), which comprised 266 freeze-dried lactic acid bacteria (LAB) that  
154 were isolated from traditionally fermented *Dahi* in Bangladesh<sup>35,36</sup> and 34 LAB strains from  
155 isolated from raw goat milk and eventually characterized.<sup>37</sup> From 300 LAB strains, 50 LAB  
156 strains were chosen. Stock cultures were made in MRS broth (de Man, Ragosa and Sharpe;  
157 Himeida, India) followed by subculturing and eventually stored at -20°C for further use.

### 158 2.2. Screening of EPS-producing strains

159 The ability of the isolates to produce EPS was assessed by using the string test, Aniline Blue  
160 (AB) agar, and Congo Red (CR) agar.<sup>38</sup> For the string test, the strains were cultured on Luria  
161 Bertani (LB) agar (Himedia, India) plates supplemented with 5% (w/v) sucrose at 37°C for 48  
162 h. Colony was lifted using an inoculation loop, and those forming a  $\geq 5$  mm string were selected.  
163 In the AB agar assay, nutrient agar (Himedia, India) was supplemented with 5% (w/v) sucrose  
164 and 0.005% (w/v) aniline blue (SRL, India). EPS-producing isolates showed slightly blue-  
165 colored colonies on the AB agar plate. Brain-heart Infusion agar (Himedia, India) was  
166 supplemented with 5% (w/v) sucrose and added with 0.08% (w/v) Congo red (Himedia, India)  
167 to make the CR agar. Light to dark black colonies indicate EPS production potential strains.  
168 Both the AB and CR plates were incubated at 37°C for  $\approx 48$  h. The strain that passed at least  
169 any one of the aforesaid assays (in total 18 strains passed) was considered for subsequent steps.

### 170 2.3. Production, extraction, and quantification of EPS

171 The screened EPS-producing isolates were incubated for 72 h at 37°C in MRS broth  
172 supplemented with 5% (w/v) sucrose on a constant orbital shaker (SCI FINETECH, Korea) at  
173 120 rpm. During this period, optical density (OD)<sub>600</sub> was measured at every 6 h using an i9  
174 Hanon UV/VIS double beam spectrophotometer at 600 nm.<sup>38,39</sup> After incubation, samples (50  
175 mL) were heated in a water bath (Daihan Scientific Bath, Korea) at  $\approx 100^\circ\text{C}$  for 50 min followed  
176 by cooling to 25°C. It was then centrifuged at 5500 $\times$ g (Neya 16R, Remi, India) for 20 min at  
177 4°C and supernatants were collected. Ice-cold ethanol was added to the supernatants (2:1) and  
178 was incubated overnight at -20°C. Then, crude EPS precipitation was collected after  
179 centrifuging the supernatant-ethanol mix at 5500 $\times$ g for 20 min at 4 °C. Eventually, it was dried  
180 overnight at 40°C (BOD incubators, Mrclab, Holon, Israel). Dry EPS weight and yield were



181 recorded and calculated, respectively. Crude EPS was purified by redissolving in distilled  
182 water, dialyzing with changing water, and lyophilized.

## 183 2.4. Functional properties of EPS

184 **2.4.1. Total carbohydrate and protein content of EPS.** Ten predominant strains were  
185 selected based on higher EPS production. The phenol-sulfuric acid method was used to  
186 evaluate the total carbohydrate content in the ten crude EPS samples.<sup>40</sup> In brief, 1% crude EPS  
187 solution was made in 0.5 mL of distilled water. Phenol (Merck, Germany) solution (6% w/v;  
188 0.5 mL) and 2.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> were added to the EPS solution. This mixture was  
189 incubated at room temperature for 20 min. Absorbance were then recorded at 490 nm  
190 (Multiskan SkyHigh Spectrophotometer, Thermo Scientific, USA). Distilled water was the  
191 blank sample, and glucose (Merck, India) was used as the standard (0 – 100 µg/mL; 20 µg/mL  
192 intervals).

193 For the estimation of total protein content, the Bradford micro-assay protocol was used.  
194 The reaction solution was made of 0.5 mL of EPS solution and 0.5 mL of Bradford protein  
195 assay reagent (Bio-Rad Quick Start™, USA). After incubation for 5 min at room temperature,  
196 absorbance was recorded at 595 nm. Distilled water and bovine serum albumin (BSA) were  
197 used as blank and standard (0 – 100 µg/mL; 20 µg/mL intervals), respectively.

198 **2.4.2. Hydroxyl radical scavenging ability of EPS.** The hydroxyl radical scavenging ability  
199 was assessed using salicylic acid methods described by Yang-Chen *et al.*,<sup>39</sup> with a few  
200 modifications. In short, salicylic acid-ethanol solution and FeSO<sub>4</sub> solution (0.4 mL of 9 mmol/L  
201 of each) were mixed separately with 0.4 mL of different concentrations (0.5, 1.0, 1.5, and 2.0  
202 mg/mL) of purified EPS in distilled water. This was followed by thorough mixing and then the  
203 addition of 0.4 mL of 8.8 mmol/L H<sub>2</sub>O<sub>2</sub> in distilled water. Finally, after incubation at 37°C for  
204 20 min, the absorbance was recorded at 510 nm. L-ascorbic acid was used as a control, and  
205 distilled water served as a blank. The hydroxyl radical scavenging activity rate was calculated  
206 using the following formula -

$$207 \quad \text{Scavenging activity (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

208 Where, A1 = absorbance of the EPS sample solution, A0 = absorbance of blank, and A2 =  
209 absorbance of the control (distilled water was used instead of H<sub>2</sub>O<sub>2</sub>).



210 **2.4.3. Antimicrobial activity of EPS.** The crude EPS antimicrobial activity was evaluated  
 211 using the agar well diffusion method, modified from Tarannum *et al.*<sup>38</sup> Six pathogenic bacteria  
 212 *viz. Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 12600, *Bacillus*  
 213 *cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, and  
 214 *Klebsiella pneumoniae* ATCC 8047 were used as indicator microorganisms against the EPS  
 215 produced by the respective organisms. These indicator strains were cultured overnight at 37°C  
 216 in LB broth. Cell density was adjusted to 10<sup>7</sup>-10<sup>8</sup> CFU/mL, and then 100 µL of bacterial  
 217 suspension was evenly spread on LB agar plates. Five millimeter-in diameter wells were made  
 218 in the agar plate. Dry extracted EPS was dissolved in Milli-Q water (0.5 and 1.0 mg/mL),  
 219 filtered through a syringe filter (0.22 µm sterilized Millipore membrane, Sigma-Aldrich), and  
 220 60 µL of the solution was added to each well. The agar plates were kept at 4°C for 1 hour, then  
 221 incubated at 37°C for 48 h. Antimicrobial activity was evaluated by measuring the inhibition  
 222 zone in diameter.

223 **2.4.4. Emulsification index (Ex) of EPS.** The biosurfactant activity of in-situ-produced crude  
 224 EPS was assayed using the emulsification index at 24 h (E<sub>24</sub>), 48 h (E<sub>48</sub>), and 96 h (E<sub>96</sub>),  
 225 following the method described by Ge *et al.*,<sup>16</sup> with few modifications. Briefly, 2 mL of 1%  
 226 (w/v) EPS in distilled water, and an equal volume of different oils (olive oil/soybean  
 227 oil/mustard oil/black cumin oil/almond oil/kerosene) or n-hexane were added and vortexed for  
 228 5 min. The mixture was allowed to stand for 24, 48, and 96 h. The said index was calculated  
 229 using the following formula:

$$230 \text{ Emulsification index (Ex)} = \frac{\text{Height of the emulsion layer formed (cm)}}{\text{Total height of the solution (cm)}} \times 100$$

231 An optical microscope was used to analyze the particle size distribution of the emulsion that  
 232 was formed as previously described by Kowsalya *et al.*<sup>41</sup> Briefly, 60 µL emulsion was placed  
 233 on a cavity slide and left to stand undisturbed for 5 min. The distribution of oil droplet sizes  
 234 was observed under a light microscope at 10× magnification.

235 **2.4.5. Water holding capacity of EPS.** The water-holding capacity of the EPS derived from  
 236 bacterial strains was assessed by the method described by Qamar *et al.*<sup>42</sup> Dried crude EPS  
 237 samples were mixed with double-distilled water (500 mg/mL) and vortexed. Then it was placed  
 238 on an orbital shaking platform at 150 rpm for 30 min at room temperature followed by  
 239 centrifugation for 20 min at 3500 rpm (Rotor A-12-5 Model, Remi NEYA 16R). The



240 supernatant was discarded and then the remnant was weighed. The water holding capacity was  
 241 calculated by using the formula stated below.

$$242 \quad \text{Water holding capacity (\%)} = \frac{\text{Weight of EPS with bound water (g)}}{\text{Initial dry weight of the EPS (g)}} \times 100$$

243 **2.4.6. Oil holding capacity of EPS.** The oil retention ability of ten crude EPS extracts was  
 244 evaluated according to Qamar *et al.*<sup>42</sup> Dried crude EPS was mixed with cottonseed oil  
 245 (500mg/mL) and thoroughly vortexed for 2 min. The mixture was then left at room temperature  
 246 for approximately 35-40 min with mild agitation (on orbital shaker, 120 rpm) after each five  
 247 min. Followed by centrifugation at 3500 rpm for 20 min at 20°C, the supernatants were  
 248 removed and weighed for quantification. The formula used to calculate the oil holding rate was  
 249 -

$$250 \quad \text{Oil holding capacity (\%)} = \frac{\text{oil bound weight (g)}}{\text{initial sample weight (g)}} \times 100$$

## 251 **2.5. Milk coagulation test of EPS strains.**

252 Highest EPS-producing strain *Lb. acidophilus* LDMB01 was introduced into a sterile  
 253 skim milk solution (w/v; 1:10). The inoculum used was 1% (v/v), and different amounts of  
 254 sucrose (0%, 3%, 6%, 9%, or 12% w/v) were added. A static culture was conducted for 24, 36,  
 255 and 48 h at a temperature of 30°C. Subsequently, the morphology of the skimmed milk was  
 256 observed at 12 h intervals starting from 24 h of incubation. A negative control was prepared  
 257 using skim milk containing 0% sugar.<sup>5</sup>

## 258 **2.6. Physiochemical properties of selected EPS-LAB strains**

259 **2.6.1. Carbohydrate fermentation.** The carbohydrate fermentation abilities of these strains  
 260 were performed in MRS broth without glucose as per the protocol of Silva *et al.*<sup>43</sup> Briefly, 14  
 261 sugars *viz.* cellobiose, dextrose, fructose, galactose, glucose, lactose, maltose, raffinose,  
 262 rhamnose, sorbitol, starch, sucrose, trehalose, and xylose were used. Carbohydrate solutions  
 263 (5%, w/v) and bromocresol purple (pH indicator; 0.025 % w/v) were prepared. Both solutions  
 264 were passed separately through a sterilized 0.22 µm Millipore membrane syringe filter. Except  
 265 for glucose, each sugar and broth medium was sterilized at 121°C for 15 min. Afterward, 0.5  
 266 mL of sterile sugar, 0.12 mL pH indicator, and 50 µL of active culture were inoculated into 4.5  
 267 mL broth in a screw-cap tube and eventually fermented at 37°C for 48 h. A positive result was



268 indicated by a color change to yellow or light brown, while non-fermenting tubes remained  
269 unchanged.

270 **2.6.2. Biochemical Tests.** Methyl red test and oxidase test were performed by the tuning Raj  
271 *et al.*<sup>17</sup> method. The methyl red test, a buffer solution was prepared by mixing peptone, dextrose,  
272 and dipotassium phosphate, and the final pH was adjusted to  $6.9 \pm 0.2$ . The pure culture was  
273 inoculated into 5 mL of this solution, followed by the addition of 5–6 drops of methyl red  
274 (MR), incubation at 37°C for 48 h and observed for color change. Red indicated a positive test,  
275 and yellow means a negative one.

276 For the oxidase test, the organisms were grown on a glucose agar plate (1% glucose),  
277 which was incubated at 37°C for 24 h. 1% tetramethyl-p-phenylenediamine dihydrochloride  
278 was dissolved in distilled water. A few drops of solution were placed on filter paper (Whatman  
279 filter paper, No.1), and a fresh colony was transferred onto it using a cotton bud. A color change  
280 within 10–30 seconds indicated a positive result; no change means negative. No color change  
281 indicates a negative test. For the catalase test, a sterile loop transferred a 24-hour culture onto  
282 a microscopic slide, and one drop of 30% H<sub>2</sub>O<sub>2</sub> was added to it. Immediate bubble formation  
283 indicated a positive result, showing oxygen release.<sup>44</sup>

284 For pectin hydrolysis, a medium was prepared by modifying Ortega-Villar *et al.*<sup>45</sup> At  
285 first, 100 mM phosphate buffer (pH 7.0), prepared with 0.4% (w/v) KH<sub>2</sub>PO<sub>4</sub>, 0.6% (w/v)  
286 NaH<sub>2</sub>PO<sub>4</sub>, and Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, which was added with 0.5% pectin, 0.2% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2%  
287 yeast extract, 0.2% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.0001% CaCl<sub>2</sub>, and 0.05% NaCl. Subsequently, followed  
288 by pH adjustment (7.0), inoculation of organisms, and incubation at 37°C for 72 h. The halo  
289 around the colony was interpreted as positive for pectin hydrolysis.

290 **2.6.3. Proteolytic activity.** Proteolytic activity was evaluated on milk agar using the method  
291 described by Rampanti *et al.*<sup>46</sup> In brief, the milk agar was prepared by supplementing Plate  
292 Count Agar with 1% skim milk powder. The agar plates with transferred inoculum were  
293 incubated for 48 h at 37°C, and colonies that formed transparent halo were identified as positive  
294 indicators of proteolytic activity.

295 **2.6.4. Lipolytic activity.** To evaluate lipolytic activity, bacterial isolates were cultured  
296 overnight in MRS broth at 30°C. Afterward, tributyrin agar (Merck) was prepared by adding  
297 10 µL of fresh culture and 10 mL/L of glycerol tributyrate. Subsequently, the agar plates were  
298 incubated at 30°C for 5 days and were examined daily for the presence of halo formation



299 surrounding the colonies. The threshold halo diameter is 2-3 mm to declare the lipolysis  
300 positive.<sup>46</sup>

## 301 2.7. Screening of LAB for conjugated linoleic acid production

302 Bacterial strains having higher EPS-producing capacity were assessed for conjugated linoleic  
303 acid (CLA) production using a spectrophotometric detection method following Barrett *et al.*,<sup>47</sup>  
304 with a few modifications. Briefly, the isolates were activated by successive sub-culturing in  
305 MRS broth and grown at 37°C (BOD incubators, Mrclab, Holon, Israel). Afterward, the strains  
306 were incubated in MRS broth containing free linoleic acid (LA; 0.5 mg/mL; Sigma-Aldrich  
307 L1376-5G) and 2% (w/v) Tween 80 (Merck, Germany). After incubation (at 37°C for 48 h), 1  
308 mL of culture was centrifuged at 20,800×g for 1 min. The pellet was discarded, and the  
309 supernatant was mixed with 2 mL of isopropanol (Merck, Germany) by vortexing and  
310 eventually allowed to stand for 3 min. Then 1.5 mL of hexane (Merck, Germany) was added  
311 and vortexed further, and stood for another 3 minutes. The CLA in the culture supernatant was  
312 assayed by dispensing 230 µL of the fat-soluble hexane layer into a UV-transparent 96-well  
313 plate (Multiskan SkyHigh Spectrophotometer, Thermo Scientific, USA), and absorbance was  
314 recorded at 233 nm. The concentration was quantified using a standard curve of conjugated  
315 linoleic acid (CLA; Sigma-Aldrich, 05507), consisting of a mixture of cis- and trans-isomers  
316 of 9,11- and 10,12-octadecadienoic acids.

## 317 2.8. Statistical analysis

318 All the experimental data were tested in triplicate, and the values were expressed as mean  
319 ± standard deviation. The statistical analysis was performed by using IBM SPSS statistical  
320 software (V27.0). One-way analysis of variance (ANOVA) was performed to test significance,  
321 and Duncan multiple range test (DMRT) was carried out to rank the results while significant  
322 differences were observed. A probability of less than 0.05 was considered statistically  
323 significant.



324

### 3. Results and discussion

325

#### 3.1. EPS Screening and Production

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

The lactic acid bacteria genera, such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella*, and *Enterococcus* can naturally synthesize diverse forms of glycans or polysaccharides. The LAB is accounted to be the largest EPS-producing bacteria.<sup>21</sup> The preliminary screening in this study was based on the EPS production potentials. Despite numerous strains yielding positive results in the string test, the inconsistency of the ropy features rendered the assay only presumptive. Consequently, the CR and AB agar plate procedures were used for confirmation, leading to the selection of 50 strains. The EPS-producing strains exhibited mucoid or ropy colonies on LB agar media that were used for the string test, characteristics express light to dark black colonies on CR agar, and an intense blue color appearance on AB agar (Table 1). The formation of a string longer than 5 mm was regarded as an initial indication of EPS production (Fig. 1). After the preliminary screening phase, EPS underwent the secondary screening phase called quantification. Eighteen strains namely— *Lactobacillus acidophilus* LDMB01, *Enterococcus faecium* D325, *Lactiplantibacillus plantarum* LDMB05, *Streptococcus thermophilus* M540-5, *Lactococcus lactis* LDMB10, *Lacticaseibacillus casei* LDMB03, *Lactobacillus del. spp. bulgaricus* M240-5, *Pediococcus pentosaceus* B225, *Lactobacillus paraplantarum* LDMB11, and *Lactobacillus del. spp. lactis* D340, *Streptococcus bovis* biotype I D125-4, *Leuconostoc mesenteroides subsp. mesenteroides* D625-3, *Lactobacillus agilis* D640-4, *Lactococcus raffinolactis* B425-4, *Lactococcus lactis* spp. *lactis* D425-4, *Streptococcus bovis* biotype II J225-1, *Leuconostoc mesenteroides subsp. dextranicum* M725-1 and *Lactobacillus fermentum* M240-4 – were selected based on higher EPS production.

347

348

349

350

351

352

353

354

355

The EPS production of 18 selected strains varied from 39.02±4.25 mg/L to 2210.18±112.14 mg/L, where LDMB01 strains showed a maximum EPS-production ability (Fig. 2). Liu *et al.*<sup>48</sup> reported that *Lb. acidophilus* produced around 2920 mg EPS/L, whereas in the present study *Lb. acidophilus* LDMB01 generated 2210.18 mg/L. The possible cause of this variation may be attributed to the variation in the bacterial strain and/or carbon sources used (glucose or lactose and sucrose, respectively). Liu *et al.*<sup>48</sup> used *Lb. acidophilus* ATCC mutan, which was obtained from Microbiology Institute of Chinese Academy (Beijing, China) but we used *Lb. acidophilus* LDMB01 isolated from raw goat milk. In another study, Hernández-Rosas *et al.*<sup>49</sup> stated that carbon source is one of the predisposing factors in EPS



356 synthesis by the LAB. This was also evident in the report of Padmanabhan *et al.*<sup>50</sup> who found  
357 that *S. thermophilus* ASCC 1275 produced approximately 430 mg/L of EPS when grown in a  
358 medium supplemented with sucrose, which was higher than the EPS yields with glucose or  
359 lactose. Afreen *et al.*<sup>51</sup> used Plackett–Burman design in their study and identified lactose, yeast  
360 extract, tryptone, and CaCl<sub>2</sub> as factors for higher EPS production. Alongside, D325-2 produced  
361 the second-highest EPS (1626.51 mg/L), followed by LDMB05 (553.80 mg/L), M540-5  
362 (422.34 mg/L), and LDMB10 (329.48 mg/L). The D425 and LDMB03 produced 118.53 mg/L  
363 and 314.13 mg/L of EPS, respectively. These findings also supported by Prete *et al.*,<sup>52</sup> who  
364 reported that *Lc. lactis* spp. and *Lac. casei* demonstrated EPS production levels ranging from  
365 30 to 600 mg/L. Moreover, EPS production of 94.76 mg/L and 56.48 mg/L was observed in  
366 *Lb. fermentum* M240-4 and *S. bovis* biotype II J225-1, respectively.

367 The growth curves of selected EPS strains are presented in Fig. 3. Starting from 18 h  
368 up to 66 h, the growth performance of LDMB05 and M540 were higher compared to the other  
369 strains; however, at > 66 h, LDMB11, LDMB01, and B225 strains exhibited higher growth.  
370 From 60 h, all selected strains had higher growth compared to the control, which can be due to  
371 the carbon source in the growth media and orbital shaking during incubation. According to  
372 Bates *et al.*,<sup>53</sup> orbital shaking exerts its effects on microbial growth through impacts on the  
373 temperature and aeration control, the two major variables in microbial growth kinetics. All  
374 strains were found to show their maximum growth potential at 72 h time period. EPS  
375 production ability depends on various critical elements affecting bacterial growth, such as  
376 relative environment, incubation time, temperature, pH, oxygen rate, optimized culture  
377 conditions, and carbon and nitrogen sources, with the quantity of EPS produced being  
378 predominantly strain-specific.<sup>54</sup> So, orbital shaking may enhance nutrient distribution, create a  
379 homogeneous environment, prevent biofilm formation, and enhance growth rates by promoting  
380 optimal cell proliferation and metabolic activity.

### 381 3.2. Biochemical properties of selective strains

382 It is worth noting that the isolates were selected based on their stock strain identity, observing  
383 the standard biochemical and morphological characteristics and their fermentation with 14  
384 sugars (Table 2). All the strains were gram-positive, non-motile, oxidase, catalase, and pectin  
385 hydrolysis test negative. Proteolytic activity was negative in M540. Similar to our findings,  
386 positive proteolytic activity was reported previously by Dong *et al.*<sup>55</sup> in *Lb. plantarum*, *Lb. del.*  
387 subsp. *bulgaricus*, *Lc. Lactis*, *Lb. casei*, *Lb. acidophilus*, *P. pentosaceus*, and *Lb. rhamnosus*



388 strains. All the screened EPS producing LABs were able to utilize glucose, dextrose and  
389 fructose, and except M240, the same was observed for galactose and maltose. None of them  
390 were able to utilize the sorbitol, rhamnose, and starch, even LDMB01 highest EPS producer.  
391 Afreen *et al.*<sup>51</sup> screened *Lactiplantibacillus paraplantarum* NCCP 962 as high EPS producer  
392 from the *doi* samples and reported their ability to utilize D-galactose, D-lactose, D-sucrose, D-  
393 mannose, D-maltose, D-fructose, and D-arabinose but was not capable to use D-sorbitol, D-  
394 ribose, and D-xylose as a carbon source.

### 395 3.3. Functional properties of EPS

396 **3.3.1. Total carbohydrate and protein content.** Fig. 4 shows the total carbohydrate and  
397 protein content of selected EPS producing LAB strains. There was a significant ( $p < 0.05$ )  
398 variation in the total carbohydrate and protein content among the EPS samples. The  
399 carbohydrate and protein content of EPS varied from  $749.89 \pm 27.25$  to  $102.06 \pm 6.39$   $\mu\text{g/mL}$  and  
400  $194.87 \pm 5.92$  to  $68.03 \pm 4.2$   $\mu\text{g/mL}$ , respectively. The highest carbohydrate content was found in  
401 D325 ( $749.89$   $\mu\text{g/mL}$ ), followed by LDMB10 ( $745.39$   $\mu\text{g/mL}$ ) and D340 ( $586.31$   $\mu\text{g/mL}$ ), and  
402 the lowest was recorded in LDMB11 ( $102.06$   $\mu\text{g/mL}$ ). On the other hand, the highest protein  
403 content was observed in M240 ( $194.87$   $\mu\text{g/mL}$ ), next was M540 ( $183.55$   $\mu\text{g/mL}$ ), and then  
404 LDMB01 ( $179.51$   $\mu\text{g/mL}$ ), and being the lowest, LDMB10 exhibited almost one-third of the  
405 M240. This variation in carbohydrate and protein content is consistent with the findings of  
406 Tarannum *et al.*,<sup>38</sup> reported variable carbohydrate in EPS from different LAB strains isolated  
407 from cow milk. However, compared to them, the carbohydrate and protein content of EPS  
408 seems higher in the present findings. In crude EPS of all bacterial strains in the current study,  
409 total carbohydrate content was found higher than total protein content. This also aligns with  
410 Tarannum *et al.*,<sup>38</sup> reported  $594.91$   $\mu\text{g/mL}$  as the highest carbohydrate content and  
411  $119.28$   $\mu\text{g/mL}$  as the highest protein content in the crude EPS. This is further supported by  
412 Arayes *et al.*,<sup>56</sup> who found higher carbohydrate content in *Alkalibacillus* sp. w3 EPS than the  
413 protein content. The purity of EPS is inversely correlated to the amount of its protein content,  
414 with the low protein content observed in this study indicating high purity.<sup>57</sup>

415 **3.3.2. Hydroxyl radical scavenging ability.** Hydroxyl radicals are the most powerful oxidants  
416 that interact with nearly all biological components, encompassing nucleic acids, lipids,  
417 carbohydrates, and proteins.<sup>58</sup> The EPS antioxidant activity is a well-established feature that  
418 has been recently investigated by scavenging hydroxyl radicals and has a significant role in  
419 protecting against non-communicable diseases such as diabetes, cardiovascular conditions, and



420 gastrointestinal ulcers.<sup>59</sup> EPS contains a sulfate group and uronic acid, which exhibit stronger  
421 hydroxyl radical scavenging activity by chelating metal ions ( $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ ).<sup>60–62</sup> In addition,  
422 according to Li *et al.*<sup>63</sup> and Wei *et al.*<sup>64</sup> being the sulfate-containing polysaccharide, EPS have  
423 antioxidant, antiviral, heavy metal adsorption, and anticoagulant activities. Moreover, the  
424 presence of protein (amino acids) could also affect free radical scavenging and the antioxidant  
425 activity of EPS.<sup>60</sup>

426 The scavenging efficacy of EPS demonstrated concentration-dependent hydroxyl  
427 radicals ranging from 0.5 mg/mL to 2 mg/mL, as shown in Fig. 5. Increasing EPS concentration  
428 from 0.5 to 2.0 mg/mL, increases the hydroxyl radical scavenging ability. This effect is  
429 probably due to the hydroxyl groups in the EPS donating more active hydrogen atoms.<sup>54</sup>  
430 However, from 1.5 to 2.0 mg/mL concentration, no remarkable changes were observed for all  
431 selected strains. For instance, EPS LDMB10 exhibited maximum scavenging activity  
432 ( $90.04 \pm 3.70\%$ ) at 1.5 mg/mL concentration, which was  $91.21 \pm 1.31\%$  at 2.0 mg/mL  
433 concentration. Other studies also confirm the potential antioxidant or scavenging ability of  
434 EPS, similar to the *Lb. rhamnosus*, *Lcb. paracasei* ssp. *paracasei*, *Lb. del.* subsp. *bulgaricus*,  
435 *Lb. helveticus*, *Lpb. plantarum*, *Lb. acidophilus*, and *Xanthomonas campestris*.<sup>54,65</sup> However,  
436 among the tested EPS, B225 had the lowest scavenging activity of 75.61% at 2.0 mg/mL  
437 concentration. Overall, the findings indicate a concentration-dependent increase in hydroxyl  
438 radical scavenging activity across all EPS tested, with LDMB10, M240, and LDMB01 EPS  
439 showing consistently high activity, suggesting that EPS from these strains possess potential  
440 antioxidant properties. However, *Limisolactobacillus fermentum* NCDC400 EPS showed only  
441 63.92% scavenging of hydroxyl groups at 3 mg/mL concentration<sup>66</sup> and *L. plantarum* R301  
442 EPS can remove 96.52% hydroxyl groups at 2 mg/mL concentration<sup>67</sup> whereas, in another  
443 study, Tarannum *et al.*<sup>38</sup> reported that scavenging activities of 90.49% and 88.30% at  
444 concentrations of 0.755 mg/mL and 0.738  $\mu\text{g/mL}$  for *Lactocaseibacillus* sp. ME17 and  
445 *Lactococcus* sp. ME7, respectively. All these findings indicate this efficacy as a multifactorial  
446 phenomenon. In the current study, *Lb. lactis* LDMB10, *Lb. del.* spp. *bulgaricus* M240, *Lb.*  
447 *acidophilus* LDMB01, *Lpb. plantarum* LDMB05, *S. thermophilus* M540 showed the variable  
448 potentials of radical scavenging activity, and revealed their suitability as food additives with  
449 naturally produced antioxidants.

450 **3.3.3. Antimicrobial activity.** As presented in Table 3, the antimicrobial activity of EPS varies  
451 depending on its concentration (0.5 vs 1.0 mg/mL). This study has documented a substantial



452 antibacterial property of various EPS against a broad range of infectious microorganisms, both  
453 Gram-positive and Gram-negative. EPS is capable of exhibiting antagonistic activity as well  
454 as mobilizing the host immune system against bacterial pathogens.<sup>68</sup> Out of all the pathogens  
455 studied, the EPS from M240, and D340 exhibited significantly ( $p < 0.05$ ) best antimicrobial  
456 activity followed by LDMB01, LDMB10. The *L. monocytogenes* ATCC 7644, and *S. aureus*  
457 ATCC 12600, are the Gram-positive bacteria that exhibit the highest susceptibility across all  
458 EPS tested and *B. cereus* ATCC 11778 were also found, except for D325 EPS. An increase in  
459 EPS concentration from 0.5 to 1.0 mg/mL resulted in enhanced antimicrobial activity in  
460 approx. 45% of the samples, while approx. 55% samples remained unaffected. There were  
461 some samples (approx. 10%) that showed highest antimicrobial activity even at the low EPS  
462 concentration. However, even at higher concentration, *E. coli* showed resistance against  
463 LDMB10, LDMB11 and LDMB01, *B. cereus* against D325 and *K. pneumoniae* against D325,  
464 LDMB03 and B225. This finding also noted that the EPS showed significant inhibition of  
465 Gram-positive bacteria *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 12600, and Gram-  
466 negative bacteria *S. typhi* ATCC 14028, even at low concentrations. The inhibitory effect  
467 against these bacteria may be attributed to the strong interactions of EPS functional groups  
468 with bacterial cell surfaces, leading to membrane disruption and inhibition of essential cellular  
469 processes. However, Rahnama *et al.*<sup>69</sup> reported *L. monocytogenes* as the most resistant  
470 organism against the EPS, and *S. aureus* and *Enterococcus faecalis* as the most susceptible  
471 one. Nehal *et al.*<sup>70</sup> reported that EPS showed a robust repressive action against *S. aureus*,  
472 *Pseudomonas aeruginosa*, *E. coli*, *L. monocytogenes*, *B. cereus*, *Proteus mirabilis*,  
473 *Acinetobacter baumannii*, *Enterobacter cloacae*, and *Candida albicans*, which is in line with  
474 the present study.

475 In addition, the present findings also indicate that the antimicrobial activity of LAB  
476 EPS is mostly strain-specific. For instance, in Fig. 6, EPS D340 showed the highest inhibitory  
477 effect against *L. monocytogenes* ATCC 7644, and LDMB01 exhibited broad-spectrum activity,  
478 showing notable inhibition zones against both *S. aureus* ATCC 12600 and *K. pneumoniae*  
479 ATCC 8047. Similarly, EPS M240 displayed superior efficacy against *B. cereus* ATCC 11778,  
480 while EPS B225 was found to be most effective against *E. coli* ATCC 25922. In addition, EPS  
481 LDMB03 showed the strongest antimicrobial activity against *S. typhi* ATCC 14028. EPS  
482 chelate metals, form a barrier, and restrict essential nutrients, disrupt cell walls and cytoplasmic  
483 membrane, inhibit mRNA and protein synthesis, disrupt cell proliferation, inactivate enzymes,  
484 generate H<sub>2</sub>O<sub>2</sub>, and accelerate DNA destruction.<sup>57,69,71,72</sup> Evidence on the structural



485 conformation and/or presence and arrangements of different functional groups in EPS from the  
486 present study could have been advantageous to explain the mechanism of their antibacterial  
487 activity, which was not covered. The use of antimicrobial compounds added directly to the  
488 food or in its packaging is one practical approach, indicating the industrial significance of EPS.

489 **3.3.4. Emulsification index (Ex).** EPS produces an in situ mechanism for potential emulsion  
490 formation, exhibiting a soluble natural polymer in enhancing food texture and developing food  
491 products.<sup>73</sup> Recently, novel applications for bacterial polysaccharides have arisen, particularly  
492 in production and emulsion stabilization. The coexistence of hydrophobic and hydrophilic  
493 properties of EPS makes it an effective emulsifying agent. The presence of OH groups  
494 contributes to the hydrophilic characteristics of the EPS, whereas its hydrophobicity is  
495 attributed to the presence of aliphatic -CH<sub>2</sub> groups.<sup>74</sup> The EPS emulsification index was  
496 assessed against various hydrophobic substances, including hydrocarbons and oils. Most of the  
497 EPS demonstrated good emulsifying properties with E<sub>24</sub> values exceeding 50% with olive oil,  
498 black cumin oil, almond oil, soybean oil, mustard oil, kerosene oil, and n-hexane. As shown in  
499 Tables 4a-g, 5, and 6, the emulsification activity of EPS was evaluated at 1% concentration  
500 with different hydrocarbons. The maximum emulsion stability ( $p < 0.05$ ) was given by B225,  
501 LDMB11, LDMB05, and LDMB01 84% and 71%, 70% and 66%, 80% and 64%, and 60% in  
502 olive oil and soybean oil, mustard oil and almond oil, black cumin oil and n-hexane, and  
503 kerosene, respectively at 24 h of storage. De *et al.*<sup>75</sup> reported 63.9% E<sub>24</sub> by *Klebsiella oxytoca*  
504 EPS in soybean oil. In another study, the EPS isolated from fermented milk and functional  
505 value-added probiotic yogurt *Lactobacillus rhamnosus* KF5 emulsified 43%, and *Lb.*  
506 *plantarum* C182 emulsified 56.23% in n-hexane.<sup>76</sup> Interestingly in black cumin oil emulsion,  
507 the EPS from LDMB05 (80.30–84.50%), LDMB03 (68.40–71.50%), M240 (67.53–69.50%)  
508 and LDMB11 (79.00–80.50%) in black cumin oil, as well as M240 in mustard oil (54.53–  
509 58.90%), showed an upward trend even at 96 h (Table 4d). The statistical analysis proved that  
510 the emulsification index varies significantly ( $p < 0.05$ ) depending on the type of EPS and  
511 hydrophobic substances, as presented in Table 5. The highest emulsification index was given  
512 by LDMB05 EPS in black cumin oil (83%) followed by 80% by LDMB11 EPS in the same  
513 system. Though not at the peak of any emulsion system, LDMB03 consistently showed more  
514 than 50% emulsification index (covering E<sub>24</sub> to E<sub>96</sub>) in all the emulsion systems in the study.  
515 According to De *et al.*,<sup>75</sup> a good emulsifier should stabilize at least 50% of the emulsion from  
516 the original volume after 24 h. It was also observed that the emulsion index of EPS gradually  
517 decreased with increasing incubation time (Table 6;  $p < 0.05$ ). The kerosene emulsion was



518 found to be more unstable, showing 25% reduction (from  $E_{24}$  to  $E_{96}$ ) in the emulsion index, followed by 21% in soybean oil and hexane and 17% in mustard oil and almond oil. The black  
519 followed by 21% in soybean oil and hexane and 17% in mustard oil and almond oil. The black  
520 cumin oil gave the most stable emulsion only with less than 2% loss in emulsion index. The  
521 factors associated with the discontinuous phase in an emulsion *viz.* viscosity, interfacial  
522 tension, polar components, hydrocarbon structures, and droplet size and distribution are  
523 involved in the emulsion stability.<sup>77-79</sup> Moreover, emulsification performance is also  
524 influenced by ionic strength, temperature, pH, salinity, and monovalent salts in the system.<sup>80</sup>

525 The gross particle size of the emulsion (*P. pentosaceus* B225 EPS in different oils) is  
526 depicted in Fig. 7. This experiment revealed that the particle size of emulsified droplets plays  
527 a critical role in determining the physical stability, including creaming rate and flocculation of  
528 different emulsions. Larger and scattered droplets in the emulsion indicate less efficiency for  
529 stability formation (Figs. 7C and 7D). On the contrary, the particle distribution showed mostly  
530 smaller, densely packed, and uniform droplets that provided a higher surface area for the EPS  
531 to adsorb, resulting in work as an effective stabilizer and resisting coalescence and  
532 sedimentation. This positive trend was also observed by Balyan *et al.*<sup>81</sup> in their work on  
533 isolating *Lactobacillus* EPS as an emulsifier to ensure the extended stability of eugenol  
534 encapsulation.

535 **3.3.5. Water holding capacity.** Water holding capacity (WHC) is an essential functional  
536 parameter that reflects the ability of polysaccharides to bind and interact with hydrodynamic  
537 water, bound water, and physically entrapped water in a system.<sup>66</sup> The WHC of LDMB03  
538 (204.23%) and LDMB05 (200.90%) were found significantly ( $p < 0.05$ ) higher compared  
539 with LDMB01 (149.17%), M540 (113.90%), LDMB10 (100.73%), M240 (98.27%), D325  
540 (46.80%), B225 (46.83%), and LDMB11 (38.37%) (Fig. 8). Qamar *et al.*<sup>42</sup> found 120.5%  
541 WHC in *Macrococcus brunensis* EPS, and Afreen *et al.*<sup>51</sup> found 475% WHC in  
542 *Lactiplantibacillus paraplantarum* NCCP 962 EPS. This is likely due to their porous structures  
543 and intermolecular hydrogen bond interactions. The compact structure, long polymer chain  
544 with a higher molecular weight and branched chains, functional group, and microstructure (e.g.  
545 porosity) in EPS are linked to increased WHC.<sup>66,82,83</sup> However, these characterizations of the  
546 produced EPS in the present study were not done. During the dough stage, EPS are typically  
547 used as hydrocolloids, with water and other dough components being tightly bound, resulting  
548 in greater moisture retention and reduced hardness of the final product.<sup>84</sup> Li *et al.*<sup>85</sup> reported a  
549 higher WHC in yoghurt from *Lb. plantarum* 70810 EPS. Similarly, Wang *et al.*<sup>86</sup> found higher



550 WHC in yogurt by using *Lb. mesenteroides* XR1 EPS and Yang *et al.*<sup>87</sup> reported that *Lb.*  
551 *rhamnosus* JAAS8 EPS improves the WHC of the fermented milk products.

552 **3.3.6. Oil holding capacity.** Oil holding capacity (OHC) is an essential characteristic of EPS  
553 and is linked to the permeable structure of the carbohydrate polymer chains. In addition, it is  
554 the ability of EPS to bind to oil molecules when no water is present.<sup>14</sup> Results showed that  
555 LDMB03 EPS exhibited the highest oil holding capacity (309.59±20.59%), whereas B225 EPS  
556 resulted in the lowest OHC (35.15±5.56%) (Fig. 8). In addition, EPS LDMB05 (191.56%),  
557 LDMB01 (159.18%), and M240 (114.22%) demonstrated notable oil-holding capacities among  
558 the tested EPS. There was a significant difference ( $p < 0.05$ ) among the oil holding capacity of  
559 various crude EPS. While the chemical composition significantly influences the OHC, the  
560 porosity of the biopolymer and its interaction with oil are also key determinants of the factor.<sup>88</sup>  
561 Trabelsi *et al.*<sup>89</sup> found only 15.96% OHC in *Lactobacillus* sp. Ca<sub>6</sub> EPS in cooked beef sausage.  
562 In the current study, *Lb. lactis* LDMB10 upheld 258.29% oil, whereas, Yang-Ren *et al.*<sup>90</sup> found  
563 that *Lb. lactis* EPS retained 325.67% and Afreen *et al.*<sup>51</sup> reported 658% OHC  
564 by *Lactiplantibacillus paraplantarum* NCCP 962 EPS. On the other hand, Nehal *et al.*<sup>70</sup>  
565 reported 9% OHC from *Lactococcus lactis* F-mou isolated from camel milk. The strain and  
566 medium variation in OHC need further detailed study. Industrially, in product or food  
567 formulations where fat adsorption is beneficial, a high oil-holding capacity contributes to a  
568 smoother, juicier, more palatable texture and better flavor retention.<sup>91</sup> The present findings  
569 anticipated that EPS LDMB03, LDMB10, LDMB01, LDMB05, and M240 could be  
570 extensively used in fermented food products, especially in fat-absorbing food, due to the higher  
571 OHC of EPS. Nowadays, biopolymers (food-grade) are expansively used as an emulsifier to  
572 stabilize the oil/water emulsion system.

### 573 3.4. Coagulation effect of EPS strain in skimmed milk

574 The coagulation/solidification effect of the superior EPS strain *Lb. acidophilus*  
575 LDMB01 with different levels of sugar (sucrose) in skimmed milk is depicted in Fig. 9. After  
576 24 h of fermentation, all the samples remained liquid, and even in 36 h, the sample with 0%  
577 sucrose exhibited a liquid appearance, while the samples with 3%, 6%, 9%, and 12% sucrose  
578 exhibited an initiation of curd formation. However, after 48 h, the sample with 0% sucrose  
579 remained unchanged, while other samples resulted in semi-solid and solid structures. Notably,  
580 the sample with 12% sucrose had a more pronounced solidification than all other samples and  
581 exhibited a complete curdling characteristic. The outcome of the present study is similar to the



582 findings of Ning *et al.*<sup>5</sup> on *Gluconobacter frateurii* HDC-08 EPS, Yu *et al.*<sup>92</sup> on  
583 *Lactiplantibacillus plantarum* HDC-01, and Afreen *et al.*<sup>51</sup> on *Lactiplantibacillus*  
584 *paraplantarum* NCCP 962 EPS, where with higher sucrose concentrations and longer  
585 fermentation periods resulted in more pronounced curd. The coagulation effect is intricately  
586 linked to the structure and molecular weight of EPS, the kind of protein present in milk, as well  
587 as the interaction of EPS and skimmed milk protein.<sup>93</sup> The findings indicated that the LAB  
588 might be capable of synthesizing EPS from sucrose, which promotes milk solidification  
589 properties in dairy products.

### 590 3.5. Conjugated linoleic acid production

591 Food-derived LAB strains are considered to be the most efficient conjugated linoleic acid  
592 (CLA) producers. This study selected the higher EPS-producing LAB strain for CLA  
593 production. The standard curve exhibited a strong linear relationship ( $R^2 = 0.9949$ ). The linear  
594 correlation between peak area and concentration was strong within the concentration range of  
595 0 to 100  $\mu\text{g/mL}$ . The CLA concentration across the strains varied, with values ranging from  
596  $9.56 \pm 1.24 \mu\text{g/mL}$  to  $30.60 \pm 1.27 \mu\text{g/mL}$  when supplied with 0.5 mg/mL LA. Among all the  
597 strains tested in the Fig. 10, the microorganism M240 exhibited the highest CLA  
598 conversion/production, reaching 30.60  $\mu\text{g/mL}$ , followed closely by LDMR01 and LDMB11  
599 with CLA yields of 29.72  $\mu\text{g/mL}$  and 28.46  $\mu\text{g/mL}$ , respectively. Wei *et al.*<sup>94</sup> found a close  
600 result for the strain *Lpb. plantarum* Lp-01, which achieved 33.47  $\mu\text{g/mL}$  of CLA when cultured  
601 in a medium supplemented with pine nut oil, indicating its capacity to convert linoleic acid  
602 present in the oil into CLA. The strain *Lb. casei* LDMB03 showed at 23.12  $\mu\text{g/mL}$  of CLA;  
603 however, in another study, using an initial linoleic acid concentration of 0.1% and incubating  
604 at 37°C for 72 h, soymilk fermented by *Lactobacillus casei* synthesized approximately 839  
605  $\mu\text{g/g}$  of CLA.<sup>95</sup> *Lactobacillus reuteri* is reported to have 0.108 mg/mL of CLA when incubated  
606 under aerobic conditions at 10°C for 30 h in the broth medium with 20 mg/L free LA. It was  
607 found that *L. reuteri* cells could grow 5.5 times faster CLA isomers (c9,t11 and t10,c12) than  
608 free-washed cells.<sup>31</sup> Jena and Choudhury<sup>96</sup> found that if the yogurt culture of *Lb. del.* subsp.  
609 *bulgaricus* LB340 and *Bifidobacterium animalis* subsp. *lactis* BLO4, BB12, B94, and HN019  
610 co-cultured with the strain of *S. thermophilus* TA040, it predominantly enhanced CLA content  
611 in yogurt during the manufacture. Baliyan *et al.*<sup>97</sup> found a variable CLA production by the EPS  
612 producing probiotic LABs isolated from raw cow's milk. Chen *et al.*<sup>28</sup> also reported 1.28  
613  $\mu\text{g/mL}$  to 54.28  $\mu\text{g/mL}$  CLA production by different LAB's.



614 The CLA production variation highlights strain-specific capabilities, which could be  
615 exhibited by the distinct metabolic pathways and enzymatic activities within each LAB species.  
616 These findings suggest that certain LAB EPS strains, particularly *Lb. del. spp. bulgaricus*  
617 M240, *Lb. acidophilus* LDMB01, and *Lb. paraplantarum* LDMB11 could be a promising strain  
618 for applications in CLA-enriched probiotic formulation. Therefore, EPS and CLA were  
619 evaluated in this study because they represent two of the most functionally significant  
620 metabolites produced by lactic acid bacteria with direct applications in dairy foods. In the study  
621 by Amiri *et al.*,<sup>98</sup> *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis* subsp. *lactis*  
622 BB12 were shown to co-produce two metabolites of EPS and CLA under the same culture  
623 conditions. This co-production suggests the potential for selecting strains that can  
624 simultaneously deliver both texture/stabilization through EPS and health benefits via CLA in  
625 fermented products.

#### 626 4. Conclusions

627 The current study provides comprehensive information about the screening, production and  
628 quantification of exopolysaccharides from selected lactic acid bacteria strains, functional  
629 properties of exopolysaccharides, and conjugated linoleic acid producing ability of higher  
630 exopolysaccharide-producing strains. The highest exopolysaccharide production was observed  
631 in *Lactobacillus acidophilus* LDMB01 EPS, and the lowest was found in *Lactococcus*  
632 *raffinolactis* B425. For conjugated linoleic acid production, *Lactobacillus delbruki* spp.  
633 *bulgaricus* M240 produced a higher amount of conjugated linoleic acid. Considering functional  
634 properties, the exopolysaccharide obtained from *Lacticaseibacillus casei* LDMB03 exhibited  
635 higher water and oil-holding activity compared to the other strains. The milk coagulation ability  
636 of the exopolysaccharide producing strain gives it a feasible application in fermented dairy  
637 products. Increasing the EPS concentration enhanced antimicrobial activity; however,  
638 approximately 11.67% of the tested samples exhibited no antimicrobial effect even at higher  
639 concentrations. Accumulated antimicrobial activity, hydroxy radical scavenging activity,  
640 emulsification index, water holding capacity, oil holding capacity, together with solidifying  
641 efficiency of exopolysaccharides, indicate numerous applications in the food industry.  
642 Considering exopolysaccharides and conjugated linoleic acid production potentials, as well as  
643 functional properties of exopolysaccharides, *Lactobacillus delbruki* subsp. *bulgaricus* M240,  
644 *Lactiplantibacillus plantarum* LDMB05, and *Lactobacillus acidophilus* LDMB01 can be  
645 recommended as suitable lactic acid bacteria strains. Furthermore, producing  
646 exopolysaccharides in functional foods manufacturing can be highly interesting due to its role



647 not only as a health-promoting agent but also as a key factor in enhancing the quality of food  
648 products. Moreover, this study is limited by spectroscopic studies like Fourier Transform  
649 Infrared spectroscopy (FTIR) and/or Nuclear Magnetic Resonance (NMR), and advanced  
650 microscopy like scanning electron microscopy (SEM), which elucidate the produced  
651 exopolysaccharides with more precise characteristics important for industrial applications.

## 652 **Author contributions**

653 Md. Sadman Tahmid: Conceptualization, Data Curation, Formal analysis, Investigation,  
654 Methodology, Software, Supervision, Validation, Visualization, Writing– original draft,  
655 Writing–review & editing. Md. Abunaser: Conceptualization, Writing–review & editing.  
656 A.K.M. Masum: Conceptualization, Investigation, Supervision, Validation, Visualization,  
657 Writing–review & editing. Mohammad Ashiqul Islam: Conceptualization, Formal Analysis,  
658 Resources, Validation, Writing–review & editing. Md. Harun-ur-Rashid: Conceptualization,  
659 Funding Acquisition, Project Administration, Resources, Supervision, Validation, Writing–  
660 review & editing.

## 661 **Conflicts of interest statement**

662 The authors declare no conflicts of interest.

## 663 **Data availability**

664 Data will be made available on request.

## 665 **Acknowledgements**

666 This work was supported by the Ministry of Education, GoB (MoE, Project ID: LS-20232873,  
667 Ref. 2023/15/MoE). The authors acknowledge the Laboratory of Animal Science and  
668 Biotechnology, BAU, for their support in providing the facility for the 96-well plate  
669 spectrophotometer analysis.

670



671 **References**View Article Online  
DOI: 10.1039/D5FB00943J

- 672 1 J. F. Ceron-Cordoba, L. C. Muñoz-Domínguez, J. U. S. Valencia and D. A. Restrepo-Molina,  
673 *Journal of Dairy Science*, DOI:10.3168/jds.2024-25908.
- 674 2 N. S. Jaffar, R. Jawan and K. P. Chong, *Front. Plant Sci.*, DOI:10.3389/fpls.2022.1047945.
- 675 3 A. I. Netrusov, E. V. Liyaskina, I. V. Kurgaeva, A. U. Liyaskina, G. Yang and V. V. Revin,  
676 *Microorganisms*, 2023, **11**, 1541.
- 677 4 F. Salimi and P. Farrokh, *World J Microbiol Biotechnol*, 2023, **39**, 213.
- 678 5 Y. Ning, H. Cao, S. Zhao, D. Gao and D. Zhao, *Polymers*, 2024, **16**, 1004.
- 679 6 Z. Q. Xiong, L.-H. Kong, P. F.-H. Lai, Y.-J. Xia, J.-C. Liu, Q.-Y. Li and L.-Z. Ai, *Journal of*  
680 *Dairy Science*, 2019, **102**, 4925–4934.
- 681 7 M. K. D. Dueholm, M. Besteman, E. J. Zeuner, M. Riisgaard-Jensen, M. E. Nielsen, S. Z.  
682 Vestergaard, S. Heidelberg, N. S. Bekker and P. H. Nielsen, *Water Research*, 2023, **229**,  
683 119485.
- 684 8 N. Kaur and P. Dey, *Research in Microbiology*, 2023, **174**, 104024.
- 685 9 A. Ponzio, A. Rebecchi, R. Zivoli and L. Morelli, *Foods*, 2024, **13**, 752.
- 686 10 J. Wu, X. Han, M. Ye, Y. Li, X. Wang and Q. Zhong, *Critical Reviews in Food Science and*  
687 *Nutrition*, 2023, **63**, 7043–7064.
- 688 11 K. Zhang, S. Liu, S. Liang, F. Xiang, X. Wang, H. Lian, B. Li and F. Liu, *International*  
689 *Journal of Biological Macromolecules*, 2024, **257**, 128733.
- 690 12 F. Stingele, J. R. Neeser and B. Mollet, *Journal of Bacteriology*, 1996, **178**, 1680–1690.
- 691 13 J.-E. Germond, M. Delley, N. D’Amico and S. J. F. Vincent, *European Journal of*  
692 *Biochemistry*, 2001, **268**, 5149–5156.
- 693 14 D. Kavitate, P. B. Devi, C. Delattre, G. B. Reddy and P. H. Shetty, *International Journal of*  
694 *Biological Macromolecules*, 2023, **226**, 111–120.
- 695 15 M. Xiao, L. Tang, X. Fu, X. Ren, J. Bi, J. Wang, D. Li, Q. Kong, H. Mou and C. Zhu, *Food*  
696 *Hydrocolloids*, 2024, **151**, 109850.
- 697 16 Z. Ge, D. Wang, F. Azi, W. Zhao, P. Wang, M. Dong, J. Wang, Y. Zhao and X. Zhao,  
698 *International Dairy Journal*, 2024, **155**, 105969.
- 699 17 A. S. Raj, G. Anup, R. Sivalingam and P. Chandran, *Bioremediation Journal*, 2024, **28**, 491–  
700 511.
- 701 18 B. G. Guerrero, K. D. L. Santos, E. S. Kamimura and C. A. F. De Oliveira, *Int J of Food Sci*  
702 *Tech*, 2024, **59**, 17–29.
- 703 19 N. S. Kiran, C. Yashaswini, S. Singh and B. G. Prajapati, *3 Biotech*, 2024, **14**, 95.



- 704 20 P. Li, Y. Bai, Y. Yang and M. Li, *Food Bioscience*, 2023, **55**, 102961.
- 705 21 H. Pourjafar, F. Ansari, A. Sadeghi, S. A. Samakkhah and S. M. Jafari, *Critical Reviews in*  
706 *Food Science and Nutrition*, 2023, **63**, 8194–8225.
- 707 22 M. Tarique, A. H. Ali, J. Kizhakkayil, S.-Q. Liu, F. Oz, E. Dertli, A. Kamal-Eldin and M.  
708 Ayyash, *Food Chemistry: X*, 2024, **21**, 101073.
- 709 23 A. A. Al-Nabulsi, Z. W. Jaradat, F. R. Al Qudsi, L. Elsalem, T. M. Osaili, A. N. Olaimat,  
710 G. Esposito, S.-Q. Liu and M. M. Ayyash, *LWT*, 2022, **167**, 113817.
- 711 24 M. Ayyash, C. Stathopoulos, B. Abu-Jdayil, G. Esposito, M. Baig, M. S. Turner, A. S. Baba,  
712 V. Apostolopoulos, A. Al-Nabulsi and T. Osaili, *LWT*, 2020, **131**, 109741.
- 713 25 M. Xiao, X. Ren, Y. Yu, W. Gao, C. Zhu, H. Sun, Q. Kong, X. Fu and H. Mou, *Food*  
714 *Chemistry: X*, 2022, **13**, 100233.
- 715 26 F. Yue, H. Han, J. Xu, X. Yao, Y. Qin, L. Zhang, X. Sun, J. Huang, F. Zhang and X. Lü,  
716 *International Journal of Biological Macromolecules*, 2025, **289**, 138803.
- 717 27 Y. Yang, X. Shi, J. Zhang, H. Xiao and C. Li, *Food Chemistry*, 2025, **465**, 142068.
- 718 28 C. Chen, F. Tong, R. Sun, Y. Zhang, Z. Pang and X. Liu, *Foods*, 2024, **13**, 1830.
- 719 29 M. Du, M. Gong, G. Wu, J. Jin, X. Wang and Q. Jin, *J. Agric. Food Chem.*, 2024, **72**, 5503–  
720 5525.
- 721 30 M. Iorizzo, C. Di Martino, F. Letizia, T. W. Crawford and G. Paventi, *Foods*, 2024, **13**, 975.
- 722 31 S. S. Li, L. Xu, J. Qing, X. Wu, H. Li, H. Chen and X. Liu, *Food Chemistry*, 2023, **409**,  
723 135257.
- 724 32 L. Wu, S. Ye, X. Deng, Z. Fu, J. Li and C. Yang, *Nutrients*, 2024, **16**, 1133.
- 725 33 R. Chourasia, M. M. Abedin, L. Chiring Phukon, D. Sahoo, S. P. Singh and A. K. Rai,  
726 *Comprehensive Reviews in Food Science and Food Safety*, 2021, **20**, 960–979.
- 727 34 M. M. Abedin, R. Chourasia, L. C. Phukon, P. Sarkar, R. C. Ray, S. P. Singh and A. K. Rai,  
728 *Critical Reviews in Food Science and Nutrition*, 2024, **64**, 10730–10748.
- 729 35 M. Rashid, K. Togo, M. Ueda and T. Miyamoto, *Pakistan Journal of Nutrition*, 2007, **6**,  
730 647–652.
- 731 36 M. Rashid, K. Togo, M. Ueda and T. Miyamoto, *World J Microbiol Biotechnol*, 2007, **23**,  
732 125–133.
- 733 37 Md. Z. Islam, Md. E. Uddin, Md. T. Rahman, M. A. Islam and Md. Harun-ur-Rashid, *Small*  
734 *Ruminant Research*, 2021, **205**, 106532.
- 735 38 N. Tarannum, T. J. Hossain, F. Ali, T. Das, K. Dhar and I. H. Nafiz, *LWT*, 2023, **186**,  
736 115263.

View Article Online  
DOI: 10.1039/D5FB00943J



- 737 39 F. Yang-Chen, J. Chen, S. Ye, Z. Liu and Y. Ding, *Process Biochemistry*, 2022, **113**, 87-96. View Article Online  
DOI: 10.1089/05FB00943J
- 738 96.
- 739 40 W. Xia, J. Han, S. Zhu, Y. Wang, W. Zhang and Z. Wu, *International Journal of Biological*
- 740 *Macromolecules*, 2023, **230**, 123177.
- 741 41 M. Kowsalya, T. Velmurugan, R. Mythili, W. Kim, K. G. Sudha, S. Ali, B. Kalpana, S.
- 742 Ramalingam and M. Prasanna Rajeshkumar, *International Journal of Biological*
- 743 *Macromolecules*, 2023, **242**, 124842.
- 744 42 S. A. Qamar, M. Asgher and M. Bilal, *Waste Biomass Valor*, 2021, **12**, 6847–6859.
- 745 43 L. C. Silva, G. B. Schmidt, L. G. O. Alves, V. S. Oliveira, R. Laureano-Melo, E. Stutz, J. F.
- 746 P. Martins, B. P. Paula, R. H. Luchese, A. F. Guerra and P. Rodrigues, *Food and Bioproducts*
- 747 *Processing*, 2020, **124**, 408–418.
- 748 44 J. Homolak, *Free Radical Research*, 2022, **56**, 343–357.
- 749 45 R. Ortega-Villar, A. Escalante, F. Astudillo-Melgar, L. Lizárraga-Mendiola, G. A. Vázquez-
- 750 Rodríguez, M. E. Hidalgo-Lara and C. Coronel-Olivares, *Microorganisms*, 2024, **12**, 1066.
- 751 46 G. Rampanti, D. N. Nedelkoska, T. Kalevska, T. Stojanovska, J. Harasym, F. Cardinali, A.
- 752 Orkusz, V. Milanović, C. Garofalo, A. Bonifazi, L. Aquilanti and A. Osimani, *Heliyon*,
- 753 DOI:10.1016/j.heliyon.2024.e37548.
- 754 47 E. Barrett, R. P. Ross, G. F. Fitzgerald and C. Stanton, *Applied and Environmental*
- 755 *Microbiology*, 2007, **73**, 2333–2337.
- 756 48 Q. Liu, X. Huang, D. Yang, T. Si, S. Pan and F. Yang, *EXCLI J*, 2016, **15**, 119–133.
- 757 49 F. Hernández-Rosas, J. D. Castilla-Marroquín, J. M. Loeza-Corte, M. A. Lizardi-Jiménez,
- 758 R. I. Hernández-Martínez, and Colegio de Postgraduados, *RMIQ*, 2021, **20**, 1–21.
- 759 50 A. Padmanabhan, Y. Tong, Q. Wu, J. Zhang and N. P. Shah, *Front. Microbiol.*, 2018, **9**,
- 760 1919.
- 761 51 A. Afreen, Z. Ahmed, N. Khalid, I. Ferheen and I. Ahmed, *Appl Microbiol Biotechnol*, 2023,
- 762 **107**, 1189–1204.
- 763 52 R. Prete, M. K. Alam, G. Perpetuini, C. Perla, P. Pittia and A. Corsetti, *Foods*, 2021, **10**,
- 764 1653.
- 765 53 M. K. Bates, D. S. Phillips and J. O'Bryan, .
- 766 54 R. Prete, F. Dell'Orco, G. Sabatini, F. Montagano, N. Battista and A. Corsetti, *Foods*, 2024,
- 767 **13**, 1663.
- 768 55 Y. Dong, J. Ronholm, I. Fliss and S. Karboune, *Probiotics & Antimicro. Prot.*,
- 769 DOI:10.1007/s12602-024-10270-y.
- 770 56 M. A. Arayes, M. E. M. Mabrouk, S. A. Sabry and B. Abdella, *Biologia*, 2023, **78**, 229–240.



- 771 57 M. Srinivash, R. Krishnamoorthi, P. U. Mahalingam and B. Malaikozhundan, *International*  
 772 *Journal of Biological Macromolecules*, 2023, **250**, 126171. View Article Online  
DOI: 10.1039/D3FB00943J
- 773 58 X. Xu, Q. Peng, Y. Zhang, D. Tian, P. Zhang, Y. Huang, L. Ma, Y. Qiao and B. Shi, *Food*  
 774 *& Nutrition Research*, 2020, **64**, 10.29219/fnr.v64.3744.
- 775 59 E. S. Khalil, M. Y. Abd Manap, S. Mustafa, A. M. Alhelli and P. Shokryazdan, *Molecules*,  
 776 2018, **23**, 398.
- 777 60 Q. Q. Li, Y. Chen, X. Liu, Y. Li, J. Xu, T. Li, W. Xiang and A. Li, *Front. Mar. Sci.*,  
 778 DOI:10.3389/fmars.2022.1097200.
- 779 61 X. Yang-Wu, J. Wu, F. An, J. Xu, M. Bat-Ochir, L. Wei, M. Li, M. Bilige and R. Wu,  
 780 *International Journal of Biological Macromolecules*, 2022, **208**, 288–298.
- 781 62 Q. Zhong, B. Wei, S. Wang, S. Ke, J. Chen, H. Zhang and H. Wang, *Marine Drugs*, 2019,  
 782 **17**, 674.
- 783 63 X. Li, Y. Gong, W. Yao, X. Chen, J. Xian, L. You and P. Fardim, *Food Chemistry: X*, 2021,  
 784 **12**, 100157.
- 785 64 Q. Wei, G. Fu, K. Wang, Q. Yang, J. Zhao, Y. Wang, K. Ji and S. Song, *Pharmaceuticals*,  
 786 2022, **15**, 581.
- 787 65 W. Zang, H. Cao, J. Ge and D. Zhao, *International Journal of Biological Macromolecules*,  
 788 2024, **263**, 130083.
- 789 66 M. Kumari, R. Kumari, B. H. Nataraj, P. A. Shelke, S. A. Ali, R. Nagpal and P. V. Behare,  
 790 *Current Research in Food Science*, 2023, **6**, 100478.
- 791 67 J. Wang, J. Zhang, H. Guo, Q. Cheng, Z. Abbas, Y. Tong, T. Yang, Y. Zhou, H. Zhang, X.  
 792 Wei, D. Si and R. Zhang, *Foods*, 2023, **12**, 2481.
- 793 68 A. K. Abdalla, M. M. Ayyash, A. N. Olaimat, T. M. Osaili, A. A. Al-Nabulsi, N. P. Shah  
 794 and R. Holley, *Front. Microbiol.*, DOI:10.3389/fmicb.2021.664395.
- 795 69 P. Rahnema Vosough, M. B. Habibi Najafi, M. R. Edalatian Dovom, A. Javadmanesh and  
 796 B. Mayo, *Food Measure*, 2021, **15**, 5221–5230.
- 797 70 F. Nehal, M. Sahnoun, S. Smaoui, B. Jaouadi, S. Bejar and S. Mohammed, *Microbial*  
 798 *Pathogenesis*, 2019, **132**, 10–19.
- 799 71 M. S. R. Rajoka, H. M. Mehwish, H. F. Hayat, N. Hussain, S. Sarwar, H. Aslam, A. Nadeem  
 800 and J. Shi, *Probiotics & Antimicro. Prot.*, 2019, **11**, 1132–1142.
- 801 72 S. K. Bhatia, P. S. Panesar and S. Mehariya, Eds., *Microbial Exopolysaccharides:*  
 802 *Production and Applications*, CRC Press, Boca Raton, 2024.
- 803 73 L. Sun, Y. Yang, P. Lei, S. Li, H. Xu, R. Wang, Y. Qiu and W. Zhang, *Carbohydrate*  
 804 *Polymers*, 2021, **261**, 117872.



- 805 74 A. Najjari, M. Jabberi, S. F. Chérif, A. Cherif, H. I. Ouzari, J. A. Linares-Pastén and H. Sghaier, *Front. Microbiol.*, DOI:10.3389/fmicb.2024.1440081. View Article Online  
DOI:10.1039/B3FB00943J
- 806
- 807 75 L. De Melo Teixeira, É. Da Silva Santos, R. S. Dos Santos, A. V. G. Ramos, D. C. Baldoqui,  
808 M. L. Bruschi, J. E. Gonçalves, R. A. C. Gonçalves and A. J. B. De Oliveira, *International*  
809 *Journal of Biological Macromolecules*, 2024, **278**, 134400.
- 810 76 V. Nemati and R. Mozafarpour, *LWT*, 2024, **199**, 116116.
- 811 77 F. Y. Ushikubo and R. L. Cunha, *Food Hydrocolloids*, 2014, **34**, 145–153.
- 812 78 X. Feng, Y. Sun, H. Tan, L. Ma, H. Dai and Y. Zhang, *Food Chemistry*, 2023, **413**, 135653.
- 813 79 T. Xiao, X. Ma, H. Hu, F. Xiang, X. Zhang, Y. Zheng, H. Dong, B. Adhikari, Q. Wang and  
814 A. Shi, *Food Chem X*, 2025, **29**, 102792.
- 815 80 D. Kavitate, S. Balyan, P. B. Devi and P. H. Shetty, *J Food Sci Technol*, 2020, **57**, 1579–  
816 1585.
- 817 81 S. Balyan, V. Dadwal and B. S. Patil, *Food Bioscience*, 2024, **61**, 104632.
- 818 82 X. Yang-Feng, J. Feng, Q. Zhu, R. Hong and L. Li, *Polymers*, 2022, **14**, 90.
- 819 83 Y. Dai, M. Xu, Z. Zhou and Y. Han, *Polymers*, 2024, **16**, 759.
- 820 84 J. Zhang, Y. Yao, J. Li, X. Ju and L. Wang, *Food Chemistry*, 2023, **425**, 136369.
- 821 85 C. Li, W. Li, X. Chen, M. Feng, X. Rui, M. Jiang and M. Dong, *LWT - Food Science and*  
822 *Technology*, 2014, **57**, 477–485.
- 823 86 L. Wang, Y. Gu and Z. Lv, *Int J Food Sci Tech*, 2022, **57**, 4076–4085.
- 824 87 Z. Yang, S. Li, X. Zhang, X. Zeng, D. Li, Y. Zhao and J. Zhang, *Journal of Bioscience and*  
825 *Bioengineering*, 2010, **110**, 53–57.
- 826 88 S. Sarkar, G. Cabrera-Barjas, R. N. Singh, J. P. Fabi, S. J. M. Breig, J. Tapia, R. K. Sani and  
827 A. Banerjee, *Sci Rep*, 2024, **14**, 25058.
- 828 89 I. Trabelsi, N. Ktari, M. Triki, I. Bkhairia, S. Ben Slima, S. Sassi Aydi, S. Aydi, A. Abdeslam  
829 and R. Ben Salah, *International Journal of Biological Macromolecules*, 2018, **111**, 11–18.
- 830 90 X. Yang-Ren, Y. Ren and L. Li, *LWT*, 2022, **153**, 112345.
- 831 91 N. Srivastava, S. Kumari, S. Kurmi, A. K. Pinnaka and A. R. Choudhury, *Arch Microbiol*,  
832 2022, **204**, 399.
- 833 92 L. Yu, G. Ye, X. Qi, Y. Yang, B. Zhou, Y. Zhang, R. Du, J. Ge and W. Ping, *Front.*  
834 *Microbiol.*, 2023, **14**, 1210302.
- 835 93 R. Du, L. Yu, N. Yu, W. Ping, G. Song and J. Ge, *International Journal of Biological*  
836 *Macromolecules*, 2022, **217**, 303–311.
- 837 94 G. Wei, G. Wu, J. Sun, Y. Qi, Q. Zhao, F. Xu, Z. Zhang and Z. Peng, *Foods*, 2024, **13**, 2472.



- 838 95 M. Sasi, S. Kumar, M. Hasan, A. S. R., E. Garcia-Gutierrez, S. Kumari, O. Prakash, I. Nain, A. Sachdev and A. Dahuja, *Critical Reviews in Food Science and Nutrition*, 2023, **63**, 9995–  
839 10013. View Article Online  
DOI: 10.1039/D3FB00943J
- 840
- 841 96 R. Jena and P. K. Choudhury, *Probiotics & Antimicro. Prot.*, DOI:10.1007/s12602-023-  
842 10189-w.
- 843 97 N. Baliyan, A. K. Maurya, A. Kumar, V. K. Agnihotri and R. Kumar, *LWT*, 2023, **176**,  
844 114553.
- 845 98 S. Amiri, M. Rezazadeh Bari, M. Alizadeh Khaledabad, R. Rezaei Mokarram and M. Sowti  
846 Khiabani, *Chemical Review and Letters*, 2021, **4**, 66–76.
- 847
- 848
- 849
- 850
- 851
- 852



853

## List of Tables

View Article Online  
DOI: 10.1039/D5FB00943J

854

**Table 1** Screening and selection of exopolysaccharide (EPS) producing lactic acid

855

bacteria strains, with 18 high-EPS producers from a total of 50 strains.

Isolates	String Test	Aniline Blue (AB) Assay	Congo Red (CR) Assay
<i>Str. bovis</i> biotype I D125-4	–	–	+
<i>Ent. faecium</i> D325-2	++	–	++
<i>Lb. delbruki</i> spp. <i>lactis</i> D340-2	–	–	+
<i>P. pentosaceus</i> B225-2	++	+	+++
<i>Leu. mesenteroides</i> spp. <i>mesenteroides</i> D625-3	–	–	+
<i>Lb. agilis</i> D640-4	–	+	–
<i>Lc. raffinolactis</i> B425-4	+	+	++
<i>Lc. lactis</i> spp. <i>lactis</i> D425-4	+	+++	+
<i>Lb. fermentum</i> M240-4	+++	–	–
<i>Lb. delbruki</i> spp. <i>bulgaricus</i> M240-5	+++	+++	+++
<i>Str. thermophilus</i> M540-5	–	–	+
<i>Str. bovis</i> biotype II J225-1	+++	–	+
<i>Lc. lactis</i> LDMB10	–	–	+++
<i>Lb. casei</i> subsp. <i>casei</i> LDMB03	++	++	+++
<i>Lpb plantarum</i> LDMB05	+++	–	+++
<i>Lb. paraplantarum</i> LDMB11	–	+	+++
<i>Lb. acidophilus</i> LDMB01	+++	+++	++
<i>Leu. mesenteroides</i> spp. <i>dextranicum</i> M725-1	+++	+++	++

856

<sup>a</sup>Strong positive: +++; moderate positive: ++; weak positive: +; negative: –

857

<sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*

858

*plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;

859

LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del.* subsp. *bulgaricus* M240; B225 = *P.*

860

*pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del.* subsp. *lactis*

861

D340; D425-4 = *Lc. lactis* spp. *lactis* D425-4; D625-3 = *Leu. mesenteroides* spp.

862

*mesenteroides* D625-3; M240-4 = *Lb. fermentum* M240-4; J225-1 = *Str. bovis* biotype II

863

J225-1; D125-4 = *Str. bovis* biotype I D125-4; M725-1 = *Leu. mesenteroides* subsp.

864

*dextranicum* M725-1; D640-4 = *Lb. agilis* D640-4; B425-4 = *Lc. raffinolactis* B425-4.

865

866

867

868

869



870 **Table 2** Biochemical characteristics of selected lactic acid bacteria strains.View Article Online  
DOI: 10.1039/D5FB00943J

Parameters	D34 0	M24 0	LDM B11	LDM B05	LDM B10	LDM B03	B225	M540	D325	LDM B01
Gram staining	+	+	+	+	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Cocci	Rod	Cocci	Cocci	Cocci	Rod
Motility Test	-	-	-	-	-	-	-	-	-	-
Glucose agar test	+	+	+	+	+	+	+	+	+	+
Proteolytic activity	±	+	+w	+w	+	±	+w	-	+	+
Lipolytic activity	±	+w	+w	+w	+w	±	-	-	-	-
Methyl red test	-	-	±	+	-	-	-	-	±	+w
Oxidase test	-	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-
Pectin hydrolysis	-	-	-	-	-	-	-	-	-	-
Fermentation										
Cellobiose	-	-	+	+	+	+	+	-	+	+
Dextrose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	-	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	-	+	+	+	-	+	+	+
Maltose	+	-	+	+	+	+	+	+	+	+
Raffinose	-	-	+	+	-	+	-	-	±	-
Rhamnose	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	+	+	-	+	-	+	+	+
Trehalose	-	-	-	+	+w	-	+	-	+	+
Xylose	-	-	+	-	+w	+	±	-	+	+

871 <sup>a</sup>Positive (+); Negative (-); Variable (±); week positive (+w).872 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
873 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
874 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
875 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
876 D340.

877

878

879

880

881

882

883

884



885 **Table 3** Antimicrobial activity of exopolysaccharides produced by bacterial strainsView Article Online  
DOI:10.1039/D5FB00943J

EPS	Gram Positive Bacteria						Gram Negative Bacteria					
	<i>L. monocytogenes</i>		<i>S. aureus</i>		<i>B. Cereus</i>		<i>E. Coli</i>		<i>S. typhi</i>		<i>K. pneumoniae</i>	
	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL	zone of inhibition (mm) in 0.5 mg/mL	zone of inhibition (mm) in 1.0 mg/mL
LDMB01	+++	+++	++	+++	+	+	-	-	++	+++	++	+++
D325	+	++	+++	+++	-	-	+	++	+	++	-	-
LDMB05	++	++	++	++	+	++	-	+	++	++	+	++
M540	++	++	++	+++	+	++	+	++	+	+	++	+++
LDMB10	+++	+++	++	+++	+	+	-	-	+++	+++	+	+
LDMB03	++	++	+	+	+	+	++	+++	+++	+++	-	-
M240	++	+++	++	++	++	+++	+	++	++	+++	++	++
B225	+	+	+++	+++	+	+	++	+++	+	++	-	-
LDMB11	++	++	++	++	+	+++	-	-	++	+++	+	++
D340	++	+++	++	++	++	++	++	++	+	++	++	+++

886 <sup>a</sup>Strong inhibition (+++) = > 15 mm; Moderate inhibition (++) = 10-15 mm; Weak inhibition  
 887 (+) = 5-10 mm; No inhibition (-) = < 5 mm.

888 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
 889 *plantarum* LDMB05; M540 = *Str. thermopilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
 890 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
 891 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
 892 D340.

893

894 **Table 4a** Effect of olive oil on the exopolysaccharides emulsification index at 24, 48,  
 895 and 96h.

Olive oil	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	69.77 <sup>bc</sup> ± 6.63	65.00 <sup>de</sup> ± 5.76	64.50 <sup>de</sup> ± 4.84
D325	60.93 <sup>ef</sup> ± 0.93	52.00 <sup>ij</sup> ± 1.90	46.50 <sup>l</sup> ± 1.70
LDMB05	61.87 <sup>e</sup> ± 3.70	54.00 <sup>ghi</sup> ± 1.85	52.50 <sup>ghij</sup> ± 0.89
M540	60.20 <sup>ef</sup> ± 5.17	57.00 <sup>fg</sup> ± 0.98	49.00 <sup>ikl</sup> ± 2.11
LDMB10	47.50 <sup>kl</sup> ± 1.44	39.00 <sup>m</sup> ± 1.31	36.20 <sup>m</sup> ± 4.39
LDMB03	63.57 <sup>de</sup> ± 0.57	63.57 <sup>de</sup> ± 1.01	63.53 <sup>de</sup> ± 0.59
M240	63.53 <sup>de</sup> ± 1.63	64.00 <sup>de</sup> ± 1.14	64.30 <sup>de</sup> ± 1.45
B225	84.17 <sup>a</sup> ± 1.99	72.90 <sup>b</sup> ± 1.04	68.23 <sup>cd</sup> ± 1.78
LDMB11	67.03 <sup>cd</sup> ± 1.59	64.00 <sup>de</sup> ± 0.96	63.53 <sup>de</sup> ± 0.97
D340	56.50 <sup>gh</sup> ± 2.10	51.00 <sup>ijkl</sup> ± 2.36	48.50 <sup>ikl</sup> ± 2.01

896 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

897 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
 898 *plantarum* LDMB05; M540 = *Str. thermopilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
 899 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
 900 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
 901 D340.

902

903



904 **Table 4b** Effect of soybean oil on the exopolysaccharides emulsification index at 24, 48, 96 h.  
 905 and 96 h.

Soybean oil	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	59.07 <sup>de</sup> ± 2.68	50.00 <sup>klmn</sup> ± 2.59	44.50 <sup>q</sup> ± 1.50
D325	57.23 <sup>efg</sup> ± 1.40	49.00 <sup>lmno</sup> ± 1.44	46.00 <sup>opq</sup> ± 1.35
LDMB05	61.47 <sup>cd</sup> ± 2.40	57.00 <sup>efg</sup> ± 1.35	50.00 <sup>klmn</sup> ± 2.07
M540	58.53 <sup>de</sup> ± 2.44	54.50 <sup>ghi</sup> ± 1.80	48.00 <sup>mno</sup> ± 1.13
LDMB10	53.00 <sup>hijk</sup> ± 1.67	45.50 <sup>pq</sup> ± 1.90	45.00 <sup>pq</sup> ± 1.35
LDMB03	62.47 <sup>c</sup> ± 1.52	53.50 <sup>hij</sup> ± 2.41	47.17 <sup>nopq</sup> ± 1.85
M240	58.50 <sup>de</sup> ± 2.36	54.93 <sup>fgh</sup> ± 1.19	50.50 <sup>klmn</sup> ± 1.41
B225	70.83 <sup>a</sup> ± 2.24	66.43 <sup>b</sup> ± 1.92	57.30 <sup>efg</sup> ± 0.89
LDMB11	63.50 <sup>bc</sup> ± 0.66	54.47 <sup>ghi</sup> ± 0.93	51.00 <sup>ijklm</sup> ± 2.21
D340	58.00 <sup>ef</sup> ± 1.37	51.50 <sup>ijkl</sup> ± 1.50	40.00 <sup>r</sup> ± 3.12

906 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

907 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
 908 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
 909 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
 910 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
 911 D340.

912

913

914

915 **Table 4c** Effect of mustard oil on the exopolysaccharides emulsification index at 24, 48,  
 916 and 96 h.

Mustard oil	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	57.30 <sup>cd</sup> ± 2.67	53.00 <sup>ef</sup> ± 1.83	50.00 <sup>fghi</sup> ± 1.64
D325	52.53 <sup>efg</sup> ± 0.92	46.50 <sup>ijk</sup> ± 1.35	41.50 <sup>lm</sup> ± 1.91
LDMB05	66.83 <sup>b</sup> ± 2.40	59.00 <sup>c</sup> ± 1.15	54.50 <sup>de</sup> ± 3.40
M540	54.10 <sup>e</sup> ± 2.13	49.53 <sup>ghij</sup> ± 1.59	44.50 <sup>kl</sup> ± 1.67
LDMB10	47.00 <sup>ijk</sup> ± 1.39	39.50 <sup>mn</sup> ± 1.41	38.50 <sup>n</sup> ± 1.95
LDMB03	59.57 <sup>c</sup> ± 1.76	58.50 <sup>c</sup> ± 1.37	58.63 <sup>c</sup> ± 1.40
M240	54.53 <sup>de</sup> ± 2.40	57.50 <sup>cd</sup> ± 1.65	58.90 <sup>c</sup> ± 0.95
B225	57.47 <sup>cd</sup> ± 0.70	50.57 <sup>fgh</sup> ± 1.12	41.77 <sup>lm</sup> ± 1.44
LDMB11	70.00 ± 1.71	50.50 <sup>fgh</sup> ± 1.57	49.00 <sup>hij</sup> ± 1.44
D340	54.00 <sup>e</sup> ± 1.67	47.00 <sup>ijk</sup> ± 1.35	42.50 <sup>lm</sup> ± 1.37

917 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

918 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
 919 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
 920 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
 921 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
 922 D340.

923



924 **Table 4d** Effect of black cumin oil on exopolysaccharides emulsification index at 24, 48, and 96 h. View Article Online  
DOI: 10.1039/D5FB00943J

Black cumin oil	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	69.07 <sup>cd</sup> ± 2.72	64.00 <sup>e</sup> ± 2.09	64.00 <sup>e</sup> ± 1.11
D325	59.13 <sup>f</sup> ± 2.70	58.00 <sup>fg</sup> ± 1.51	57.47 <sup>gh</sup> ± 1.59
LDMB05	80.30 <sup>b</sup> ± 2.70	84.00 <sup>a</sup> ± 1.44	84.50 <sup>a</sup> ± 1.37
M540	59.50 <sup>f</sup> ± 2.55	53.50 <sup>i</sup> ± 1.73	50.50 <sup>j</sup> ± 2.62
LDMB10	55.50 <sup>ghi</sup> ± 0.79	54.50 <sup>hi</sup> ± 1.57	50.00 <sup>j</sup> ± 2.26
LDMB03	68.40 <sup>cd</sup> ± 1.47	70.60 <sup>cd</sup> ± 1.44	71.50 <sup>c</sup> ± 1.47
M240	67.53 <sup>d</sup> ± 0.68	68.20 <sup>d</sup> ± 1.74	69.50 <sup>cd</sup> ± 1.57
B225	57.00 <sup>gh</sup> ± 0.96	59.60 <sup>f</sup> ± 2.36	60.07 <sup>f</sup> ± 1.40
LDMB11	79.00 <sup>b</sup> ± 1.41	81.00 <sup>b</sup> ± 0.70	80.50 <sup>b</sup> ± 0.70
D340	59.00 <sup>f</sup> ± 1.25	58.50 <sup>fg</sup> ± 1.70	48.00 <sup>j</sup> ± 1.14

926 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

927 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
928 *plantarum* LDMB05; M540 = *Str. thermopilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
929 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
930 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
931 D340.

932

933

934 **Table 4e** Effect of almond oil on exopolysaccharides emulsification index at 24, 48, and  
935 96 h.

Almond oil	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	57.17 <sup>bcde</sup> ± 2.75	48.00 <sup>kl</sup> ± 2.55	41.00 <sup>o</sup> ± 2.78
D325	54.57 <sup>efg</sup> ± 1.32	51.03 <sup>hijk</sup> ± 1.12	51.00 <sup>hijk</sup> ± 1.28
LDMB05	63.83 <sup>a</sup> ± 3.48	58.50 <sup>bc</sup> ± 1.47	53.50 <sup>gh</sup> ± 1.44
M540	50.67 <sup>hijkl</sup> ± 2.80	47.50 <sup>lm</sup> ± 1.14	44.50 <sup>mn</sup> ± 1.01
LDMB10	50.00 <sup>ijkl</sup> ± 1.11	47.50 <sup>lm</sup> ± 0.44	43.00 <sup>no</sup> ± 1.93
LDMB03	58.00 <sup>bcd</sup> ± 2.02	55.57 <sup>cdef</sup> ± 1.48	49.70 <sup>ikl</sup> ± 1.15
M240	57.10 <sup>bcde</sup> ± 2.02	53.10 <sup>fghi</sup> ± 1.76	47.40 <sup>lm</sup> ± 1.56
B225	63.43 <sup>a</sup> ± 1.62	54.97 <sup>defg</sup> ± 1.12	49.43 <sup>jkl</sup> ± 0.71
LDMB11	65.50 <sup>a</sup> ± 0.46	59.50 <sup>b</sup> ± 0.66	52.00 <sup>ghij</sup> ± 2.96
D340	56.00 <sup>cdef</sup> ± 1.25	54.00 <sup>efgh</sup> ± 1.11	49.50 <sup>ikl</sup> ± 1.80

936 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

937 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
938 *plantarum* LDMB05; M540 = *Str. thermopilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
939 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
940 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
941 D340.

942



943 **Table 4f** Effect of n-hexane on exopolysaccharides emulsification index at 24, 48, and 96 h. View Article Online  
DOI: 10.1039/D5FB00943J

n-Hexane	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	45.43 <sup>bc</sup> ± 5.75	32.10 <sup>ef</sup> ± 3.01	26.37 <sup>ij</sup> ± 3.47
D325	49.60 <sup>ef</sup> ± 4.11	39.00 <sup>ij</sup> ± 0.95	33.00 <sup>lm</sup> ± 2.07
LDMB05	63.80 <sup>a</sup> ± 2.60	54.50 <sup>cd</sup> ± 2.36	50.00 <sup>ef</sup> ± 1.28
M540	57.63 <sup>bc</sup> ± 2.06	56.50 <sup>bc</sup> ± 0.85	47.50 <sup>fg</sup> ± 1.11
LDMB10	39.47 <sup>ij</sup> ± 1.59	37.00 <sup>jk</sup> ± 2.25	30.00 <sup>mn</sup> ± 2.25
LDMB03	58.03 <sup>bc</sup> ± 2.90	56.70 <sup>bc</sup> ± 1.71	55.10 <sup>c</sup> ± 0.82
M240	51.47 <sup>de</sup> ± 2.56	48.27 <sup>ef</sup> ± 0.70	44.57 <sup>gh</sup> ± 2.10
B225	42.53 <sup>hi</sup> ± 1.66	34.93 <sup>kl</sup> ± 2.90	27.60 <sup>n</sup> ± 1.11
LDMB11	60.00 <sup>b</sup> ± 1.91	56.00 <sup>c</sup> ± 1.97	51.00 <sup>def</sup> ± 1.85
D340	42.50 <sup>hi</sup> ± 1.30	38.47 <sup>jk</sup> ± 2.55	31.83 <sup>lm</sup> ± 1.84

945 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

946 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
947 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
948 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
949 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
950 D340.

951

952

953

954 **Table 4g** Effect of kerosene oil on exopolysaccharides emulsification index at 24, 48, and  
955 96 h.

Kerosene	E <sub>24</sub> % (Mean ± SD)	E <sub>48</sub> % (Mean ± SD)	E <sub>96</sub> % (Mean ± SD)
LDMB01	59.60 <sup>a</sup> ± 1.49	45.00 <sup>gh</sup> ± 1.67	39.00 <sup>i</sup> ± 1.51
D325	48.63 <sup>ef</sup> ± 5.36	38.00 <sup>jk</sup> ± 1.51	35.00 <sup>kl</sup> ± 1.01
LDMB05	56.60 <sup>ab</sup> ± 2.50	49.50 <sup>ef</sup> ± 1.57	49.43 <sup>ef</sup> ± 1.66
M540	33.00 <sup>l</sup> ± 3.66	28.50 <sup>m</sup> ± 2.44	20.50 <sup>n</sup> ± 1.67
LDMB10	54.00 <sup>bcd</sup> ± 1.25	51.60 <sup>de</sup> ± 0.92	43.90 <sup>hi</sup> ± 0.95
LDMB03	55.50 <sup>bc</sup> ± 3.54	52.43 <sup>cde</sup> ± 2.60	50.17 <sup>ef</sup> ± 1.35
M240	48.90 <sup>ef</sup> ± 1.41	41.00 <sup>ij</sup> ± 1.06	38.00 <sup>jk</sup> ± 0.87
B225	50.10 <sup>ef</sup> ± 1.10	39.23 <sup>i</sup> ± 1.91	26.40 <sup>m</sup> ± 1.65
LDMB11	57.50 <sup>ab</sup> ± 2.56	43.50 <sup>hi</sup> ± 1.41	40.50 <sup>ij</sup> ± 1.08
D340	47.50 <sup>fg</sup> ± 1.37	44.00 <sup>hi</sup> ± 0.92	39.50 <sup>i</sup> ± 1.37

956 <sup>a</sup>means of different superscripts significantly differ within row and column ( $p < 0.05$ ).

957 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
958 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
959 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
960 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
961 D340.

962



963 **Table 5** Emulsification index of different exopolysaccharides with various oils

View Article Online

DOI: 10.1039/D5FB00943J

EPS	Olive oil	Soybean oil	Mustard oil	Black cumin oil	Almond oil	n-hexane	Kerosene
LDMB01	66.42 <sup>b</sup>	51.19 <sup>e</sup>	53.43 <sup>c</sup>	65.69 <sup>e</sup>	48.72 <sup>e</sup>	48.73 <sup>c</sup>	47.87 <sup>c</sup>
D325	53.14 <sup>ef</sup>	50.74 <sup>e</sup>	46.84 <sup>f</sup>	58.20 <sup>f</sup>	52.20 <sup>d</sup>	40.53 <sup>d</sup>	40.54 <sup>e</sup>
LDMB05	56.12 <sup>d</sup>	56.16 <sup>bc</sup>	60.11 <sup>a</sup>	82.93 <sup>a</sup>	58.61 <sup>a</sup>	56.10 <sup>a</sup>	51.84 <sup>a</sup>
M540	55.40 <sup>de</sup>	53.68 <sup>d</sup>	49.38 <sup>de</sup>	54.50 <sup>gh</sup>	47.56 <sup>ef</sup>	53.88 <sup>b</sup>	27.33 <sup>g</sup>
LDMB10	41.61 <sup>g</sup>	47.83 <sup>f</sup>	41.67 <sup>g</sup>	53.33 <sup>h</sup>	46.83 <sup>f</sup>	35.49 <sup>f</sup>	49.83 <sup>b</sup>
LDMB03	63.56 <sup>c</sup>	54.38 <sup>cd</sup>	58.90 <sup>a</sup>	70.17 <sup>c</sup>	54.42 <sup>bc</sup>	56.61 <sup>a</sup>	52.70 <sup>a</sup>
M240	63.94 <sup>bc</sup>	54.64 <sup>bcd</sup>	56.98 <sup>b</sup>	68.41 <sup>d</sup>	52.53 <sup>d</sup>	48.10 <sup>c</sup>	42.63 <sup>d</sup>
B225	75.10 <sup>a</sup>	64.86 <sup>a</sup>	49.93 <sup>d</sup>	58.89 <sup>f</sup>	55.94 <sup>b</sup>	35.02 <sup>f</sup>	38.58 <sup>f</sup>
LDMB11	64.86 <sup>bc</sup>	56.32 <sup>b</sup>	56.50 <sup>b</sup>	80.17 <sup>b</sup>	59.00 <sup>a</sup>	55.67 <sup>ab</sup>	47.17 <sup>c</sup>
D340	52.00 <sup>f</sup>	49.83 <sup>e</sup>	47.83 <sup>ef</sup>	55.17 <sup>g</sup>	53.17 <sup>cd</sup>	37.60 <sup>e</sup>	43.67 <sup>d</sup>
<i>p</i> value	0.00	0.00	0.00	0.00	0.00	0.00	0.00

964 <sup>a</sup>values with different lowercase letters within a column indicate significantly differences ( $p <$   
 965 0.05).

966 <sup>b</sup>LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb.*  
 967 *plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10;  
 968 LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P.*  
 969 *pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis*  
 970 D340.

971

972

973

974 **Table 6** Effect of incubation time (24, 48, and 96 h) on emulsification index using  
 975 different oils.

Time	Olive oil	Soybean oil	Mustard oil	Black cumin oil	Almond oil	n-hexane	Kerosene
E <sub>24</sub>	63.51 <sup>a</sup>	60.26 <sup>a</sup>	57.33 <sup>a</sup>	65.44 <sup>a</sup>	57.62 <sup>a</sup>	52.32 <sup>a</sup>	51.13 <sup>a</sup>
E <sub>48</sub>	58.24 <sup>b</sup>	53.68 <sup>b</sup>	51.16 <sup>b</sup>	65.19 <sup>a</sup>	52.97 <sup>b</sup>	46.98 <sup>b</sup>	43.28 <sup>b</sup>
E <sub>96</sub>	55.89 <sup>c</sup>	47.94 <sup>c</sup>	47.98 <sup>c</sup>	63.60 <sup>b</sup>	48.10 <sup>c</sup>	41.01 <sup>c</sup>	38.24 <sup>c</sup>
<i>p</i> value	0.00	0.00	0.00	0.00	0.00	0.00	0.00

976 <sup>a</sup>values with different lowercase letters within a column indicate significantly differences ( $p <$   
 977 0.05).

978 <sup>b</sup>E<sub>24</sub> = Emulsification index at 24 h; E<sub>48</sub> = Emulsification index at 48 h; E<sub>96</sub> = Emulsification  
 979 index at 96 h.

980

981

982

983



984

## List of Figures

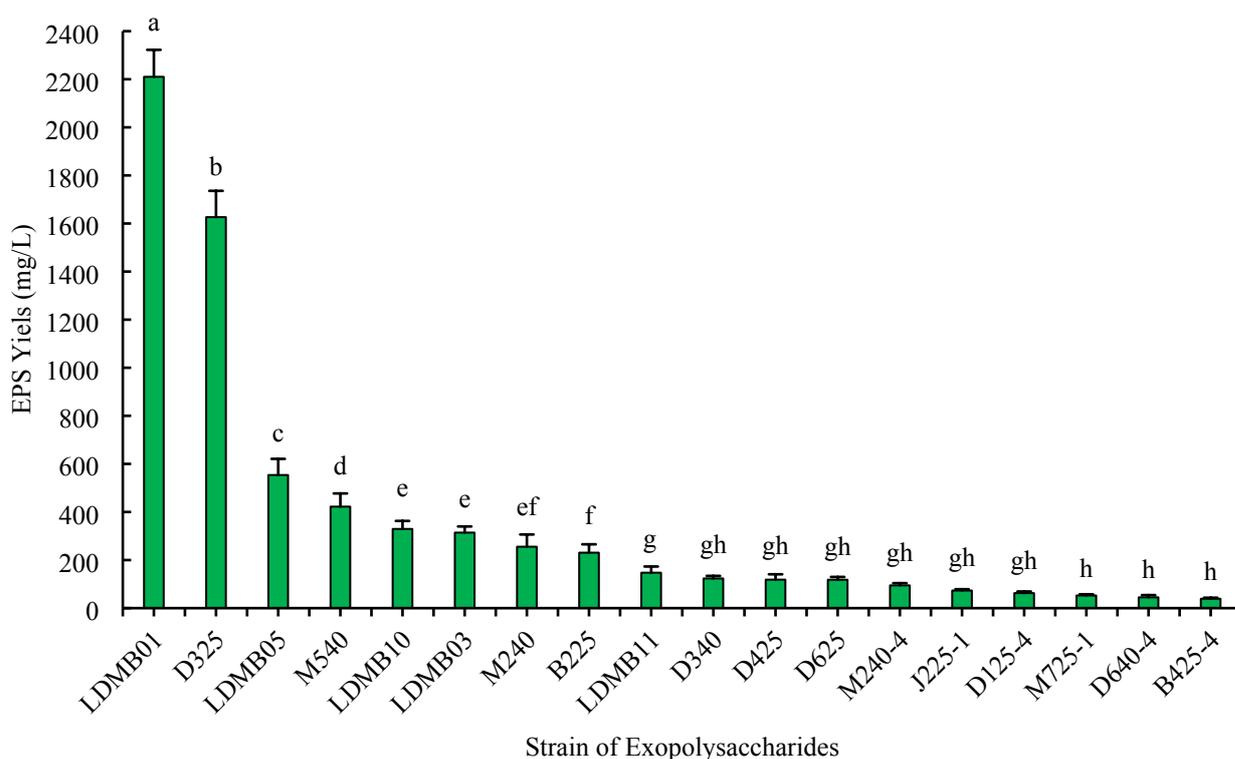
View Article Online  
DOI: 10.1039/D5FB00943J

985

986

**Fig. 1** String test of exopolysaccharides produced by bacterial strains.

987



988

**Fig. 2** Exopolysaccharides production by selected lactic acid bacterial strains. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis* D340; D425-4 = *Lc. lactis* spp. *lactis* D425-4; D625-3 = *Leu. mesenteroides* spp. *mesenteroides* D625-3; M240-4 = *Lb. fermentum* M240-4; J225-1 = *Str. bovis* biotype II J225-1; D125-4 = *Str. bovis* biotype I D125-4; M725-1 = *Leu. mesenteroides* subsp. *dextranicum* M725-1; D640-4 = *Lb. agilis* D640-4;

986



997 B425-4 = *Lc. raffinolactis* B425-4. Different letters atop the bars indicate significant difference  
 998 at  $p < 0.05$  (mean  $\pm$ SD).

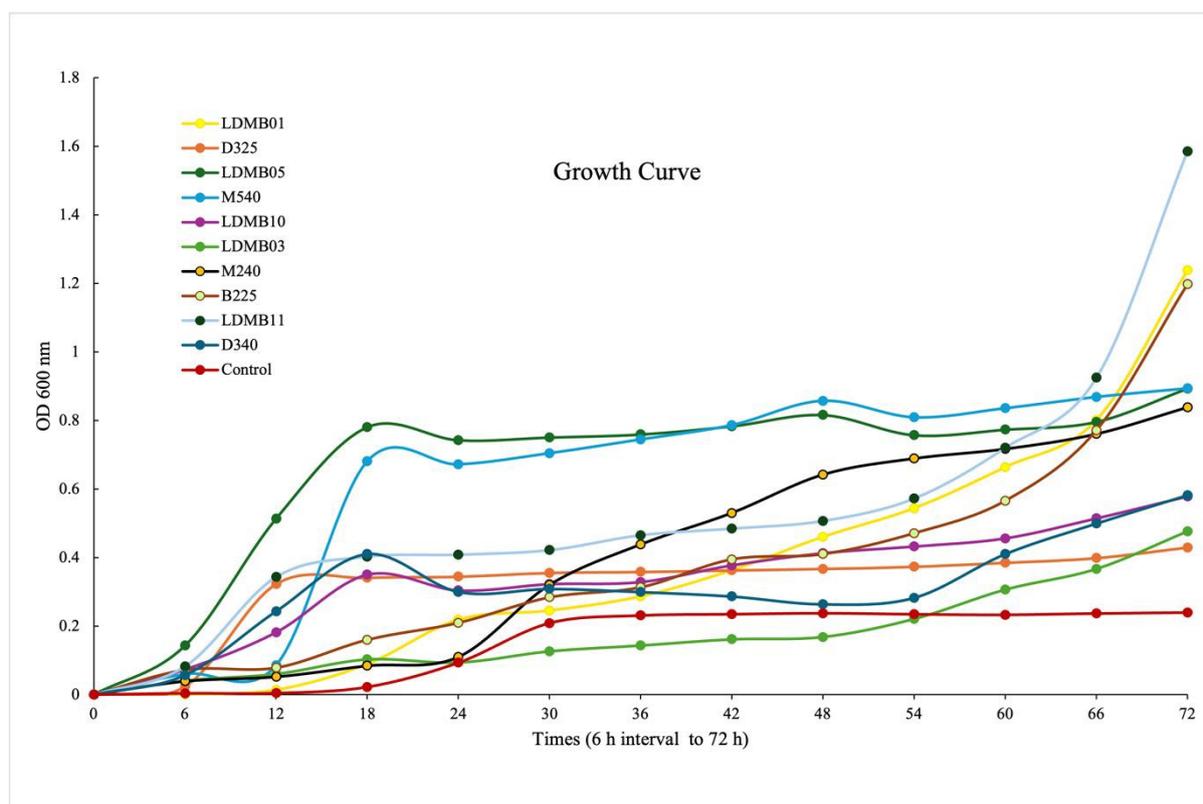
View Article Online

DOI: 10.1039/D5FB00943J

999

1000

1001



1002

1003

1004

1005

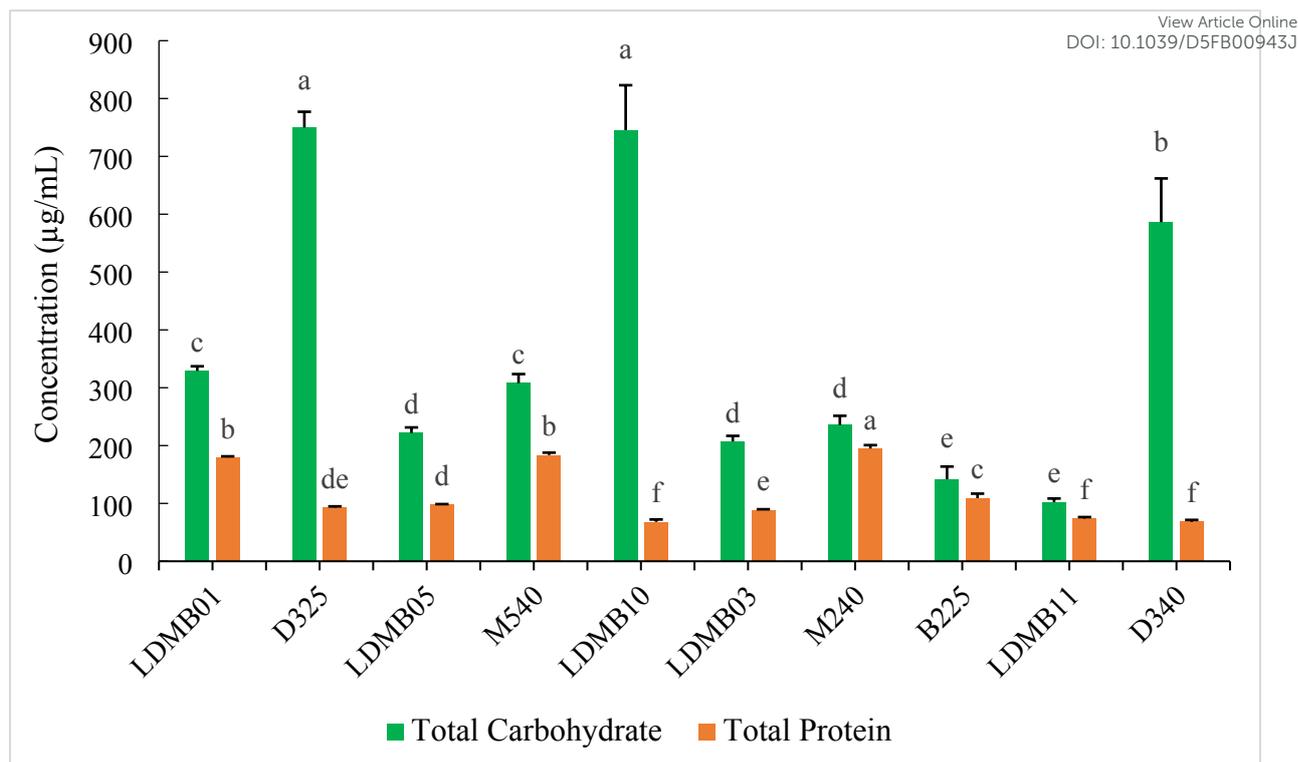
1006

1007

1008

**Fig. 3** Growth curve of exopolysaccharides producing strains and control strain during the 72 h growth period with 6 h intervals. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis* D340.





1009

1010

1011

1012

1013

1014

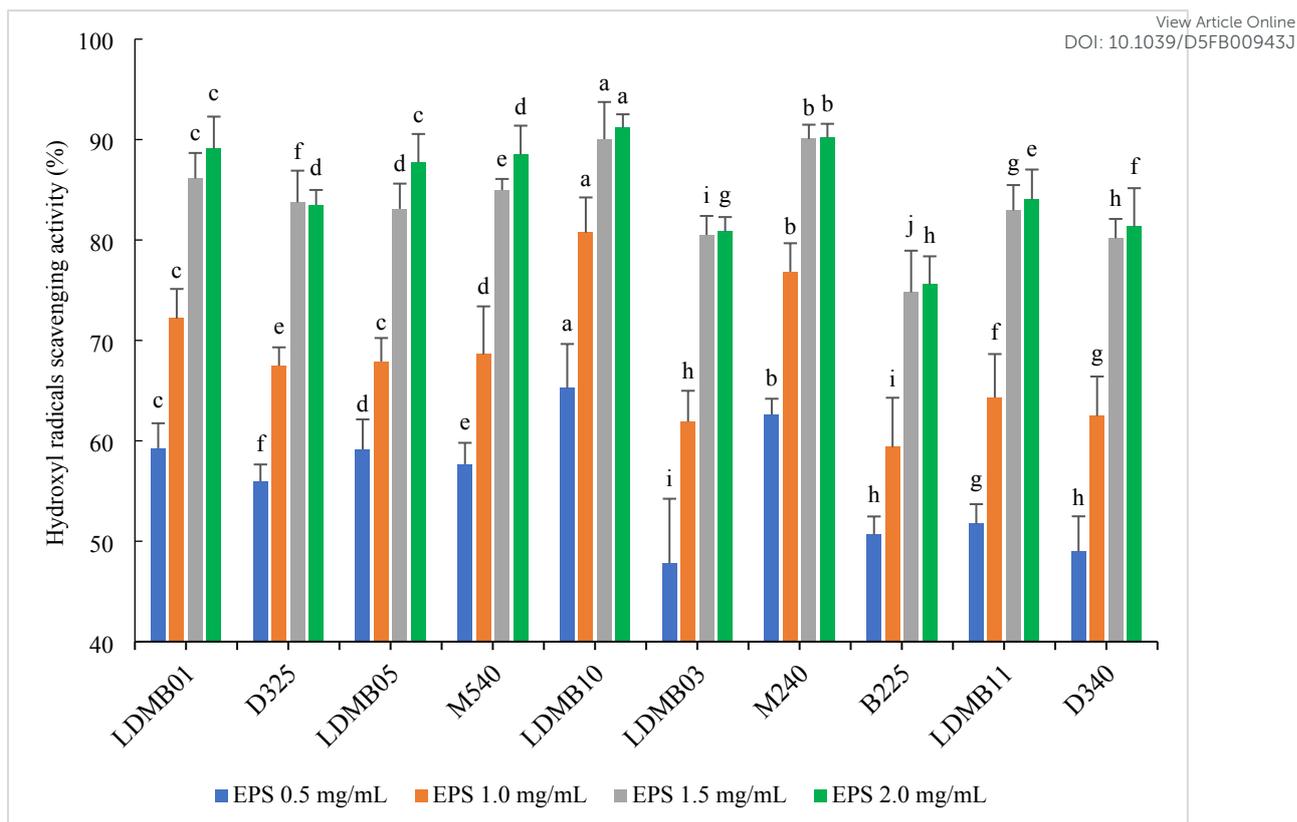
1015

1016

1017

**Fig. 4** Total carbohydrate and protein content (Mean  $\pm$  SD) of exopolysaccharides. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis* D340. Different letters among the bars in same category show significant difference ( $p < 0.05$ ).





1018

1019

1020

1021

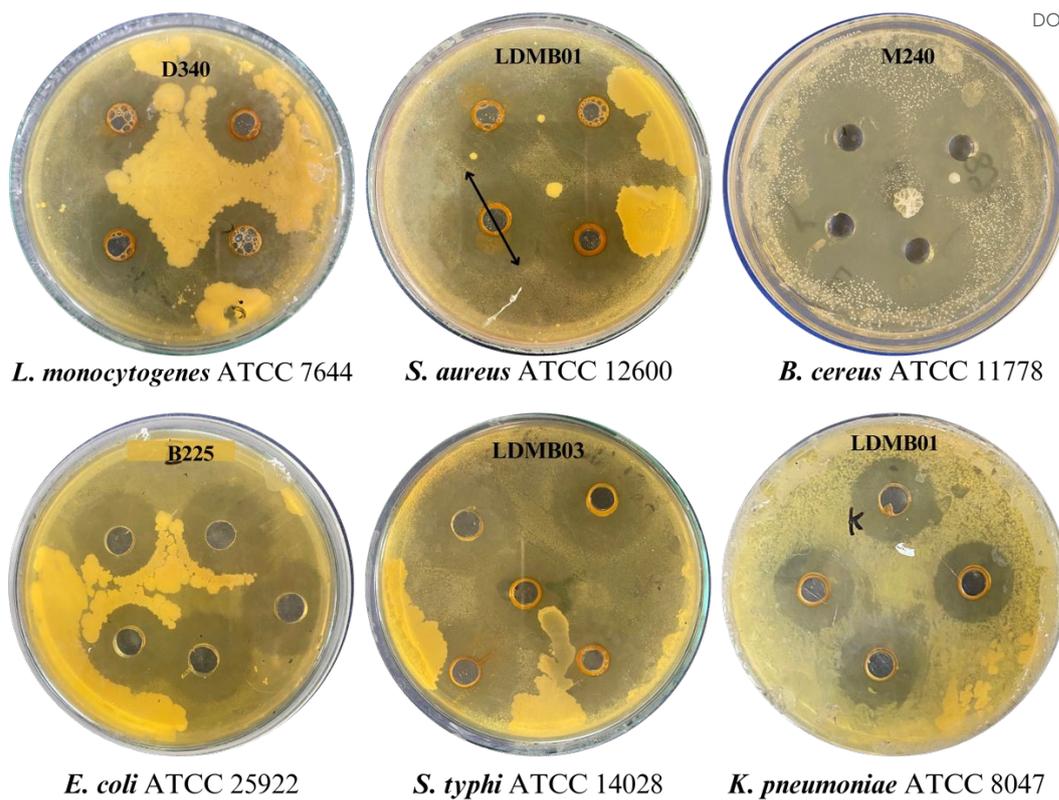
1022

1023

1024

**Fig. 5** Hydroxyl radical scavenging activity of different exopolysaccharides in different concentrations. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. del. subsp. bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. del. subsp. lactis* D340. Different letters atop the bars indicate significant difference at  $p < 0.05$  (mean  $\pm$ SD).





**Fig. 6** Antimicrobial activity of exopolysaccharides (EPS) from selected highest EPS producers using agar assay technique. D340 = *Lb. del.* subsp. *lactis* D340; LDMB01 = *Lb. acidophilus* LDMB01; M240 = *Lb. del.* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB03 = *Lb. casei* LDMB03.

1025

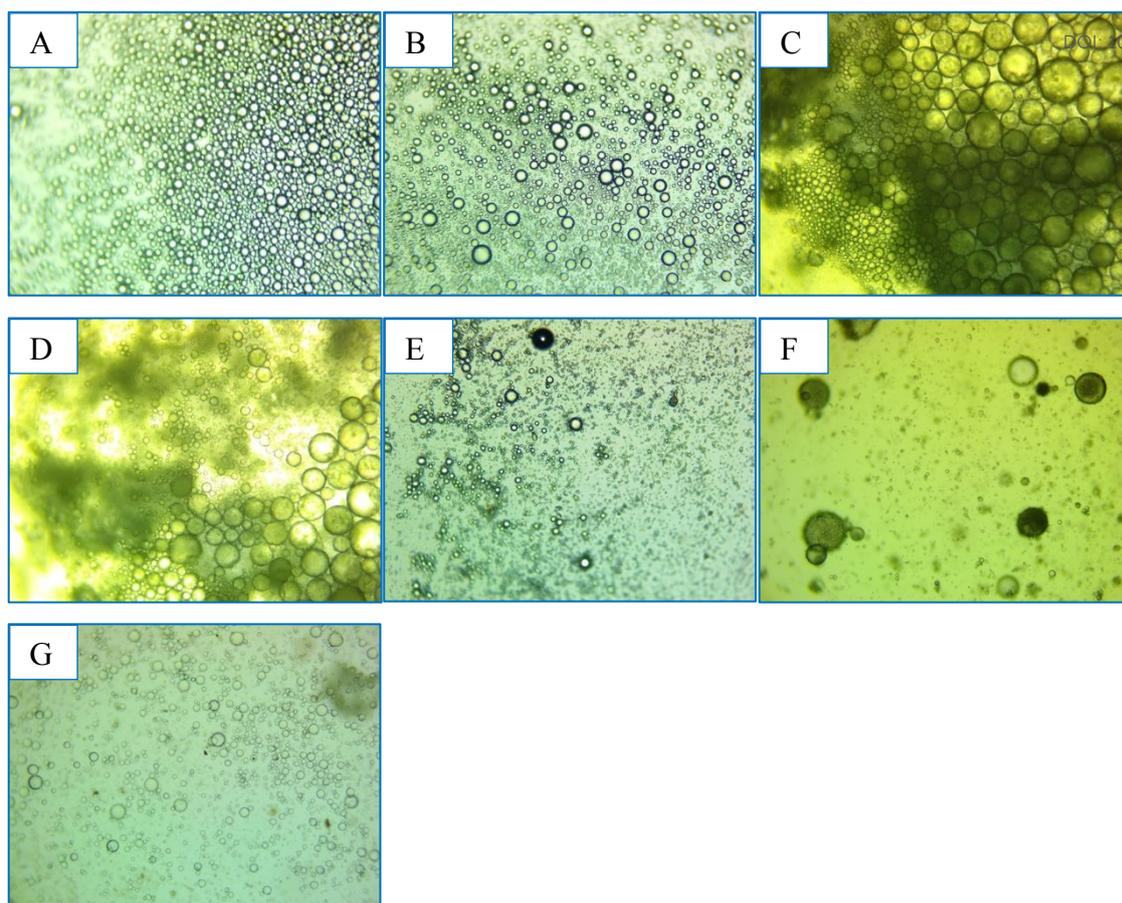
1026

1027

1028

1029



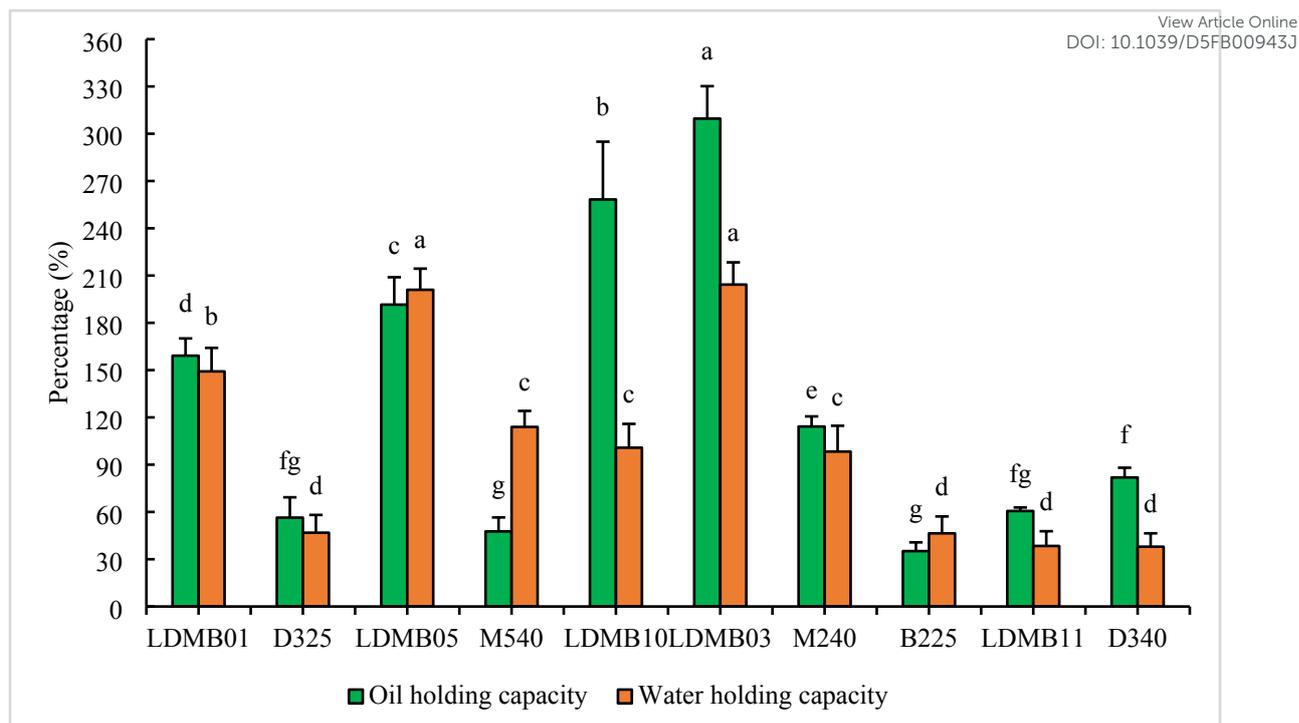


1030

1031 **Fig. 7** Optical microscopic (10×) image of exopolysaccharide (from *P. pentosaceus* B225) in  
1032 the emulsion with olive oil (A), soybean oil (B), mustard oil (C), black cumin oil (D), almond  
1033 oil (E), n-hexane (F), and kerosene oil (G).

1034





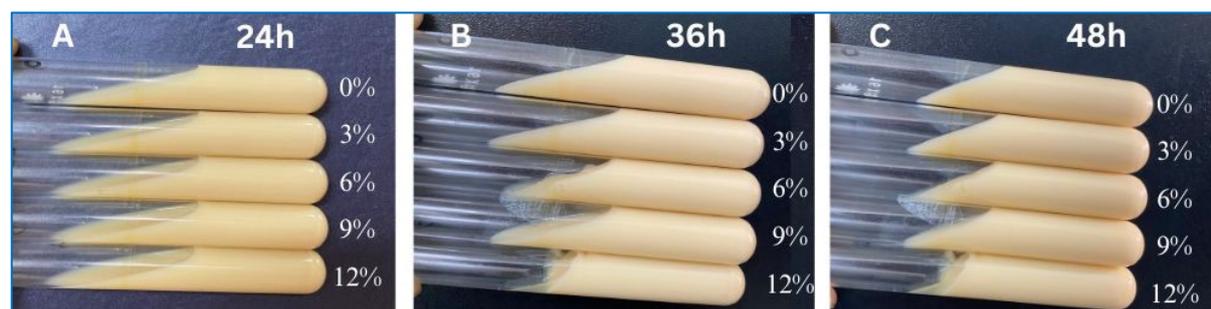
1035

1036 **Fig. 8** Oil and water holding capacity of exopolysaccharides. LDMB01 = *Lb. acidophilus*  
 1037 LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str.*  
 1038 *thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 =  
 1039 *Lb. del. subsp. bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum*  
 1040 LDMB11; D340 = *Lb. del. subsp. lactis* D340. Different letters atop the bars indicate a  
 1041 significant difference at  $p < 0.05$  (mean  $\pm$ SD).

1042

1043

1044

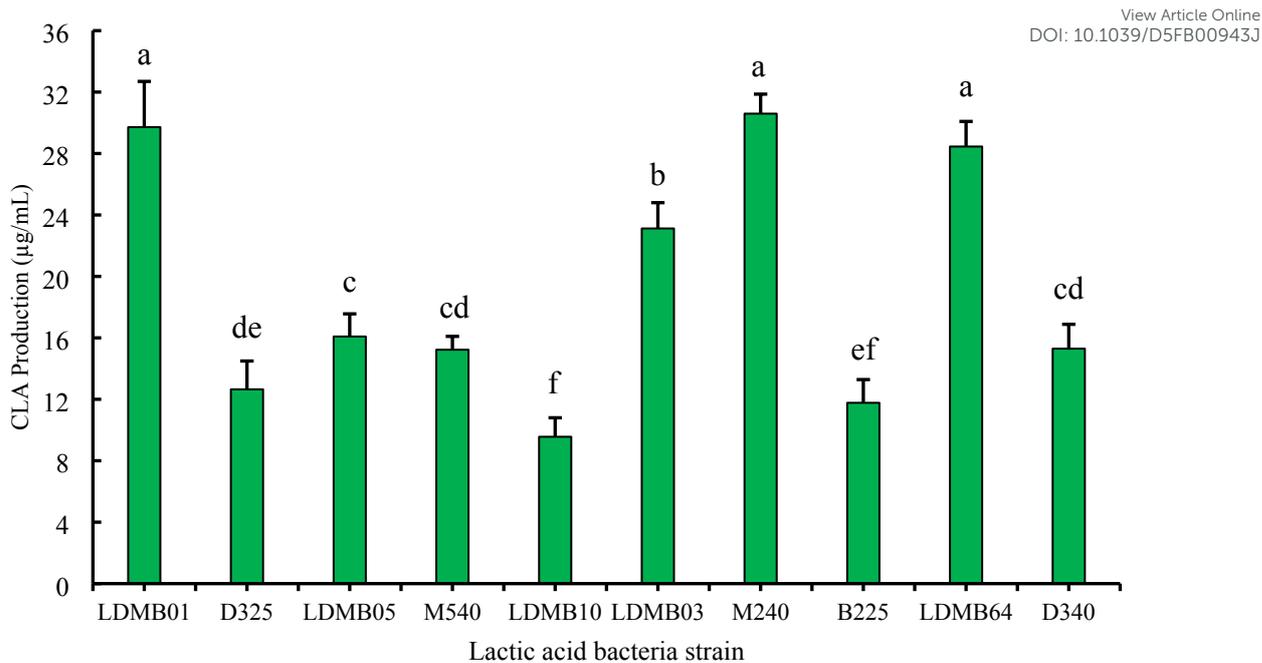


1045

1046 **Fig. 9** Coagulation effect of highest EPS producing strain (*Lb. acidophilus* LDMB01) on  
 1047 10% skimmed milk supplemented with varying sucrose concentrations (0, 3, 6, 9 and 12%) at  
 1048 24 h (A), 36 h (B), and 48 h (C) of incubation.

1049





1050

1051 **Fig. 10** Yield of conjugated linoleic acid (CLA) from selected lactic acid bacterial strains.  
 1052 LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum*  
 1053 LDMB05; M540 = *Str. thermophilus* M540; LDMB10 = *Lc. lactis* LDMB10; LDMB42 = *Lb.*  
 1054 *casei* LDMB42; M240 = *Lb. del. spp. bulgaricus* M240; B225 = *P. pentosaceus* B225;  
 1055 LDMB64 = *Lb. paraplantarum* LDMB64; D340 = *Lb. del. spp. lactis* D340. Different letters  
 1056 atop the bars indicate significant difference at  $p < 0.05$  (mean  $\pm$ SD).

1057



### Data Availability Statement

Data are available from the corresponding author upon reasonable request.

