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Exopolysaccharides and conjugated linoleic acids produced by selected lactic acid bacteria: physicochemical attributes, antimicrobial activity, and techno-functional properties of exopolysaccharides

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Lactic acid bacteria (LAB) exopolysaccharides (EPS) are high-value biopolymers that have predominant applications in the food sector owing to their thickening, gelling, stabilizing, texturizing, emulsifying, and flocculating properties, while conjugated linoleic acids (CLAs) have potential health benefits for humans. This study aims to screen and isolate bacterial strains based on their EPS and CLA production as well as to characterize the carbohydrate and protein content, water and oil holding capacity, emulsification, antimicrobial activity, and hydroxyl radical scavenging activity of EPS. In this study, 50 out of 300 bacterial strains stocked in the laboratory were screened using agar media, and 18 strains were selected based on their EPS production. Afterward, the CLA production and EPS techno-functional properties of the 10 bacterial strains with the highest EPS-producing capacity (*Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactiplantibacillus plantarum*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Lacticaseibacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Pediococcus pentosaceus*, *Lactobacillus paraplantarum*, and *Lactobacillus delbrueckii* subspp. *lactis*) were evaluated. The EPS production varied significantly, from 39.02 mg L^{-1} in *Lactococcus raffinolactis* B425 to 2210.18 mg L^{-1} in *Lactobacillus acidophilus* LDMB01. For CLA production, *Lactobacillus del.* spp. *bulgaricus* M240 exhibited the highest yield (30.60 μg mL^{-1}). In terms of techno-functional properties, *Enterococcus faecium* D325 had the highest carbohydrate content (749.89 μg mL^{-1}) while *Lactobacillus delbrueckii* subsp. *bulgaricus* M240 exhibited the highest protein content (194.87 μg mL^{-1}). *Lacticaseibacillus casei* LDMB03 demonstrated the highest water-holding (204.28%) and oil-holding (309.59%) capacity. The 10% EPS exhibited the highest antimicrobial activity against the tested pathogenic bacteria, even at relatively low concentrations. *Pediococcus pentosaceus* B225 had the strongest emulsification index while the highest hydroxyl radical scavenging activity (94.40%) was recorded for *Lactococcus lactis* LDMB10. These findings highlight the diverse functional properties of LAB-derived EPS, underscoring their potential for the development of functional foods, pharmaceuticals, and cosmetic products.

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Sustainability spotlight

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

1 Introduction

Lactic acid bacteria (LAB) are generally recognized as safe (GRAS), facultatively anaerobic or microaerophilic, non-motile, Gram-positive, catalase-negative, and non-spore-forming bacterial strains that produce organic acids, bacteriocins, and exopolysaccharides during their metabolic activities.[1] LAB metabolic activities vary significantly across genera and even strains within the same species owing to their genetic makeup and enzyme repertoires.[2] LABs are capable of producing microbial exopolysaccharides (EPS), which are non toxic

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antioxidants and high molecular weight biodegradable natural carbohydrate polymers. In addition to bacteria, yeasts, fungi, algae, and both eukaryotic and prokaryotic organisms with diverse biological activities have the potential to secrete EPS.[3–5] On the basis of its monosaccharide composition, EPS can be classified into homopolysaccharides and heteropolysaccharides.[6] According to Dueholm *et al.*,[7] EPS is mostly produced intracellularly, and different pathways are involved in its secretion. It is synthesized *via* any of the following four biosynthesis pathways: (1). the ATP-binding cassette (ABC) transporter-dependent, (2). Wzx/Wzy-dependent, (3). extracellular, and (4). synthase-dependent pathways.[8–10] The biosynthetic pathway involved in EPS production by LAB significantly influences the functional and structural properties of EPS. Structural (chemical and biomolecular) modifications of EPS can improve its yield and also enhance the desired physicochemical and biological activities.[6,10,11]

The molecules of *Leuconostoc mesenteroides*, *Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*, and *Streptococcus salivarius* produce alternan, dextran, reuteran, and mutan types of EPS *via* extracellular biosynthetic pathways; however, the properties of these compounds are significantly different. Similarly, the properties of commercial EPS, including that produced by *Xanthomonas campestris*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum*, are distinct, though these bacteria use the Wzx/Wzy-dependent pathway. Stingele *et al.*[12] mentioned that the ST11 and YS4 strains of *Str. thermophilus* is non-ropy while the *Str. thermophilus* Sfi6 strain is ropy. Differences exist even between *Str. thermophilus* Sfi6 and *Str. thermophilus* Sfi39, and the variation in their gene level is evident from the studies by Stingele *et al.*[12] and Germond *et al.*[13] In addition to the bacterial strain, exopolysaccharide production, structural characterization, biosynthesis, biological properties, physicochemical properties, and effective applications depend on the composition of the fermentation medium, including carbon, nitrogen, salts, and the condition of the culture, such as pH, time, and temperature.[14,15] The *Lactobacillus helveticus* MB2-1 strain, under its optimum growing conditions, produced 340 mg L^{-1} of EPS at an optimal temperature of 37 °C.[16] In contrast, under similar conditions (25% (w/w) dipotassium hydrogen phosphate, 30% (w/w) ethanol, 2% (w/w) 1-butyl-3-methylimidazolium octyl sulfate, and a pH of 10), the EPS yields of *Lactobacillus acidophilus* and *Limosilactobacillus reuteri* were found to be 63.30 μg mL^{-1} and 146.48 μg mL^{-1}, respectively.[17]

The industrial EPS production is costly, with 40% of expenses slated for microbial growth substrates.[18] The extraction of EPS from LAB with distinctive bioactivities is becoming progressively attractive, especially for industrial applications. They are increasingly used in agriculture, medicine, cosmetics, and food because of their beneficial properties, such as stability, solubility, viscosity, elasticity, water holding capacity, oil holding capacity, emulsification property, biocompatibility, gelation, and thickening agent.[15,19] During fermentation, the produced EPS enhances texture and flavor and provides health benefits.[14] *Streptococcus thermophilus* EPS is valuable in dairy, improving texture stability, handling tolerance, and melting property while reducing chewiness and hardness in low-fat Mozzarella cheese.[20] EPS-producing probiotics significantly enhance food safety, quality, shelf life, and functionality. This is because EPS enhances the rheological properties and water-holding capacity of food matrices, improves emulsion and foam stability, facilitates the formation of protective biofilms that suppress spoilage and pathogenic microorganisms, and functions as prebiotic and immunomodulatory compounds that confer additional health benefits.[19,21] LAB EPS regulates gut health, thereby influencing microbiome composition and short-chain fatty acid production, which impacts intestinal function and energy metabolism.[22] EPS also offers biological effects, including anti-cholesterol, antibacterial, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antiviral, antioxidant, hypoglycemic, immunomodulatory, and anti-*Helicobacter pylori* activities.[23–25] *Lactobacillus plantarum* KX041 EPS favors molecular mechanisms against inflammation and obesity[26] and *Lactiplantibacillus plantarum* LCC-605 increases the stability of fermented yoghurt, as well as antioxidant and cholesterol-reducing abilities.[27]

Like EPS, conjugated linoleic acid (CLA) has received significant attention as a health-promoting compound that prevents atherosclerosis, regulates gut microbiota and immunity, reduces fat or lipid deposition, promotes bone formation, and exerts anti-cardiovascular, antioxidant, anti-obesity, anti-diabetic, and anti-carcinogenic effects.[28–31] According to Wu *et al.*,[32] CLA can enhance insulin signalling, fatty acid oxidation, and glucose transport in adipose tissue or the liver. Moreover, EPS-producing LAB have the ability to convert free linoleic acid (LA) into conjugated linoleic acid.[33,34] Among LAB, it has been found that many food-grade LAB strains, including *Lactobacillus lactis*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* can produce CLA in yogurt, cheese, and milk products.[34]

Given the variability among LAB strains and their diverse metabolic end products, it was hypothesized that the LAB selected for this study would exhibit different potentials for EPS production with distinct physicochemical and techno-functional properties of the produced EPS. Similarly, the selected LAB strains were expected to differ in their CLA production capabilities, which was considered an additional health and well-being-promoting feature of EPS-producing LAB. Therefore, the aim of the study was to screen EPS-producing LAB strains from the selected LAB lot, evaluate the produced EPS, and assess the CLA production potential of the selected EPS-LAB. The set objectives were to quantify the EPS and CLA produced by the screened LAB strains, followed by monitoring, recording, and assessing the physicochemical and techno-functional properties of the produced EPS, *viz.*, total carbohydrate and protein content, hydroxyl radical scavenging activity, antimicrobial activity, emulsification index, oil-holding capacity, water-holding capacity, and solidification ability of EPS. It is anticipated that the outcome of this study will provide valuable insights into the techno-functional properties of EPS produced by bacterial strains and their potential applications in the food industry, especially with regard to product development and the well-being of consumers.

2 Materials and methods

2.1. Selection of bacteria and processing

Bacterial strains were obtained from the Laboratory of Dairy Microbiology and Biotechnology (LDMB), which comprised 266 freeze-dried lactic acid bacteria (LAB) that were isolated from traditionally fermented Dahi in Bangladesh[35,36] and 34 LAB strains isolated from raw goat milk and eventually characterized.[37] From the 300 LAB strains, 50 LAB strains were chosen. Stock cultures were made in MRS broth (de Man, Rogosa and Sharpe; HiMedia, India), followed by subculturing and eventual storage at −20 °C for further use.

2.2. Screening of EPS-producing strains

The ability of the isolates to produce EPS was assessed using the string test, Aniline Blue (AB) agar assay, and Congo Red (CR) agar assay.[38] For the string test, the strains were cultured on Luria Bertani (LB) agar (HiMedia, India) plates supplemented with 5% (w/v) sucrose at 37 °C for 48 h. A colony was lifted using an inoculation loop, and those forming a ≥5 mm string were selected. In the AB agar assay, nutrient agar (HiMedia, India) was supplemented with 5% (w/v) sucrose and 0.005% (w/v) aniline blue (SRL, India). EPS-producing isolates showed slightly blue colonies on the AB agar plate. Brain-heart infusion agar (HiMedia, India) was supplemented with 5% (w/v) sucrose and added to 0.08% (w/v) Congo red (HiMedia, India) to make the CR agar. Light to dark black colonies indicate potential EPS-producing strains. Both the AB and CR plates were incubated at 37 °C for ∼48 h. Strains that passed at least any one of the aforementioned assays (a total of 18 strains passed) were considered for subsequent steps.

2.3. Production, extraction, and quantification of EPS

The screened EPS-producing isolates were incubated for 72 h at 37 °C in MRS broth supplemented with 5% (w/v) sucrose on a constant orbital shaker (SCI Finetech, Korea) at 120 rpm. During this period, optical density $(OD)_{600}$ was measured every 6 h using an i9 Hanon UV-VIS double beam spectrophotometer at 600 nm.[38,39] After incubation, the samples (50 mL) were heated in a water bath (Daihan Scientific Bath, Korea) at ∼100 °C for 50 min and then cooled to 25 °C. They were then centrifuged at 5500×*g* (Neya 16R, Remi, India) for 20 min at 4 °C, and the supernatants were collected. Ice-cold ethanol was added to the supernatants (2 : 1), and the mixtures were incubated overnight at −20 °C. Then, crude EPS precipitation was collected after centrifuging the supernatant–ethanol mixtures at 5500×*g* for 20 min at 4 °C. Eventually, they were dried overnight at 40 °C (BOD incubators, MRC Lab, Holon, Israel). The dry EPS weight and yield were recorded and calculated, respectively. Crude EPS was purified by redissolving in distilled water, dialyzing with changing water, and lyophilizing.

2.4. Functional properties of EPS

2.4.1. Total carbohydrate and protein content of EPS. Ten predominant strains were selected based on their high EPS production. The phenol-sulfuric acid method was used to evaluate the total carbohydrate content in the ten crude EPS samples.[40] Briefly, 1% crude EPS solution was made in 0.5 mL of distilled water. A phenol (Merck, Germany) solution (6% w/v; 0.5 mL) and 2.5 mL of concentrated H_2SO_4 were added to the EPS solution. This mixture was incubated at room temperature for 20 min. Absorbance was then recorded at 490 nm (Multiskan SkyHigh Spectrophotometer, Thermo Scientific, USA). Distilled water was the blank sample, and glucose (Merck, India) was used as the standard (0–100 μg mL^{-1}; 20 μg mL^{-1} intervals).

To estimate the total protein content, the Bradford micro-assay protocol was used. The reaction solution was made of 0.5 mL of EPS solution and 0.5 mL of the Bradford protein assay reagent (Bio-Rad Quick Start™, USA). After incubation for 5 min at room temperature, absorbance was recorded at 595 nm. Distilled water and bovine serum albumin (BSA) were used as the blank and standard (0–100 μg mL^{-1}; 20 μg mL^{-1} intervals), respectively.

2.4.2. Hydroxyl radical scavenging ability of EPS. The hydroxyl radical scavenging ability was assessed using salicylic acid methods described by Yang-Chen *et al.*,[39] with a few modifications. In short, a salicylic acid–ethanol solution and $FeSO_4$ solution (0.4 mL of 9 mmol L^{-1} solutions of each) were mixed separately with 0.4 mL of different concentrations (0.5, 1.0, 1.5, and 2.0 mg mL^{-1}) of purified EPS in distilled water. This was followed by thorough mixing and the addition of 0.4 mL of 8.8 mmol L^{-1} H_2O_2 in distilled water. Finally, after incubation at 37 °C for 20 min, the absorbance was recorded at 510 nm. L-ascorbic acid was used as a control, and distilled water served as a blank. The hydroxyl radical-scavenging activity rate was calculated using the following formula:

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

where A_1 = absorbance of the EPS sample solution, A_0 = absorbance of the blank, and A_2 = absorbance of the control (distilled water was used instead of H_2O_2).

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

2.4.4. Emulsification index of EPS. The biosurfactant activity of the in-situ-produced crude EPS was assayed using the

emulsification index at 24 h (E_{24}), 48 h (E_{48}), and 96 h (E_{96}), following a method described by Ge *et al.*,[16] with a few modifications. Briefly, 2 mL of 1% (w/v) EPS in distilled water and an equal volume of different oils (olive oil/soybean oil/mustard oil/black cumin oil/almond oil/kerosene) or *n*-hexane were added separately and vortexed for 5 min. The mixture was allowed to stand for 24, 48, and 96 h. The emulsification index was calculated using the following formula:

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

An optical microscope was used to analyze the particle-size distribution of the emulsions that were formed, as previously described by Kowsalya *et al.*[41] Briefly, 60 μL of each emulsion was placed on a cavity slide and left to stand undisturbed for 5 min. The distribution of oil droplet sizes was observed under a light microscope at 10× magnification.

2.4.5. Water holding capacity of EPS. The water-holding capacity of the EPS derived from bacterial strains was assessed using a method described by Qamar *et al.*[42] Dried crude EPS samples were mixed with double-distilled water (500 mg mL^{-1}) and vortexed. Then, the samples were placed on an orbital shaking platform at 150 rpm for 30 min at room temperature, followed by centrifugation for 20 min at 3500 rpm (Rotor A-12-5 Model, Remi NEYA 16R). The supernatants were discarded, and the remnants were weighed. The water-holding capacity was calculated using the formula stated below:

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

2.4.6. Oil holding capacity of EPS. The oil retention ability of 10 crude EPS extracts was evaluated according to Qamar *et al.*[42] Dried crude EPS was mixed with cottonseed oil (500 mg mL^{-1}) and thoroughly vortexed for 2 min. The mixture was then left at room temperature for approximately 35–40 min with mild agitation (on an orbital shaker, 120 rpm) every five min. Followed by centrifugation at 3500 rpm for 20 min at 20 °C, the supernatants were removed and weighed for quantification. The following formula was used to calculate the oil-holding capacity:

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

2.5. Milk coagulation test of EPS strains

The strain *Lb. acidophilus* LDMB01, with the highest EPS production, was introduced into a sterile skim milk solution (w/v; 1 : 10). The inoculum used was 1% (v/v), and different amounts of sucrose (0%, 3%, 6%, 9%, or 12% w/v) were added. A static culture was created for 24, 36, and 48 h at 30 °C. Subsequently, the morphology of the skim milk was observed at 12 h intervals starting from 24 h of incubation. A negative control was prepared using skim milk containing 0% sugar.[5]

2.6. Physiochemical properties of selected EPS-LAB strains

2.6.1. Carbohydrate fermentation. The carbohydrate fermentation properties of the strains were examined in MRS broth without glucose as per the protocol of Silva *et al.*[43] Briefly, 14 sugars, namely cellobiose, dextrose, fructose, galactose, glucose, lactose, maltose, raffinose, rhamnose, sorbitol, starch, sucrose, trehalose, and xylose, were used. Carbohydrate solutions (5%, w/v) and bromocresol purple (pH indicator; 0.025% w/v) were prepared. Both solutions were passed separately through a sterilized 0.22 μm Millipore membrane syringe filter. Except for glucose, each sugar and broth medium was sterilized at 121 °C for 15 min. Afterward, 0.5 mL of the sterile sugar, 0.12 mL of the pH indicator, and 50 μL of the active culture were inoculated into a 4.5 mL broth in a screw-cap tube and fermented at 37 °C for 48 h. A positive result was indicated by a color change to yellow or light brown, while non-fermenting tubes remained unchanged.

2.6.2. Biochemical tests. Methyl red test and oxidase test were performed using the tuning method reported by Raj *et al.*[17] For the methyl red test, a buffer solution was prepared by mixing peptone, dextrose, and dipotassium phosphate, and the final pH was adjusted to 6.9 ± 0.2. The pure culture was inoculated into 5 mL of this solution, followed by the addition of 5–6 drops of methyl red (MR), incubation at 37 °C for 48 h and observation for color change. Red indicates a positive test result, and yellow meant negative.

For the oxidase test, the organisms were grown on a glucose agar plate (1% glucose), which was incubated at 37 °C for 24 h. 1% tetramethyl-*p*-phenylenediamine dihydrochloride was dissolved in distilled water. A few drops of this solution were placed on filter paper (Whatman filter paper, No.1), and a fresh colony was transferred onto it using a cotton bud. A color change within 10–30 seconds indicated a positive result; no change means negative. For the catalase test, a sterile loop was used to transfer a 24 hours culture onto a microscopic slide, and one drop of 30% H_2O_2 was added to it. Immediate bubble formation indicated a positive result, showing oxygen release.[44]

For pectin hydrolysis, a medium was prepared using the method of Ortega-Villar *et al.*, with a few modifications.[45] At first, a 100 mM phosphate buffer (pH = 7.0) was prepared with 0.4% (w/v) KH_2PO_4, 0.6% (w/v) NaH_2PO_4, and $Na_2HPO_4 \cdot 7H_2O$, and 0.5% pectin, 0.2% $(NH_4)_2SO_4$, 0.2% yeast extract, 0.2% $MgSO_4 \cdot 7H_2O$, 0.0001% $CaCl_2$, and 0.05% NaCl were added. This was followed by pH adjustment (to 7.0), inoculation of organisms, and incubation at 37 °C for 72 h. A halo around the colony was interpreted as positive for pectin hydrolysis.

2.6.3. Proteolytic activity. Proteolytic activity was evaluated on milk agar using a method described by Rampanti *et al.*[46] Briefly, milk agar was prepared by supplementing Plate Count Agar with 1% skim milk powder. The agar plates with the transferred inoculum were incubated for 48 h at 37 °C, and

colonies that formed a transparent halo were identified as positive indicators of proteolytic activity.

2.6.4. Lipolytic activity. To evaluate lipolytic activity, bacterial isolates were cultured overnight in MRS broth at 30 °C. Afterward, tributyrin agar (Merck) was prepared by adding 10 μL of fresh culture and 10 mL L^{-1} of glycerol tributyrate. Subsequently, the agar plates were incubated at 30 °C for 5 days and examined daily for the presence of halo formation around the colonies. The threshold halo diameter to declare the lipolysis positive is 2–3 mm.[46]

2.7. Screening of LAB for conjugated linoleic acid production

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

2.8. Statistical analysis

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

3 Results and discussion

3.1. EPS screening and production

Lactic acid bacteria genera, such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella*, and *Enterococcus*, can naturally synthesize diverse forms of glycans or polysaccharides. LAB account for the largest EPS-producing bacteria.[21] The preliminary screening in this study was based on their potential for EPS production. 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

Table 1 Screening and selection of exopolysaccharide (EPS)-producing lactic acid bacterial strains, with 18 high-EPS producers from a total of 50 strains[a,b]

Isolates	String test	Aniline blue (AB) assay	Congo red (CR) assay
Str. bovis biotype I D125-4	—	—	+
Ent. faecium D325-2	+ +	—	+ +
Lb. delbrueckii subsp. *lactis* D340-2	—	—	+
P. pentosaceus B225-2	+ +	+	+ + +
Leu. mesenteroides subsp. *mesenteroides* D625-3	—	—	+
Lb. agilis D640-4	—	+	—
Lc. raffinolactis B425-4	+	+	+ +
Lc. lactis subsp. *lactis* D425-4	+	+ + +	+
Lb. fermentum M240-4	+ + +	—	—
Lb. delbrueckii subsp. *bulgaricus* M240-5	+ + +	+ + +	+ + +
Str. thermophilus M540-5	—	—	+
Str. bovis biotype II J225-1	+ + +	—	+
Lc. lactis LDMB10	—	—	+ + +
Lb. casei subsp. *casei* LDMB03	+ +	+ +	+ + +
Lpb plantarum LDMB05	+ + +	—	+ + +
Lb. paraplantarum LDMB11	—	+	+ + +
Lb. acidophilus LDMB01	+ + +	+ + +	+ +
Leu. mesenteroides subsp. *dextranicum* M725-1	+ + +	+ + +	+ +

[a] Strong positive: + + +; moderate positive: + +; weak positive: +; and negative: −. [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. delbrueckii* subsp. *lactis* D340; D425-4 = *Lc. lactis* subsp. *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; and B425-4 = *Lc. raffinolactis* B425-4.

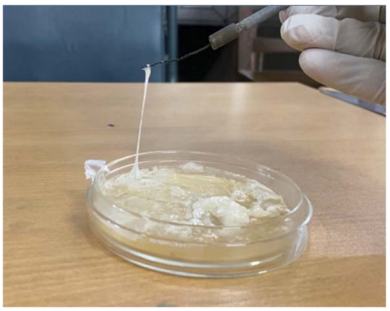

Fig. 1 String test of the exopolysaccharides produced by bacterial strains.

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, 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 a 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 delbrueckii* subsp. *bulgaricus* M240-5, *Pediococcus pentosaceus* B225, *Lactobacillus paraplantarum* LDMB11, and *Lactobacillus delbrueckii* subsp. *lactis* D340, *Streptococcus bovis* biotype I D125-4, *Leuconostoc mesenteroides* subsp.*mesenteroides* D625-3, *Lactobacillus agilis* D640-4, *Lactococcus raffinolactis* B425-4, *Lactococcus lactis* subsp. *lactis* D425-4, *Streptococcus bovis* biotype II J225-1, *Leuconostoc mesenteroides* subsp. *dextranicum* M725-1 and *Lactobacillus fermentum* M240-4, were selected based on their high EPS production.

The EPS production of the 18 selected strains varied from 39.02 ± 4.25 mg L^{-1} to 2210.18 ± 112.14 mg L^{-1}, where the LDMB01 strain showed a maximum EPS-production capacity (Fig. 2). Liu *et al.*[48] reported that *Lb. acidophilus* produced around 2920 mg EPS L^{-1}, while in the present study, *Lb. acidophilus* LDMB01 generated 2210.18 mg L^{-1}. 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.*[48] used *Lb. acidophilus* ATCC mutan, which was obtained from the 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.*[49] stated that the carbon source is one of the predisposing factors in EPS synthesis by LAB. This was also evident in a report by Padmanabhan *et al.*[50] who found that *S. thermophilus* ASCC 1275 produced approximately 430 mg L^{-1} of EPS when grown in a medium supplemented with sucrose, which was higher than the EPS yields with glucose or lactose. Afreen *et al.*[51] used the Plackett–Burman design in their study and identified lactose, yeast extract, tryptone, and $CaCl_2$ as factors for higher EPS production. D325-2 produced the second-highest EPS (1626.51 mg L^{-1}), followed by LDMB05 (553.80 mg L^{-1}), M540-5 (422.34 mg L^{-1}), and LDMB10 (329.48 mg L^{-1}). D425 and LDMB03 produced 118.53 and 314.13 mg L^{-1} of EPS, respectively. These findings are also supported by Prete *et al.*,[52] who reported that *Lc. lactis* spp. and *Lac. casei* demonstrated EPS production levels ranging from 30 to 600 mg L^{-1}. Moreover, EPS production levels of 94.76 and 56.48 mg L^{-1} were observed in *Lb. fermentum* M240-4 and *S. bovis biotype* II J225-1, respectively.

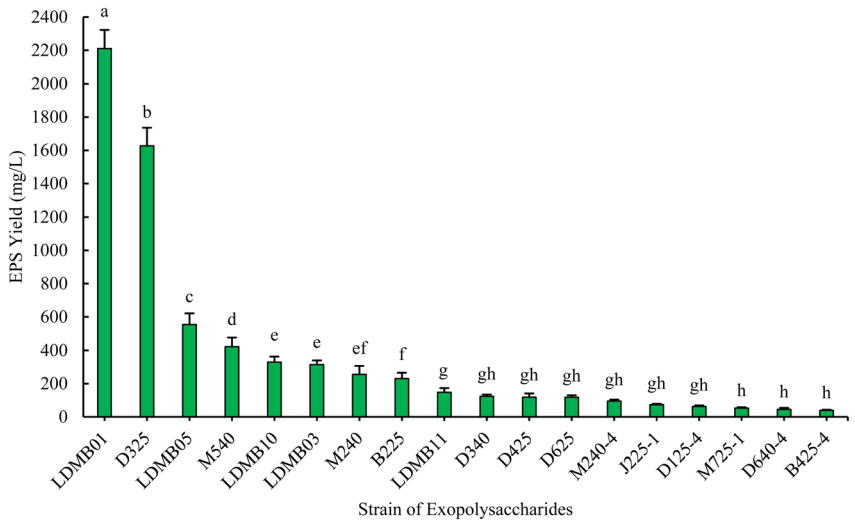

Fig. 2 Exopolysaccharide production by the selected lactic acid bacterial strains. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; D340 = *Lb. delbrueckii* subsp. *lactis* D340; D425-4 = *Lc. lactis* subsp. *lactis* D425-4; D625-3 = *Leu. mesenteroides* subsp. *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; and B425-4 = *Lc. raffinolactis* B425-4. Different letters atop the bars indicate a significant difference at $p < 0.05$ (mean ± SD).

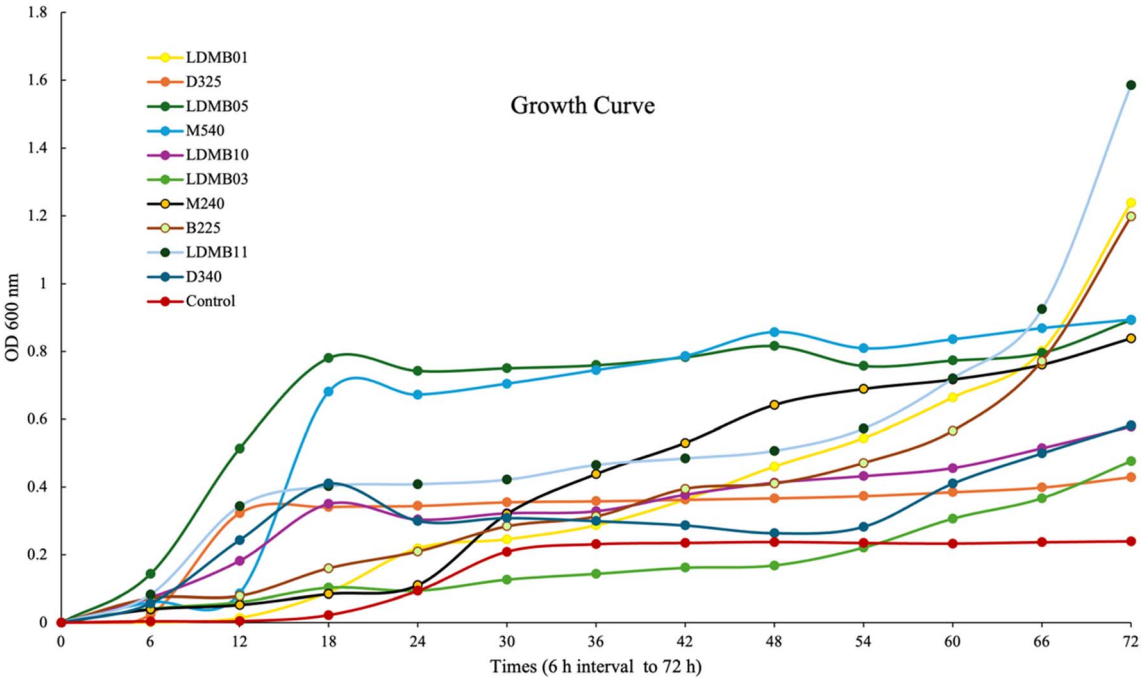

Fig. 3 Growth curves of the exopolysaccharide-producing strains and control strain during the 72 h growth period with 6 h intervals. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

The growth curves of selected EPS strains are presented in Fig. 3. Starting from 18 h up to 66 h, the growth performances of LDMB05 and M540 were higher than those of the other strains. However, after 66 h, the LDMB11, LDMB01, and B225 strains exhibited higher growth. From 60 h onward, all selected strains had higher growth compared to the control, which could have been due to the carbon source in the growth media and orbital shaking during incubation. According to Bates *et al.*,[53] orbital shaking exerts effects on microbial growth through impacts on the temperature and aeration control, the two major variables in microbial growth kinetics. All strains were found to show their maximum growth potential at 72 h. EPS production ability depends on various critical elements affecting bacterial growth, such as relative environment, incubation time, temperature, pH, oxygen rate, optimized culture conditions, and carbon and nitrogen sources, with the quantity of EPS produced being predominantly strain-specific.[54] So, orbital shaking may enhance nutrient distribution, create a homogeneous environment, prevent biofilm formation, and enhance growth rates by promoting optimal cell proliferation and metabolic activity.

3.2. Biochemical properties of selective strains

It is worth noting that the isolates were selected based on their stock strain identity, observing the standard biochemical and morphological characteristics and their fermentation with the 14 sugars (Table 2). All the strains were Gram-positive, non-motile, oxidase, catalase, and pectin hydrolysis test-negative. Proteolytic activity was negative in M540. Similar to our findings, positive proteolytic activity had been reported previously by Dong *et al.*[55] in *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lc. Lactis*, *Lb. casei*, *Lb. acidophilus*, *P. pentosaceus*, and *Lb. rhamnosus* strains. All the screened EPS-producing LABs were able to utilize glucose, dextrose, galactose, maltose and fructose, except M240. None of the LABs were able to utilize sorbitol, rhamnose, and starch, even LDMB01, the highest EPS producer. Afreen *et al.*[51] screened *Lactiplantibacillus paraplantarum* NCCP 962 as a high EPS producer from the *doi* samples and reported their ability to utilize D-galactose, D-lactose, D-sucrose, D-mannose, D-maltose, D-fructose, and D-arabinose, but it was not able to utilize D-sorbitol, D-ribose, and D-xylose as carbon sources.

3.3. Functional properties of EPS

3.3.1. Total carbohydrate and protein content. Fig. 4 shows the total carbohydrate and protein content of selected EPS-producing LAB strains. There was a significant ($p < 0.05$) variation in the total carbohydrate and protein contents among the EPS samples. The carbohydrate and protein contents of EPS varied from 749.89 ± 27.25 to 102.06 ± 6.39 μg mL^{-1} and 194.87 ± 5.92 to 68.03 ± 4.2 μg mL^{-1}, respectively. The highest carbohydrate content was found in D325 (749.89 μg mL^{-1}), followed by LDMB10 (745.39 μg mL^{-1}) and D340 (586.31 μg mL^{-1}), and the lowest was recorded in LDMB11 (102.06 μg mL^{-1}). On the other hand, the highest protein content was observed in M240 (194.87 μg mL^{-1}), followed by M540 (183.55 μg mL^{-1}), then LDMB01 (179.51 μg mL^{-1}), and, being the lowest, LDMB10 exhibited almost one-third that of M240. This variation in the carbohydrate and protein content is consistent

Table 2 Biochemical characteristics of the selected lactic acid bacterial strains[a,b]

Parameters	D340	M240	LDMB11	LDMB05	LDMB10	LDMB03	B225	M540	D325	LDMB01
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	+	±	—	+	+

[a] Positive (+); negative (−); variable (±); and weak positive (+w). [b] 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. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

with the findings of Tarannum *et al.*,[38] who reported variable carbohydrate contents in EPS from different LAB strains isolated from cow milk. However, the carbohydrate and protein contents of EPS seem higher in the present study. In the crude EPS of all bacterial strains in the current study, the total carbohydrate content was higher than the total protein content. This also aligns with the findings of Tarannum *et al.*,[38] who reported 594.91 μg mL^{-1} as the highest carbohydrate content and 119.28 μg mL^{-1} as the highest protein content in the crude EPS. This is further supported by Arayes *et al.*,[56] who found higher carbohydrate content in the EPS of *Alkalibacillus* sp. w3 than the protein content. The purity of EPS is inversely

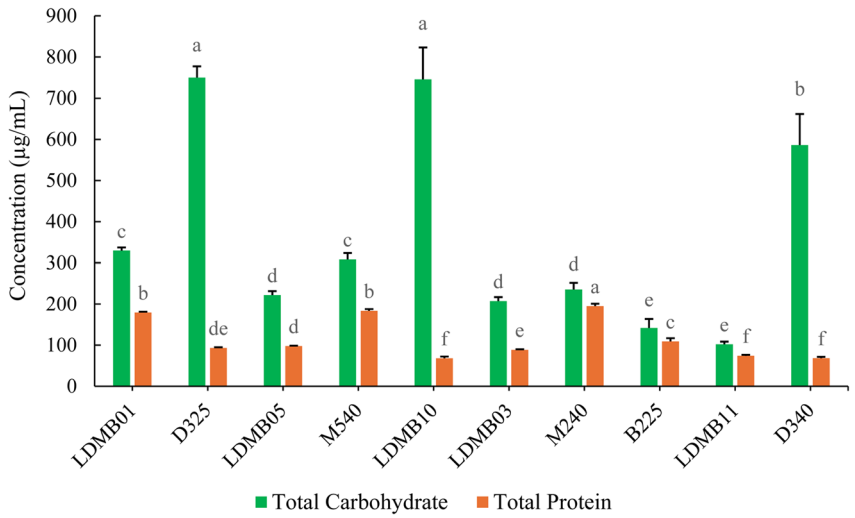

Fig. 4 Total carbohydrate and protein content (mean ± SD) of exopolysaccharides. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340. Different letters atop the bars in the same category show a significant difference ($p < 0.05$).

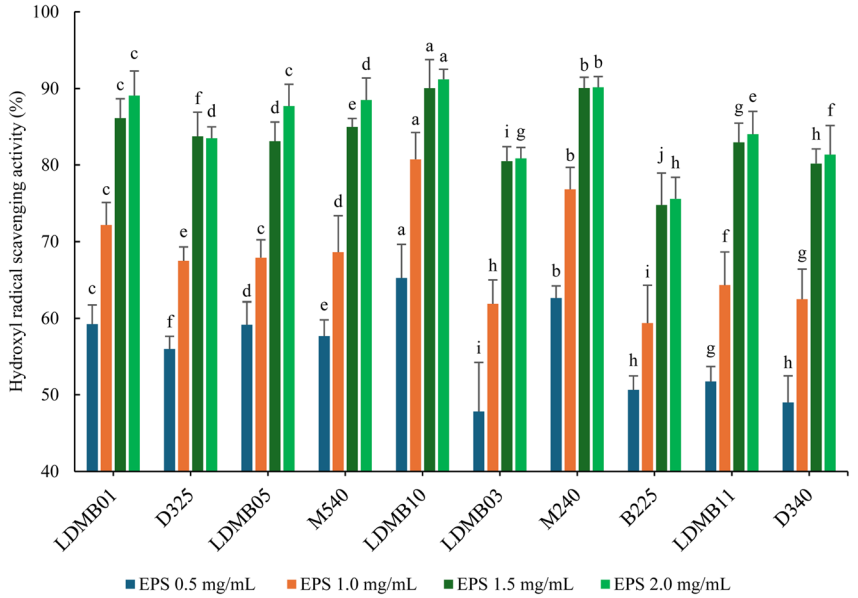

Fig. 5 Hydroxyl radical-scavenging activity of different exopolysaccharides in different concentrations. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240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

correlated to its protein content, with the low protein content observed in this study indicating high purity.[57]

3.3.2. Hydroxyl radical scavenging ability. Hydroxyl radicals are the most powerful oxidants that interact with nearly all biological components, encompassing nucleic acids, lipids, carbohydrates, and proteins.[58] The EPS antioxidant activity is a well-established feature that has been recently investigated by scavenging hydroxyl radicals and has a significant role in protection against non-communicable diseases, such as diabetes, cardiovascular conditions, and gastrointestinal ulcers.[59] EPS contains a sulfate group and uronic acid, which exhibit strong hydroxyl radical-scavenging activity by chelating metal ions (Fe^{2+} and Cu^{2+}).[60–62] In addition, according to Li *et al.*[63] and Wei *et al.*,[64] being the sulfate-containing polysaccharide, EPS exhibits antioxidant, antiviral, heavy metal adsorption, and anticoagulant activities. Moreover, the presence of protein (amino acids) could affect free radical scavenging and the antioxidant activity of EPS.[60]

The scavenging efficacy of EPS revealed concentration-dependent hydroxyl radicals ranging from 0.5 mg mL^{-1} to 2 mg mL^{-1}, as shown in Fig. 5. Increasing the EPS concentration from 0.5 to 2.0 mg mL^{-1} increased the hydroxyl radical-scavenging ability. This effect is probably due to the hydroxyl groups in the EPS donating more active hydrogen atoms.[54] However, from 1.5 to 2.0 mg mL^{-1}, no remarkable changes were observed for all the selected strains. For instance, EPS LDMB10 exhibited maximum scavenging activity (90.04 ± 3.70%) at 1.5 mg mL^{-1}, which was 91.21 ± 1.31% at 2.0 mg mL^{-1}. Other studies also confirm the potential antioxidant or scavenging ability of EPS, similar to *Lb. rhamnosus*, *Lcb. paracasei* ssp. *paracasei*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, *Lpb. plantarum*, *Lb. acidophilus*, and *Xanthomonas campestris*.[54,65] However, among the tested EPS, B225 had the lowest scavenging activity of 75.61% at a 2.0 mg mL^{-1} concentration. Overall, the findings indicate a concentration-dependent increase in hydroxyl radical-scavenging activity across all the EPS tested, with LDMB10, M240, and LDMB01 EPS showing consistently high activity, suggesting that the EPS from these strains possess potential antioxidant properties. However, *Limisolactobacillus fermentum* NCDC400 EPS showed only 63.92% scavenging of hydroxyl groups at a 3 mg mL^{-1} concentration,[66] and *L. plantarum* R301 EPS can remove 96.52% hydroxyl groups at a 2 mg mL^{-1} concentration.[67] However, in another study, Tarannum *et al.*[38] reported scavenging activities of 90.49% and 88.30% at concentrations of 0.755 and 0.738 μg mL^{-1} for *Lacticaseibacillus* sp. ME17 and *Lactococcus* sp. ME7, respectively. All these findings indicate this efficacy as a multifactorial phenomenon. In the current study, *Lb. lactis* LDMB10, *Lb. delbrueckii* spp. *bulgaricus* M240, *Lb. acidophilus* LDMB01, *Lpb. plantarum* LDMB05, *S. thermophilus* M540-4 showed potentials for radical scavenging activity and revealed their suitability as food additives with naturally produced antioxidants.

3.3.3. Antimicrobial activity. This paper documented the substantial antibacterial properties of various EPS against a broad range of infectious microorganisms, both Gram-positive and Gram-negative, as presented in Table 3. EPS is capable of exhibiting antagonistic activity as well as mobilizing the host immune system against bacterial pathogens.[68] Out of all the pathogens studied, the EPS from M240 and D340 exhibited the best significant ($p < 0.05$) antimicrobial activity, followed by LDMB01 and LDMB10. The *L. monocytogenes* ATCC 7644, and *S. aureus* ATCC 12600, are the Gram-positive bacteria that exhibited the highest susceptibility across all EPS tests, *B.*

Table 3 Antimicrobial activity of the exopolysaccharides produced by bacterial strains[a,b]

	Gram-positive bacteria						Gram-negative bacteria					
	L. monocytogenes		*S. aureus*		*B. cereus*		*E. coli*		*S. typhi*		*K. pneumoniae*	
EPS	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}	Zone of inhibition (mm) in 0.5 mg mL^{-1}	Zone of inhibition (mm) in 1.0 mg mL^{-1}
LDMB01	+++	+++	++	+++	+	+	—	—	++	+++	++	+++
D325	+	++	+++	+++	—	—	+	++	+	++	—	—
LDMB05	++	++	++	++	+	++	—	+	++	++	+	++
M540	++	++	++	+++	+	++	+	++	+	+	++	+++
LDMB10	+++	+++	++	+++	+	+	—	—	+++	+++	+	+
LDMB03	++	++	+	+	+	+	++	+++	+++	+++	—	—
M240	++	+++	++	++	++	+++	+	++	++	+++	++	++
B225	+	+	+++	+++	+	+	++	+++	+	++	—	—
LDMB11	++	++	++	++	+	+++	—	—	++	+++	+	++
D340	++	+++	++	++	++	++	++	++	+	++	++	+++

[a] Strong inhibition (+++) = >15 mm; moderate inhibition (++) = 10–15 mm; weak inhibition (+) = 5–10 mm; and no inhibition (–) = <5 mm. [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

cereus ATCC 11778 was also impacted but D325 was not. An increase in the EPS concentration from 0.5 to 1.0 mg mL^{-1} resulted in enhanced antimicrobial activity in approx. 45% of the samples, while approx. 55% of the samples remained unaffected. There were some samples (approx. 10%) that showed the highest antimicrobial activity even at a low EPS concentration. However, even at a higher concentration, *E. coli* showed resistance against LDMB10, LDMB11 and LDMB01, *B. cereus* resisted D325 and *K. pneumoniae* resisted D325, LDMB03 and B225. This finding also noted that the EPS showed a significant inhibition of Gram-positive bacteria *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 12600, and Gram-negative bacteria *S. typhi* ATCC 14028, even at low concentrations. The inhibitory effect against these bacteria may be attributed to the strong interactions of EPS functional groups with bacterial cell surfaces, leading to membrane disruption and inhibition of essential cellular processes. However, Rahnama *et al.*[69] reported *L. monocytogenes* as the most resistant organism against the EPS and reported *S. aureus* and *Enterococcus faecalis* as the most susceptible organisms. Nehal *et al.*[70] reported that EPS showed a robust repressive action against *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, *L. monocytogenes*, *B. cereus*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Enterobacter cloacae*, and *Candida albicans,* which is in line with the present study.

In addition, the present findings indicate that the antimicrobial activity of LAB EPS is mostly strain-specific. For instance, Fig. 6 shows that EPS D340 showed the highest inhibitory effect against *L. monocytogenes* ATCC 7644, and LDMB01 exhibited broad-spectrum activity, showing notable inhibition zones against both *S. aureus* ATCC 12600 and *K. pneumoniae* ATCC 8047. Similarly, EPS M240 displayed superior efficacy against *B. cereus* ATCC 11778, while EPS B225 was found to be most effective against *E. coli* ATCC 25922. In addition, EPS LDMB03 showed the strongest antimicrobial activity against *S. typhi* ATCC 14028. EPS chelates metals, forms barriers, restricts essential nutrients, disrupts cell walls and cytoplasmic membranes, inhibits mRNA and protein synthesis, disrupts cell proliferation, inactivates enzymes, generates H_2O_2, and accelerates DNA destruction.[57,69,71,72] Evidence of the structural conformation and/or presence and arrangements of different functional groups in EPS from the present study could have been advantageous in explaining the mechanism of their antibacterial activity, which was not covered. The use of antimicrobial compounds added directly to food or its packaging is one practical approach, indicating the industrial significance of EPS.

3.3.4. Emulsification index (Ex). EPS produces an *in situ* mechanism for potential emulsion formation, exhibiting a soluble natural polymer in enhancing food texture and developing food products.[73] Recently, novel applications for bacterial polysaccharides have emerged, particularly in production and emulsion stabilization. The coexistence of the hydrophobic and hydrophilic properties of EPS makes it an effective emulsifying agent. The presence of OH groups contributes to the hydrophilic characteristics of the EPS, while its hydrophobicity is attributed to the presence of aliphatic $-CH_2$ groups.[74] The EPS emulsification index was assessed

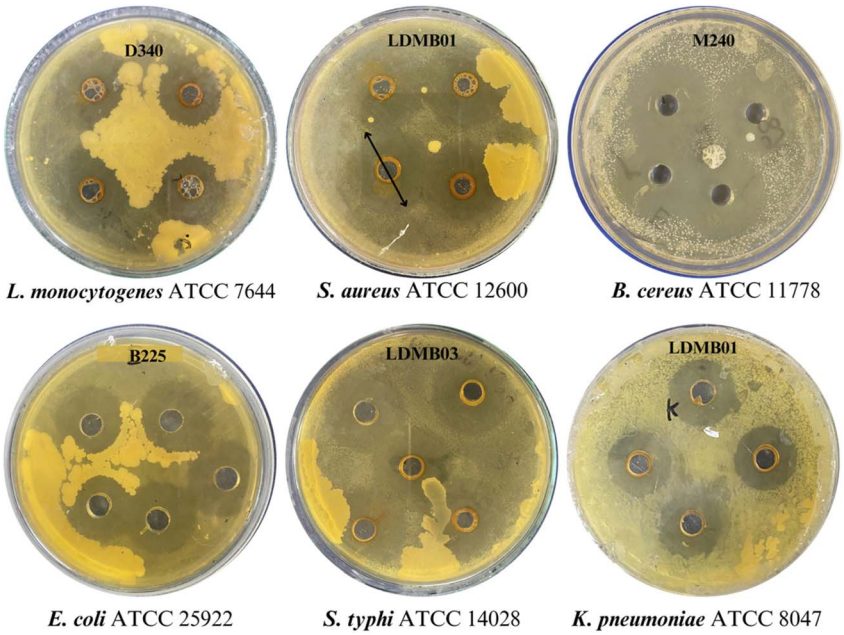

Fig. 6 Antimicrobial activity of the exopolysaccharides (EPS) from the selected highest EPS producers using the agar assay technique. D340 = *Lb. delbrueckii* subsp. *lactis* D340; LDMB01 = *Lb. acidophilus* LDMB01; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; and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

against various hydrophobic substances, including hydrocarbons and oils. Most of the EPS demonstrated good emulsifying properties with E_{24} values exceeding 50% with olive oil, black cumin oil, almond oil, soybean oil, mustard oil, kerosene oil, and *n*-hexane. As shown in Tables 4–12, the emulsification activity of EPS was evaluated at 1% concentration with different hydrocarbons. The maximum emulsion stability ($p < 0.05$) was recorded for B225, LDMB11, LDMB05, and LDMB01 at 84% and 71%, 70% and 66%, 80% and 64%, and 60% in olive oil and soybean oil, mustard oil and almond oil, black cumin oil and *n*-hexane, and kerosene, respectively, at 24 h of storage. De *et al.*[75] reported 63.9% E_{24} by *Klebsiella oxytoca* EPS in soybean oil. In another study, the EPS isolated from fermented milk and functional value-added probiotic yogurt *Lactobacillus rhamnosus* KF5 had an emulsification index of 43%, and that of *Lb. plantarum* C182 was 56.23% in *n*-hexane.[76] Interestingly, the EPS from LDMB05 (80.30–84.50%), LDMB03 (68.40–71.50%), M240 (67.53–69.50%) and LDMB11 (79.00–80.50%) in black cumin oil emulsion, and M240 in mustard oil emulsion (54.53–58.90%), showed an upward trend even at 96 h (Table 7). Statistical analysis proved that the emulsification index varies significantly ($p < 0.05$) with the type of EPS and hydrophobic substances, as presented in Table 11. The highest emulsification index was recorded for LDMB05 EPS in black cumin oil emulsion (83%), followed by 80% for LDMB11 EPS in the same system. Although not at the peak of any emulsion system, LDMB03 consistently showed more than a 50% emulsification index (covering E_{24} to E_{96}) in all the emulsion systems in this study. According to De *et al.*,[75] a good emulsifier should stabilize at least 50% of the emulsion from the original volume after 24 h. It was also observed that the emulsification index of EPS gradually decreased with an increase in the incubation time (Table 12; $p < 0.05$). The kerosene emulsion was found to be the most unstable, showing 25% reduction (from E_{24} to E_{96}) in the emulsification index, followed by 21% in soybean oil and *n*-hexane and 17% in mustard and almond oils. The black cumin oil exhibited the most stable emulsion, with less than 2% loss in the emulsification index. The factors associated with the discontinuous phase in an emulsion, namely viscosity, interfacial tension, polar components, hydrocarbon structures, and droplet size and distribution, are involved in the emulsion stability.[77–79] Moreover, emulsification performance is influenced by ionic strength, temperature, pH, salinity, and monovalent salts in the system.[80]

Table 4 Effect of olive oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 5 Effect of soybean oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

The gross particle size of the emulsions (*P. pentosaceus* B225 EPS in different oils) is depicted in Fig. 7. This experiment revealed that the particle size of emulsified droplets plays a critical role in determining the physical stability, including the creaming rate and flocculation, of different emulsions. Larger and scattered droplets in the emulsion indicate low efficiency of stability formation (Fig. 7C and D). On the contrary, the particle distribution showed mostly smaller, densely

Table 6 Effect of mustard oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] 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. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 7 Effect of black cumin oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 8 Effect of almond oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

packed, and uniform droplets that provided a higher surface area for the EPS to adsorb, resulting in it working as an effective stabilizer and resisting coalescence and sedimentation. This positive trend was also observed by Balyan *et al.*[81] in their work on isolating *Lactobacillus* EPS as an emulsifier to ensure the extended stability of eugenol encapsulation.

3.3.5. Water holding capacity. Water holding capacity (WHC) is an essential functional parameter that reflects the ability of polysaccharides to bind and interact with hydrodynamic water, bound water, and physically entrapped water in a system.[66] The WHC of LDMB03 (204.23%) and LDMB05 (200.90%) were significantly ($p < 0.05$) higher than that of LDMB01 (149.17%), M540 (113.90%), LDMB10 (100.73%), M240 (98.27%), D325 (46.80%), B225 (46.83%), and LDMB11 (38.37%) (Fig. 8). Qamar *et al.*[42] recorded 120.5% WHC for *Macrococcus brunensis* EPS, and Afreen *et al.*[51] recorded 475% WHC for *Lactiplantibacillus paraplantarum* NCCP 962 EPS. This is likely due to their porous structures and intermolecular hydrogen bond interactions. The compact structure, long polymer chain with a higher molecular weight, branched chains, functional group, and microstructure (*e.g.* porosity) in EPS are linked to an increased WHC.[66,82,83] However, these characterizations were not conducted for the produced EPS in the present study. During the dough stage, EPS are typically used as hydrocolloids, with water and other dough components being tightly bound, resulting in greater moisture retention and reduced hardness of the final product.[84] Li *et al.*[85] reported a higher WHC for yoghurt from *Lb. plantarum* 70810 EPS. Similarly, Wang *et al.*[86] reported a higher WHC in yogurt using *Lb. mesenteroides* XR1 EPS, and Yang *et al.*[87] reported that *Lb. rhamnosus* JAAS8 EPS improved the WHC of the fermented milk products.

3.3.6. Oil holding capacity. Oil holding capacity (OHC) is an essential characteristic of EPS and is linked to the permeable structure of the carbohydrate polymer chains. In addition, it is the ability of EPS to bind to oil molecules when no water is present.[14] The results showed that LDMB03 EPS exhibited the highest oil-holding capacity (309.59% ± 20.59%), while B225 EPS had the lowest OHC (35.15% ± 5.56%) (Fig. 8). In addition,

Table 9 Effect of *n*-hexane on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 10 Effect of kerosene oil on the exopolysaccharide emulsification index at 24, 48, and 96 h[a,b]

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

[a] Means of different superscripts significantly differ within row and column ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 11 Emulsification index of different exopolysaccharides with various oils[a,b]

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

[a] Values with different lowercase letters within a column indicate significant differences ($p < 0.05$). [b] LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340.

Table 12 Effect of incubation time (24, 48, and 96 h) on the emulsification index using different oils[a,b]

Time	Olive oil	Soybean oil	Mustard oil	Black cumin oil	Almond oil	*n*-hexane	Kerosene
E_{24}	63.51^{a}	60.26^{a}	57.33^{a}	65.44^{a}	57.62^{a}	52.32^{a}	51.13^{a}
E_{48}	58.24^{b}	53.68^{b}	51.16^{b}	65.19^{a}	52.97^{b}	46.98^{b}	43.28^{b}
E_{96}	55.89^{c}	47.94^{c}	47.98^{c}	63.60^{b}	48.10^{c}	41.01^{c}	38.24^{c}
p Value	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Values with different lowercase letters within a column indicate significant differences ($p < 0.05$). [b] E_{24} = Emulsification index at 24 h; E_{48} = Emulsification index at 48 h; and E_{96} = Emulsification index at 96 h.

EPS LDMB05 (191.56%), LDMB01 (159.18%), and M240 (114.22%) demonstrated notable oil-holding capacities among the tested EPS. There was a significant difference ($p < 0.05$) in the oil-holding capacities of various crude EPS samples. While the chemical composition significantly influences the OHC, the porosity of the biopolymer and its interaction with oil are also key determinants of the OHC.[88] Trabelsi *et al.*[89] recorded only 15.96% OHC for *Lactobacillus* sp. Ca_6 EPS in cooked beef sausage. In the current study, *Lb. lactis* LDMB10 retained 258.29% oil, while Yang-Ren *et al.*[90] found that *Lb. lactis* EPS retained 325.67%, and Afreen *et al.*[51] reported 658% OHC for *Lactiplantibacillus paraplantarum* NCCP 962 EPS. On the other hand, Nehal *et al.*[70] reported 9% OHC for *Lactococcus lactis* F-mou EPS isolated from camel milk. The strain and medium variation in OHC need further detailed study. Industrially, in product or food formulations where fat adsorption is beneficial, a high oil-holding capacity contributes to a smoother, juicier, more palatable texture and better flavor retention.[91] The present findings show that EPS LDMB03, LDMB10, LDMB01, LDMB05, and M240 could be extensively used in fermented food products, especially in fat-absorbing food, owing to the higher OHC of EPS. Nowadays, biopolymers (food grade) are extensively used as emulsifiers to stabilize oil/water emulsion systems.

3.4. Coagulation effect of EPS strain in skim milk

The coagulation/solidification effect of the superior EPS strain *Lb. acidophilus* LDMB01 with different levels of sugar (sucrose) in skim milk is depicted in Fig. 9. After 24 h of fermentation, all the samples remained liquid, and even at 36 h, the sample with 0% sucrose exhibited a liquid appearance, while the samples with 3%, 6%, 9%, and 12% sucrose exhibited an initiation of curd formation. However, after 48 h, the sample with 0% sucrose remained unchanged, while other samples exhibited semi-solid and solid structures. Notably, the sample with 12% sucrose had a more pronounced solidification than all other samples and exhibited a complete curdling characteristic. The outcome of the present study is similar to the findings of Ning *et al.*[5] for *Gluconobacter frateurii* HDC-08 EPS, Yu *et al.*[92] for *Lactiplantibacillus plantarum* HDC-01, and Afreen *et al.*[51] for *Lactiplantibacillus paraplantarum* NCCP 962 EPS, where higher sucrose concentrations and longer fermentation periods resulted in more pronounced curd formation. The coagulation effect is intricately linked to the structure and molecular weight of EPS, the kind of protein present in the milk, as well as the interaction between the EPS and skim milk protein.[93] The findings indicated that the LAB might be capable of synthesizing EPS from sucrose, which promotes milk solidification properties in dairy products.

3.5. Conjugated linoleic acid production

Food-derived LAB strains are considered to be the most efficient conjugated linoleic acid (CLA) producers. This study selected the LAB strains with the highest EPS-producing capacity for CLA production. The standard curve exhibited a strong linear

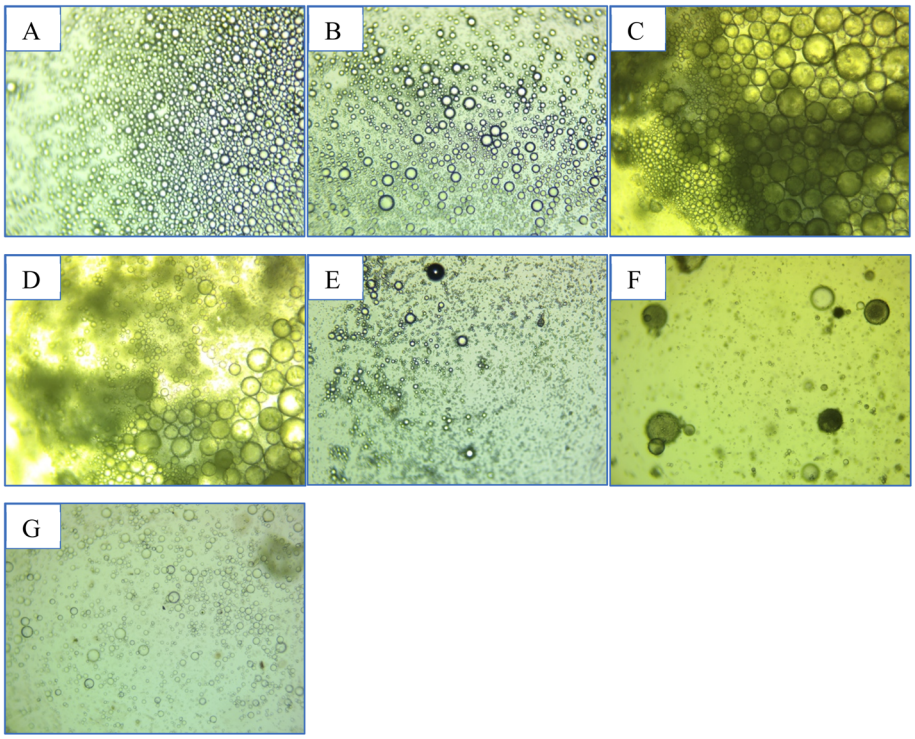

Fig. 7 Optical microscopic (10×) images of the exopolysaccharide from *P. pentosaceus* B225 in olive oil (A), soybean oil (B), mustard oil (C), black cumin oil (D), almond oil (E), *n*-hexane (F), and kerosene oil (G) emulsions.

relationship ($R^2 = 0.9949$). The linear correlation between the peak area and concentration was strong within the concentration range of 0 to 100 μg mL^{-1}. The CLA concentration across the strains varied, with values ranging from 9.56 ± 1.24 μg mL^{-1} to 30.60 ± 1.27 μg mL^{-1} when supplied with 0.5 mg mL^{-1} LA. Among all the strains tested (Fig. 10), the microorganism M240 exhibited the highest CLA conversion/production, reaching 30.60 μg mL^{-1}, followed closely by LDMR01 and LDMB11 with CLA yields of 29.72 and 28.46 μg mL^{-1}, respectively. Wei *et al.*[94] obtained a close result for the *Lpb. plantarum* Lp-01 strain, which achieved 33.47 μg mL^{-1} of CLA when cultured in a medium supplemented with pine nut oil, indicating its capacity to convert linoleic acid present in the oil into CLA. The *Lb. casei* LDMB03 strain exhibited 23.12 μg mL^{-1} of CLA; however, in another study, using an initial linoleic acid concentration of 0.1% and incubating at 37 °C for 72 h, soymilk

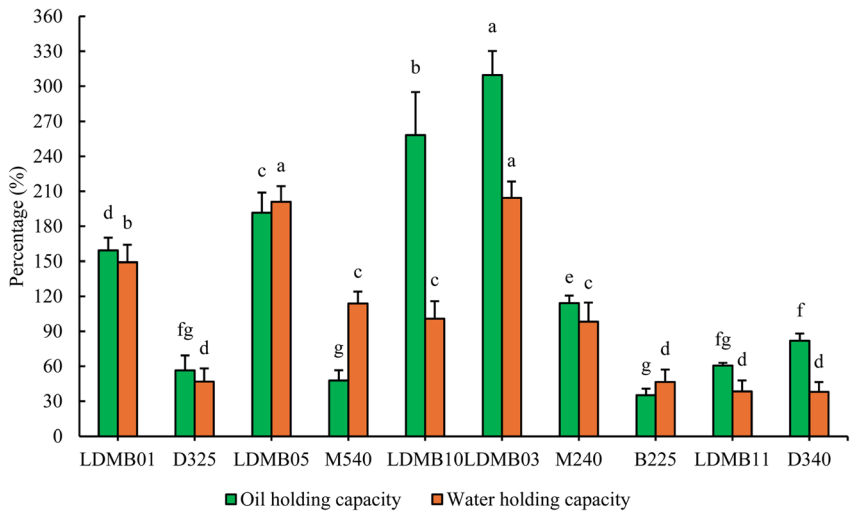

Fig. 8 Oil- and water-holding capacity of exopolysaccharides. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB03 = *Lb. casei* LDMB03; M240 = *Lb. delbrueckii* subsp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB11 = *Lb. paraplantarum* LDMB11; and D340 = *Lb. delbrueckii* subsp. *lactis* D340. Different letters atop the bars indicate a significant difference at $p < 0.05$ (mean ± SD).

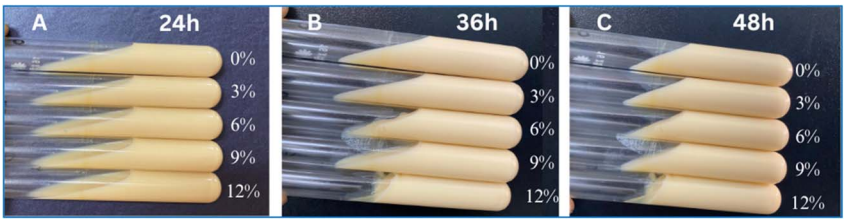

Fig. 9 Coagulation effect of the highest EPS-producing strain (*Lb. acidophilus* LDMB01) on 10% skim milk supplemented with varying sucrose concentrations (0%, 3%, 6%, 9% and 12%) at 24 h (A), 36 h (B), and 48 h (C) of incubation.

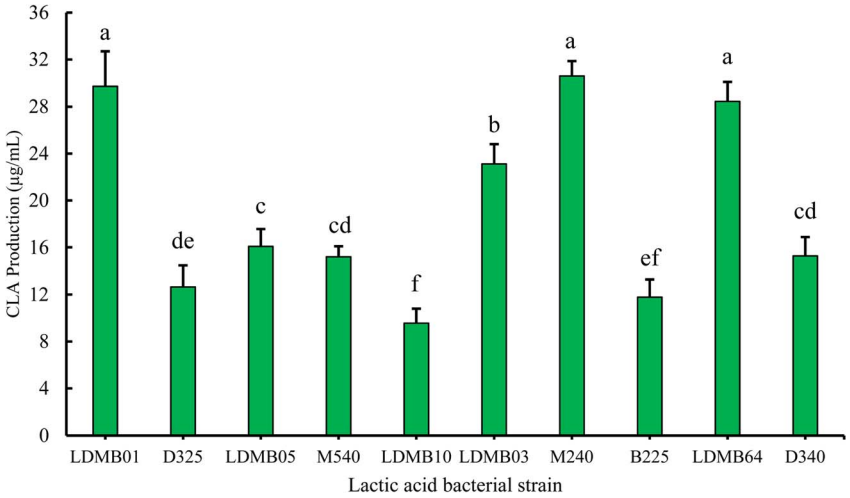

Fig. 10 Yield of the conjugated linoleic acid (CLA) from the selected lactic acid bacterial strains. LDMB01 = *Lb. acidophilus* LDMB01; D325 = *Ent. faecium* D325; LDMB05 = *Lpb. plantarum* LDMB05; M540 = *Str. thermophilus* M540-5; LDMB10 = *Lc. lactis* LDMB10; LDMB42 = *Lb. casei* LDMB42; M240 = *Lb. delbrueckii* spp. *bulgaricus* M240; B225 = *P. pentosaceus* B225; LDMB64 = *Lb. paraplantarum* LDMB64; and D340 = *Lb. delbrueckii* spp. *lactis* D340. Different letters atop the bars indicate a significant difference at $p < 0.05$ (mean ± SD).

fermented by *Lactobacillus casei* synthesized approximately 839 μg g^{-1} of CLA.[95] *Lactobacillus reuteri* was reported to have 0.108 mg mL^{-1} of CLA when incubated under aerobic conditions at 10 °C for 30 h in a broth medium with 20 mg L^{-1} free LA. It was observed that *L. reuteri* cells could grow 5.5 times faster CLA isomers (c9, t11 and t10, c12) than free-washed cells.[31] Jena and Choudhury[96] found that the yogurt culture of *Lb. del*. subsp. *bulgaricus* LB340 and *Bifidobacterium animalis* subsp. *lactis* BLO4, BB12, B94, and HN019 co-cultured with the *S. thermophilus* TA040 strain predominantly enhanced CLA content in yogurt during the manufacture. Baliyan *et al.*[97] observed variable CLA production by EPS-producing probiotic LABs isolated from raw cow milk. Chen *et al.*[28] also reported 1.28 to 54.28 μg mL^{-1} CLA production by different LABs.

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

4 Conclusions

This study provides comprehensive information about the screening, production and quantification of exopolysaccharides from selected lactic acid bacterial strains, as well as the functional properties of exopolysaccharides and the conjugated linoleic acid producing property of strains with high exopolysaccharide-production capacity. The highest exopolysaccharide production was observed in *Lactobacillus acidophilus* LDMB01 EPS, and the lowest was found in *Lactococcus raffinolactis* B425. *Lactobacillus delbrueckii* subsp. *bulgaricus* M240 produced the highest amount of conjugated linoleic acid. For the functional properties, the exopolysaccharide obtained from *Lacticaseibacillus casei* LDMB03 exhibited the highest water- and oil-holding capacity. Owing to the milk coagulation

ability of the exopolysaccharide-producing strains, they have potential application in fermented dairy products. Increasing the EPS concentration enhanced antimicrobial activity; however, approximately 11.67% of the tested samples exhibited no antimicrobial effect even at higher concentrations. Accumulated antimicrobial activity, hydroxy radical scavenging activity, emulsification index, water-holding capacity, oil-holding capacity, together with the solidifying efficiency of exopolysaccharides, imply numerous EPS applications in the food industry. Regarding exopolysaccharides and conjugated linoleic acid production, as well as the functional properties of exopolysaccharides, *Lactobacillus delbrueckii* subsp. *bulgaricus* M240, *Lactiplantibacillus plantarum* LDMB05, and *Lactobacillus acidophilus* LDMB01 can be recommended as suitable lactic acid bacterial strains. Furthermore, producing exopolysaccharides in functional food manufacturing can be highly interesting because of their role not only as health-promoting agents but also as a key factor in enhancing the quality of food products. The future scope of this study should include spectroscopic analyses like Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), and advanced microscopy techniques like scanning electron microscopy (SEM), which can be used to examine the produced exopolysaccharides with more precise characteristics important for industrial applications.

Author contributions

Md. Sadman Tahmid: conceptualization, data curation, formal analysis, investigation, methodology, software, supervision, validation, visualization, writing – original draft, and writing – review and editing. Md. Abunaser: conceptualization and writing – review and editing. A. K. M. Masum: conceptualization, investigation, supervision, validation, visualization, and writing – review and editing. Mohammad Ashiqul Islam: conceptualization, formal analysis, resources, validation, and writing – review and editing. Md. Harun-ur-Rashid: conceptualization, funding acquisition, project administration, resources, supervision, validation, and writing – review and editing.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data are available from the corresponding author upon reasonable request.

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