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# Exopolysaccharides derived from probiotic *Limosilactobacillus fermentum* GD29: Structural insights and antibacterial potential

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ABSTRACT

*Background:* Extracellular polymeric substances (EPS) of microbial origin have gained significant attention due to their low toxicity, high biodegradability, and diverse functional properties compared to synthetic alternatives. They serve as emulsifiers, stabilizers, thickeners, texturizers, flocculants, and gelling agents.
*Objective:* This study aims to evaluate EPS production by lactic acid bacteria (LAB), particularly strains with Generally Recognized as Safe (GRAS) status, including members of the *Lactobacillus* group.
*Methodology:* Eight LAB isolates *Lactobacillus plantarum* GDS82, *Limosilactobacillus fermentum* GD29, *Lactobacillus plantarum* GD26, *Lactobacillus helveticus* GD10, *Lactiplantibacillus plantarum* GD14, *Limosilactobacillus fermentum* GD24, *Lactobacillus helveticus* GD2SC, and *Limosilactobacillus fermentum* GD13—were screened for EPS production. Among these, *L. fermentum* GD29 exhibited the highest yield, producing up to 17 g/L of EPS with elevated carbohydrate, protein, and reducing sugar content. FT-IR analysis identified various functional groups, including alcohols, aldehydes, alkenes, sulfonates, vinyl ethers, and phenols, while GC–MS analysis revealed metabolites such as esters, aldehydes, ketones, and terpenes associated with food and industrial applications. The EPS demonstrated notable antibacterial activity, with inhibition zones against *Escherichia coli* (14 mm) and *Salmonella* spp. (12 mm), and also showed activity against *Vibrio cholerae* and *Staphylococcus aureus*. Additionally, *L. plantarum* GD26 exhibited an inhibition zone of 11 mm against *E. coli*.
*Conclusion:* The findings indicate that EPS derived from *Limosilactobacillus fermentum* GD29 and *Lactobacillus plantarum* GD26 possess significant antibacterial activity against foodborne pathogens. In particular, EPS from *L. fermentum* GD29 demonstrates strong potential as a multifunctional, LAB-derived biopolymer with promising applications in food and industrial sectors.

## 1. Introduction

Extracellular polymeric substances (EPS) are complex biomolecular matrices secreted by microorganisms, including beneficial probiotic bacteria. These substances play a crucial role in the formation and stability of biofilms, which are structured microbial communities attached to surfaces and embedded within a self-produced extracellular matrix. In the human oral cavity, EPS production by probiotic bacteria is increasingly recognized as an important factor in maintaining oral health and preventing disease (Zaghloul et al., 2023).

EPS are extracellular polymeric matrices composed of a mixture of biomolecules, primarily polysaccharides along with proteins, lipids, and nucleic acids, produced by certain lactic acid bacteria (LAB). In recent years, microbial EPS have attracted considerable attention due to their expanding applications as natural polymers in various industries. Their unique structural and functional properties make them valuable in fields such as medicine, biotechnology, and the food industry (Korcz and Varga, 2021). In food applications, EPS are widely used as thickeners, stabilizers, and emulsifiers to improve texture, viscosity, and overall product quality. EPS produced by LAB are of particular interest as potential alternatives to synthetic additives due to their favorable physicochemical characteristics (Prete et al., 2021; Kirubakaran et al., 2025).

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However, despite extensive identification of EPS-producing LAB strains, their specific functional roles and health benefits, especially under in vivo conditions, remain insufficiently explored (Sorensen et al., 2022).

Oral probiotics, including species of *Lactobacillus* and *Streptococcus*, produce EPS that enhance their ability to adhere to oral surfaces, establish beneficial biofilms, and suppress the growth of pathogenic microorganisms. The EPS-mediated inhibition of pathogenic biofilm formation contributes to the prevention of oral diseases such as dental caries and periodontitis by maintaining microbial balance within the oral cavity (Guhanraj and Dhanasekaran, 2024).

LAB are well known for their capacity to synthesize EPS during fermentation processes, where these compounds contribute to improved texture and consistency in fermented foods. In addition to EPS, postbiotics refer to bioactive compounds released by microorganisms either during active metabolism or following cell lysis (Asif et al., 2023). These include short-chain fatty acids (SCFAs), enzymes, extracellular polysaccharides, microbial cell components, and cell wall-derived molecules such as teichoic acids, lipoteichoic acids, and peptidoglycans. Postbiotics are also defined as non-viable microbial products or cell-free bioactive compounds derived from food-grade microorganisms that can provide health benefits to the host (Monteiro et al. 2023; Aguilar-Toalá et al., 2018).

EPS are increasingly recognized for their ability to modify the rheological properties of food matrices. However, further intervention-based studies are required to validate their health-promoting effects under practical conditions (Sorensen et al., 2022; Brishti et al., 2025; Radhamanalan et al., 2026). Although EPS production by various lactic acid bacteria (LAB) has been widely reported, the relationship between EPS synthesis and their functional properties remains incompletely understood. In particular, EPS-producing LAB, especially those belonging to the *Lactobacillus* genus, show significant potential for improving the technological properties of dairy products, including texture and viscosity (Ale et al., 2020). Overall, LAB-derived EPS hold considerable promise for enhancing food quality and functionality; however, additional research is necessary to fully elucidate their benefits and optimize their applications in both industrial and health-related contexts (Guérin et al., 2020). Fourier Transform Infrared (FT-IR) spectroscopy was employed to identify the functional groups present in purified EPS, providing insights into its chemical composition. This technique enables the characterization of chemical bonds and functional groups within EPS samples, thereby contributing to a better understanding of their molecular structure and properties (Wu et al., 2021; Nidamarthi et al., 2026).

Extensive studies have examined EPS production in fermented dairy products across various regions. In contrast, the present study provides the first comprehensive investigation of EPS-producing oral probiotic isolates obtained from the Tiruchirappalli region of Tamil Nadu, India. The study focuses on selected LAB strains, including *Lactobacillus plantarum* GDS82, *Limosilactobacillus fermentum* GD29, *Lactobacillus plantarum* GD26, *Lactobacillus helveticus* GD10, *Lactiplantibacillus plantarum* GD14, *Limosilactobacillus fermentum* GD24, *Lactobacillus helveticus* GD2SC, and *Limosilactobacillus fermentum* GD13, to evaluate their EPS production. The investigation includes extraction, quantification, and chemical characterization using FT-IR, GC–MS, and NMR, along with the assessment of antibacterial activity.

The significance of this study lies in the identification of potent EPS-producing probiotic strains with notable antimicrobial properties, particularly *Limosilactobacillus fermentum* GD29. Although biofilm formation was not directly investigated, EPS are well-established as key structural and functional components of microbial biofilms, contributing to matrix integrity, surface adhesion, and microbial interactions. Therefore, the findings of this study provide foundational insights into EPS characteristics that may be relevant to biofilm-related processes. These results may support future research exploring the role of probiotic-derived EPS in modulating biofilm formation and controlling pathogenic microorganisms, particularly in oral environments.

## 2. Materials and methods

### 2.1. LAB bacterial isolates collection and cultivation

*Lactobacillus* species were obtained from the Bioprocess Technology Laboratory, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. A total of eight lactic acid bacteria (LAB) isolates were selected, namely *Lactobacillus plantarum* GDS82, *Limosilactobacillus fermentum* GD29, *Lactobacillus plantarum* GD26, *Lactobacillus helveticus* GD10, *Lactiplantibacillus plantarum* GD14, *Limosilactobacillus fermentum* GD24, *Lactobacillus helveticus* GD2SC, and *Limosilactobacillus fermentum* GD13. The LAB isolates were cultured on de Man, Rogosa, and Sharpe (MRS) agar and incubated at 37 °C for 48 h. Morphologically distinct mucoid or ropy colonies were subsequently selected and streaked onto MRS agar supplemented with 5% sucrose, 10 g/L tryptone, 10 g/L meat extract, 5 g/L yeast extract, 5 g/L sodium acetate, 2 g/L disodium phosphate, 2 g/L triammonium citrate, 0.1 g/L $MgSO_4$, and 0.05 g/L $MnSO_4$ (pH 6.5). Colonies exhibiting typical *Lactobacillus* characteristics, such as large, white, and smooth appearances, were further purified by repeated streaking (Chen et al., 2013). These purified isolates were then evaluated for their ability to produce extracellular polymeric substances (EPS).

### 2.2. Morphological characterization of the EPS producing Lactobacillus spp

Eight polysaccharide-producing strains were individually streaked onto MRS agar plates and incubated at 37 °C for 48 h. Based on preliminary screening, the strain exhibiting the highest level of EPS production was selected for further analysis. The selected EPS-producing strain was subsequently cultured on Tryptic Soy Agar (TSA) composed of tryptone (17 g/L), soya peptone (3 g/L), sodium chloride (5 g/L), dextrose (2.5 g/L), dipotassium hydrogen phosphate (2.5 g/L), and agar (15 g/L). The plates were incubated at 37 °C for 48 h to promote optimal growth and EPS production. This procedure ensured suitable conditions for the expression of EPS-producing characteristics, facilitating subsequent analysis and potential applications (Razack et al., 2013; Guhanraj and Dhanasekaran, 2024).

#### 2.2.1. Visualization of EPS producing oral probiont – Crystal Violet Staining

Crystal violet staining was performed on *Lactobacillus* cultures grown in MRS medium supplemented with 2% (w/v) sucrose and incubated at 37 °C for 48 h under anaerobic conditions. A small volume of the culture was spread onto a clean microscope slide and allowed to air-dry. The smear was then stained with 1% (w/v) crystal violet for 2 min, followed by gentle washing with 20% (w/v) copper sulfate solution. After air-drying, the prepared slide was examined under a light microscope (McCutcheon and Southam, 2018).

#### 2.2.2. Ropy EPS-producing LAB

Each LAB isolate was cultured on MRS agar plates supplemented with 10% (w/v) sucrose and incubated at 37 °C for 72 h under anaerobic conditions. Following incubation, the viscous material from a single colony was gently lifted using a sterile loop, and the length of the ropy extension was measured in millimeters.

### 2.3. Preparation of inoculum and characterization

For EPS production, an inoculum of LAB was prepared by inoculating 100 mL of MRS medium in 250 mL Erlenmeyer flasks with a loopful of culture. The inoculated flasks were incubated at 37 °C for 18 h. During incubation, the growth of the culture was monitored by measuring absorbance at 620 nm. An inoculum with a cell density of approximately $10^8$ CFU/mL (colony-forming units per milliliter) was used for subsequent EPS production (Radhamanalan and Dharumadurai, 2023).

#### 2.3.1. Production, extraction and quantification of EPS

MRS medium was inoculated with *Lactobacillus* spp. at a concentration of 10% (v/v) and incubated at 37 °C for 72 h. Following incubation, 100 mL cultures of *Limosilactobacillus fermentum* GD29, *Lactobacillus plantarum* GD26, *Lactobacillus helveticus* GD10, *Limosilactobacillus fermentum* GD13, *Lactiplantibacillus plantarum* GD14, *Limosilactobacillus fermentum* GD24, *Lactobacillus plantarum* GD5, and *Lactobacillus helveticus* GD2SC were subjected to heat treatment in a boiling water bath (~100 °C) for 20 min, followed by cooling to room temperature (~25 °C). The culture supernatants were collected by centrifugation at 11,500 × g for 15 min at 4 °C. For EPS precipitation, an equal volume of chilled 90% ethanol was added to the supernatants, and the mixture was incubated overnight at −20 °C. The resulting crude EPS was then recovered by centrifugation at 11,772 × g for 20 min at 4 °C. The collected EPS was dried at 4 °C overnight, and its dry weight and yield were determined. Finally, the dried EPS was re-dissolved in distilled water for subsequent chemical characterization and functional analysis (Imran et al., 2016; Yadav et al., 2024).

#### 2.3.2. Appearance and solubility of the EPS

The physical properties of the EPS, including color, odor, and texture, were evaluated to characterize its appearance and sensory attributes. In addition, the solubility of the EPS powder in water was assessed.

### 2.4. Estimation of carbohydrates in Lactobacillus spp. EPS using the phenol-sulfuric acid method

The total carbohydrate content of the EPS was determined using the phenol–sulfuric acid method. In this assay, concentrated sulfuric acid hydrolyzes glycosidic linkages in polysaccharides, releasing monosaccharides that undergo partial dehydration to form furfural or related derivatives. These compounds subsequently react with phenol to produce a colored complex, the intensity of which is measured spectrophotometrically at 490 nm. Briefly, 1 mL of the sample was mixed with 1 mL of 5% (w/v) phenol, followed by the addition of 5 mL of concentrated sulfuric acid. The reaction mixture was maintained on ice during acid addition and then incubated at room temperature for 20 min. Absorbance was measured at 490 nm using a spectrophotometer. A standard calibration curve was prepared using glucose solutions in the range of 0–100 μg/mL, derived from a 1 mg/mL stock solution, by plotting absorbance against concentration. A blank sample containing no glucose was treated identically to correct for background absorbance (Lee et al., 2022).

### 2.5. Estimation of protein on Lactobacillus spp. EPS by Lowry's assay

The protein content of the postbiotic extracts was determined using the Lowry method. In this assay, 0.1 mL of the postbiotic extract (1 mg/mL) and varying concentrations of bovine serum albumin (BSA) standard (0.5 mg/mL) were added to separate test tubes. Distilled water was then added to each tube to make the final volume up to 0.2 mL. Subsequently, 1 mL of Reagent C (prepared by mixing 2% $Na_2CO_3$ in 0.1 N NaOH with 0.5% $CuSO_4$ in 1% potassium sodium tartrate) was added to each tube. The mixtures were thoroughly mixed and incubated at room temperature for 10 min. Following this, 0.3 mL of Reagent D (1 N Folin–Ciocalteu reagent) was added, mixed immediately, and the tubes were incubated in the dark for 60 min at room temperature. The absorbance of the resulting blue-colored complex was measured at 660 nm using a spectrophotometer. Protein concentration in the samples was determined by comparison with a standard calibration curve generated using known concentrations of BSA. This method is based on the reaction of proteins with cupric ions under alkaline conditions (biuret reaction), followed by the reduction of the Folin reagent, leading to the formation of a blue-colored complex proportional to the protein concentration (Zaghloul and Ibrahim, 2022).

### 2.6. Dinitro Salicylic Acid (DNS) method for reducing sugar analysis

The reducing sugar content of the samples was determined using the DNS (3,5-dinitrosalicylic acid) method. In this assay, the aldehyde groups of reducing sugars react with 3,5-dinitrosalicylic acid under alkaline conditions, resulting in its reduction to 3-amino-5-nitrosalicylate and the formation of a brown-colored complex. The intensity of this color is proportional to the concentration of reducing sugars and is measured spectrophotometrically at 575 nm. The DNS reagent was prepared by dissolving sodium hydroxide and 3,5-dinitrosalicylic acid in distilled water at approximately 80 °C until a clear solution was obtained. Subsequently, the remaining components were added to complete the reagent preparation. The solution was then filtered and stored in amber-colored bottles at room temperature to prevent photo-oxidation and ensure stability. This method provides a reliable estimation of reducing sugar concentration based on the absorbance of the developed color, which is directly proportional to the amount of reducing sugars present in the sample (Thorakkattu et al., 2022).

### 2.7. Functional group characterization on EPS

#### 2.7.1. Fourier transform infrared (FT-IR) analysis

The extracellular polymeric substances (EPS) obtained from *Limosilactobacillus fermentum* GDS82, *Lactobacillus plantarum* GD5, *Lactobacillus helveticus* GD2SC, and *Limosilactobacillus fermentum* GD6 were analyzed using Fourier transform infrared (FT-IR) spectroscopy. The analysis was performed using a Shimadzu-8400S FT-IR spectrophotometer.

FT-IR spectroscopy was employed to identify the functional groups present in the EPS samples by recording absorption spectra over the range of 4000–400 $cm^{-1}$ . The obtained spectra enabled the detection of characteristic functional groups, including hydroxyl, carboxyl, and other relevant chemical moieties. This technique provides valuable information on molecular structure and chemical composition, thereby facilitating the characterization of EPS derived from bacterial isolates for potential research and industrial applications (Do et al., 2020).

#### 2.7.2. Gas chromatography-mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) was employed to analyze the chemical composition of extracellular polymeric substances (EPS) produced by *Limosilactobacillus fermentum*. The procedure began with the extraction and purification of EPS from bacterial cultures to ensure the removal of impurities. For GC–MS analysis, samples were subjected to derivatization, when required, to convert non-volatile compounds into volatile derivatives suitable for analysis. Trimethylsilyl (TMS) derivatization was performed for sugar components. The GC–MS system was calibrated according to the manufacturer's instructions, and an appropriate capillary column was selected to achieve optimal separation of EPS constituents. The prepared samples were injected into the GC system, where compounds were separated based on programmed temperature gradients. The separated components were subsequently introduced into the mass spectrometer, where they were ionized and detected based on their mass-to-charge ($m/z$) ratios. Mass spectra were recorded over a range of 50–800 $m/z$. Compound identification was performed by comparing retention times and mass spectra with reference standards and spectral libraries. Quantification was carried out using peak area normalization, and the relative abundance of each component was expressed as a percentage of the total chromatographic peak area (Rupprecht et al., 2022; Suresh et al., 2026).

#### 2.7.3. Nuclear magnetic resonance (NMR) analysis

Nuclear magnetic resonance (NMR) spectroscopy was employed to elucidate the structural features and composition of extracellular polymeric substances (EPS) produced by *Limosilactobacillus fermentum*. Prior to analysis, EPS samples were purified and concentrated to eliminate impurities that could interfere with spectral interpretation. The purified

EPS was dissolved in deuterium oxide ($D_2O$) at an appropriate concentration to ensure optimal signal detection. Structural analysis was initially performed using one-dimensional proton ($^1H$) NMR spectroscopy to examine the hydrogen atoms present in the EPS structure. For detailed structural characterization, two-dimensional NMR techniques, including correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC), were employed where necessary. All spectra were recorded under controlled experimental conditions, including temperature and solvent environment. The resulting chemical shifts (δ values), signal intensities, and coupling patterns were analyzed to identify functional groups, sugar residues, and structural linkages within the EPS matrix (Vinothini et al., 2019; Dharumadurai et al., 2014; Surya et al., 2026; Kafle, 2019).

### 2.8. Antibacterial activity of LAB using postbiotics

The antibacterial activity of EPS was evaluated using selected LAB strains, including *Limosilactobacillus fermentum* GDS82, *Lactobacillus helveticus* GD2SC, *Lactobacillus plantarum* GD26, and *Limosilactobacillus fermentum* GD29. The isolates were cultured in MRS broth and incubated at 37 °C for 24 h under anaerobic conditions. Following incubation, the cultures were centrifuged at 1508 × g for 20 min to obtain the cell-free supernatant. The supernatant was then heat-treated at 55 °C for 30 min. Subsequently, two volumes of ethanol were added, and the mixture was incubated overnight at 4 °C to facilitate EPS precipitation. After incubation, the mixture was centrifuged at 1508 × g for 30 min, and the resulting supernatant was filtered using a sterile 0.45 μm membrane filter. Mueller–Hinton agar (MHA) plates were prepared, and test pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Vibrio cholerae*, were evenly swabbed onto the agar surface. Wells were created in the agar, and each well was loaded with 30 μL of the prepared EPS extract. The plates were incubated at 37 °C for 24 h. After incubation, the antibacterial activity was assessed by measuring the zones of inhibition around the wells (Radhamanalan and Dharumadurai, 2023; Abitha Eswari et al., 2023; Radhamanalan and Dharumadurai, 2024; Dayana et al., 2026; Radhamanalan et al., 2026).

### 2.9. Statistical analysis

All experimental data were analyzed using OriginPro 8.0, Microsoft Excel, and SPSS software. The results are presented as the mean ± standard deviation (SD) based on three independent replicates. Statistical significance was determined at a confidence level of $P < 0.05$.

## 3. Results

### 3.1. Isolation and characterization

A total of eight lactic acid bacteria (LAB), namely *Lactobacillus plantarum* GDS82, *Limosilactobacillus fermentum* GD29, *Lactobacillus plantarum* GD26, *Lactobacillus helveticus* GD10, *Lactiplantibacillus plantarum* GD14, *Limosilactobacillus fermentum* GD24, *Lactobacillus helveticus* GD2SC, and *Limosilactobacillus fermentum* GD13, were preliminarily characterized based on their morphological and biochemical properties. All isolates grown on MRS agar exhibited cream-white, slimy colonies that ranged from small to pinpoint in size. Gram staining revealed that all LAB isolates were Gram-positive and appeared as rod-shaped bacteria **(**Fig. 1**).**

### 3.2. Preliminary screening of extracellular polymeric substances (EPS)

#### 3.2.1. Qualitative estimation of EPS

All eight strains exhibited mucous formation on the agar surface, indicating their ability to produce extracellular polymeric substances (EPS). Therefore, these isolates were preliminarily confirmed as EPS-producing probiotic bacteria.

#### 3.2.2. Quantitative estimation of EPS production

All eight *Lactobacillus* species demonstrated the ability to produce extracellular polymeric substances (EPS). The highest EPS yield was recorded at 0.235 g/mL (Fig. 2**).** Among the eight isolates, four strains showed comparatively higher EPS production. Notably, *Limosilactobacillus fermentum* GD29 exhibited the highest yield, followed by *Limosilactobacillus fermentum* GDS82, *Limosilactobacillus fermentum* GD6, and *Lactobacillus plantarum* GD26.

#### 3.2.3. Purification of EPS

EPS was purified using the ethanol precipitation method. During the fermentation period, the highest EPS production was observed as a white, mucoid substance. The recovered exopolysaccharide was obtained as a dry powder **(**Fig. 3**).** Subsequently, EPS was extracted by cold ethanol precipitation, yielding approximately 12 g/L. Under optimized conditions, the potent postbiotic strains—*Limosilactobacillus fermentum* GD29, *Limosilactobacillus fermentum* GDS82, *Limosilactobacillus fermentum* GD6, and *Lactobacillus plantarum* GD26—were cultured at a larger scale (5 L). The maximum EPS yield was observed after 3 days of incubation. Among the isolates, *Limosilactobacillus fermentum* GD29 exhibited the highest EPS production (17 g/L), followed by

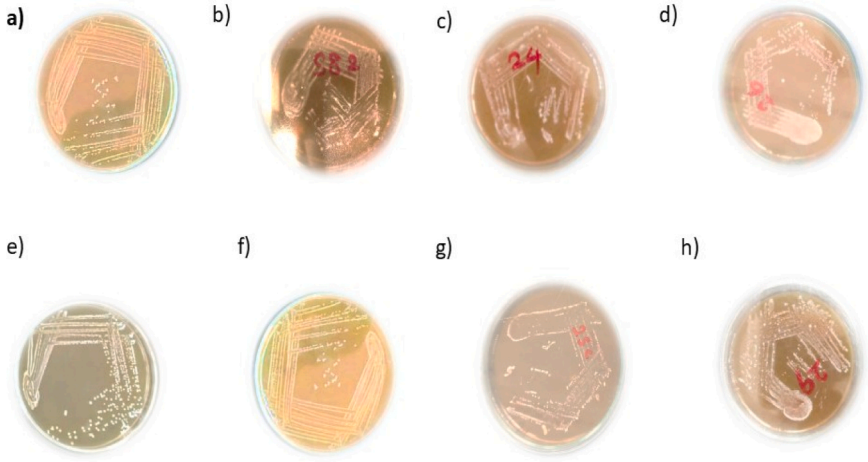


**Fig. 1. Cultural morphology of LAB isolates on MRS agar.** a)*Limosilactobacillus fermentum* GD13, b) *Lactobacillus plantarum* GDS82, c) *Limosilactobacillus fermentum* GD24, d) *Lactobacillus plantarum* GD26, e). *Lactobacillus helviticus* GD10, f). *Lactiplantibacillus plantarum* GD14, g). *Lactobacillus helviticus* GD2SC, h) *Limosilactobacillus fermentum* GD29.

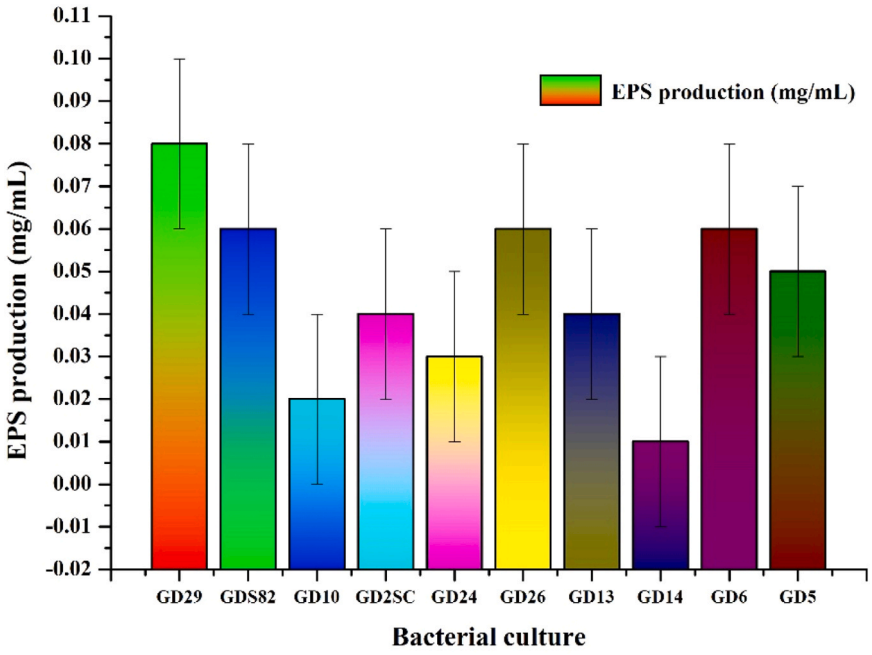


**Fig. 2.** EPS production yield from *Lactobacillus* spp.

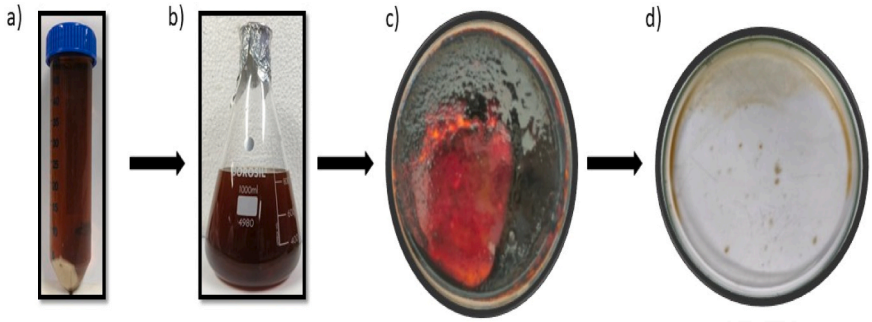


**Fig. 3.** EPS production medium and crude EPS recovery from ethanol precipitation a) EPS precipitation, b) Large scale production medium, c) Crude EPS, d) Dry EPS powder.

*Limosilactobacillus fermentum* GDS82 (13.2 g/L), *Limosilactobacillus fermentum* GD6 (8.7 g/L), and *Lactobacillus plantarum* GD26 (6.89 g/L).

### 3.2.4. Appearance and solubility of the EPS

Among the eight *Lactobacillus* spp., the extracted extracellular polymeric substances (EPS) obtained after ethanol precipitation exhibited a gel-like appearance and were readily soluble in water.

### 3.2.5. Estimation of carbohydrates on bacterial EPS

The total carbohydrate content of the eight postbiotic EPS samples was determined using the phenol–sulfuric acid method. Among the eight EPS-producing cultures, *Limosilactobacillus fermentum* GD29, *Lactobacillus helveticus* GD10, and *Lactiplantibacillus plantarum* GD14 exhibited higher carbohydrate content compared to the other isolates. Glucose was used as the standard, and the results are presented in Fig. 4**a.**

### 3.2.6. Reducing sugar analysis by Dinitro Salicylic Acid (DNS)

Reducing sugars react with the DNS alkaline reagent to form a colored complex, which is measured spectrophotometrically at 575 nm. Among the isolates, *Limosilactobacillus fermentum* GD29 exhibited the highest reducing sugar content in the EPS samples (Fig. 4**b).**

### 3.2.7. Estimation of protein on bacterial EPS

The total protein content of the eight bacterial cultures was determined using the Lowry method. Among the eight cultures, *Limosilactobacillus fermentum* GD29 and *Lactobacillus helveticus* GD2SC exhibited the highest protein content **(**Fig. 4**c).**

## 3.3. Functional characterisation of potent EPS molecules

Both qualitative and quantitative analyses were conducted to evaluate extracellular polymeric substances (EPS) production in five potent EPS-producing LAB strains, namely *Limosilactobacillus fermentum* GD29, *Limosilactobacillus fermentum* GDS82, *Limosilactobacillus fermentum* GD6, *Lactobacillus helveticus* GD2SC, and *Lactobacillus plantarum* GD26. These strains were selected based on their comparatively high EPS yields.

To further investigate the chemical characteristics of the produced EPS, advanced analytical techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography–Mass Spectrometry (GC–MS), and Nuclear Magnetic Resonance (NMR), were employed.

### 3.3.1. Fourier transform infrared (FT-IR)

The functional groups present in the purified extracellular polymeric substances (EPS) were analyzed using FT-IR spectroscopy, revealing a diverse range of chemical bonds **(**Fig. 5**).** Strong absorption bands

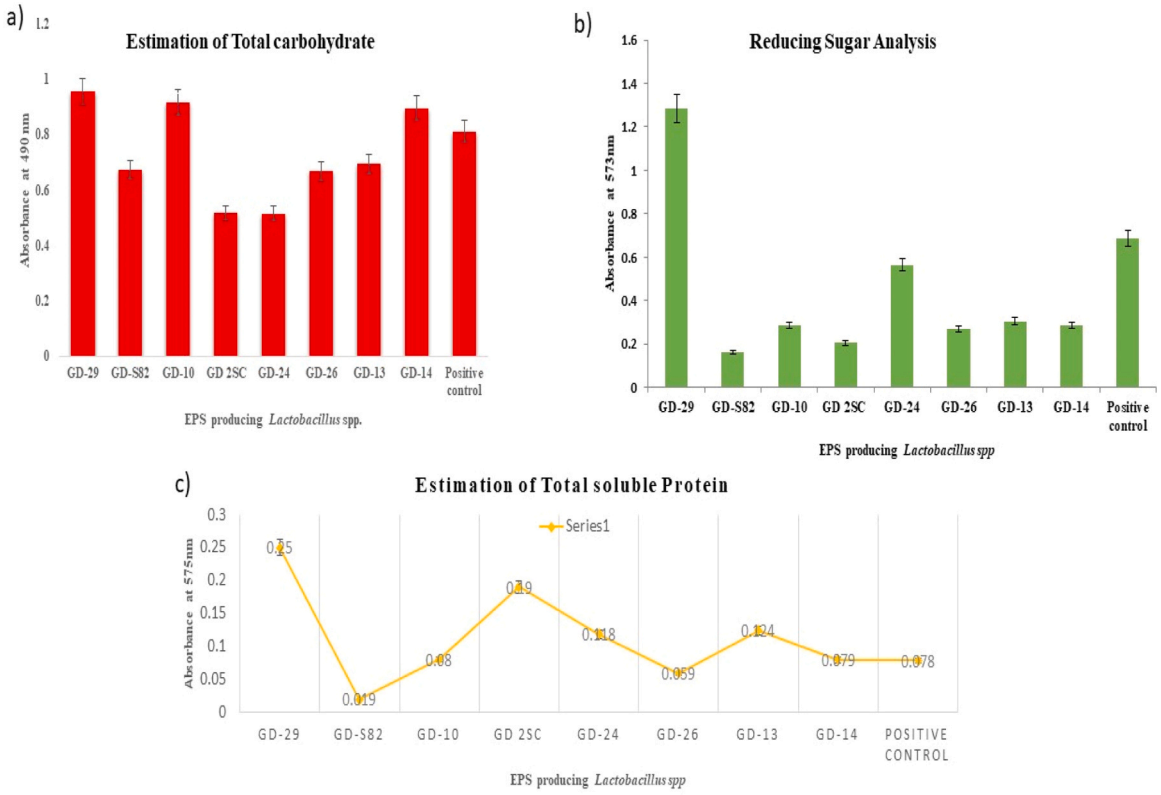


**Fig. 4.** a) Phenol-Sulphuric Acid Method for EPS detection (Total carbohydrate) b) Dinitro Salicyclic Acid (DNS) method for Reducing Sugar Analysis c) Lowry's Assay for Total Soluble Protein.

corresponding to hydroxyl (O–H) stretching vibrations, indicative of alcohol groups, were observed at multiple wavenumbers, including 3782.69, 3845.36, 3788.47, 3726.76, 3786.00, 3340.10, 3338.18, 3350.71, and 3310.21 $cm^{-1}$ . Peaks associated with secondary alcohols and N–H stretching vibrations of aliphatic primary amines were detected in the region of 2362.37–2336.69 $cm^{-1}$ , while a prominent signal corresponding to carbon dioxide (O═C═O stretching) was observed near 2359.48 $cm^{-1}$ . The presence of sulfonate (S═O) groups was identified at 1371.14 $cm^{-1}$ , and peaks corresponding to vinyl ether (C–O) and secondary alcohol groups appeared at 1220.72 and 1221.68 $cm^{-1}$ , respectively. Alkane-related vibrations, including C–H stretching and bending, were observed at 2898.49, 2980.45, 1452.14, and 1482.99 $cm^{-1}$ . A characteristic peak for imine/oxime (C═N stretching) was detected at 1642.09 $cm^{-1}$ , while sulfonyl-related vibrations appeared at 1410.67 $cm^{-1}$ . Aromatic amine (C–N stretching) was identified at 1321.96 $cm^{-1}$ . Additionally, peaks corresponding to primary alcohols and alkyl aryl ethers (C–O stretching) were observed at 1273.75, 1272.79, 1275.68, 1085.73, and 1083.80 $cm^{-1}$ . The presence of aldehyde groups (C═O stretching) was indicated by a peak at 1733.69 $cm^{-1}$ , while alkene-associated vibrations (C═C stretching and bending) were observed at 1638.23 and 878 $cm^{-1}$ . Signals corresponding to isothiocyanate (N = C═S) were detected at 2112.64 and 1045.23 $cm^{-1}$ , and phenolic groups (O–H bending) appeared at 1044.26 and 1373.07 $cm^{-1}$ . Furthermore, peaks corresponding to nitro compounds (N–O) and anhydride groups (CO–O–CO) were observed at 1530.24 $cm^{-1}$ **(**Table 1**).**

The extracellular polymeric substances (EPS) produced by postbiotic strains exhibited a complex chemical composition, characterized by a diverse range of functional groups, including alcohols, carbon dioxide-related groups, isothiocyanates, aldehydes, alkenes, sulfonates, vinyl ethers, and phenolic compounds.

### 3.3.2. *Gas chromatography-mass spectrometry*

GC–MS analysis of the potent EPS-producing strain *Limosilactobacillus fermentum* GD29 revealed multiple peaks corresponding to a diverse range of organic compounds **(**Fig. 6**a;** Table 2**).** The chromatogram displayed several distinct peaks at different retention times, with prominent signals observed at 6.750 min, 37.685 min, and additional intermediate regions. Compound identification was performed using the WILEY8 spectral library. The major compounds detected included 4-(dimethylamino)azoestrone 3-methyl ether ($C_{21}H_{27}NO_2$), 5-(7′-chloroquinolin-4′-ylamino)-2-(pyrrolidin-1″-ylmethyl)phenol ($C_{18}H_{18}ClN_3O$), 1-acetyl-3-[(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)carbonyl]indole ($C_{18}H_{18}N_2O_2$), dodecane, 1,1-difluoro- ($C_{12}H_{24}F_2$), (3 R,4S)-3-ethyl-4-methyltetrahydrofuran-3-ol ($C_7H_{14}O_2$), pentadecane ($C_{15}H_{32}$), and cyclopentasiloxane, decamethyl- ($C_{10}H_{30}O_5Si_5$) **(**Fig. 6**b).** Compound identification was based on mass spectral data, including molecular ion peaks and fragmentation patterns, which were matched with entries in the spectral library. The results indicate that the EPS extract comprises a complex mixture of organic compounds that collectively contribute to its chemical profile. Notably, 4-(dimethylamino)azoestrone 3-methyl ether was identified as one of the prominent compounds, characterized by a structurally complex framework containing an estrone core modified with a dimethylamino group and an azo linkage. Its distinct structure and retention behavior highlight its significance among the detected components.

### 3.3.3. *Nuclear magnetic resonance spectrometer (NMR) analysis of EPS*

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for identifying the types and relative abundance of monosaccharide units present in the EPS backbone. By analyzing chemical shifts and coupling patterns corresponding to different sugar residues, the carbohydrate composition of EPS can be elucidated (Fontana and Widmalm, 2023). The $^{1}H$ and $^{1}$ $^{3}C$ NMR spectra of the EPS sample provide important insights into its molecular structure **(**Fig. 7**).** In the $^{1}H$

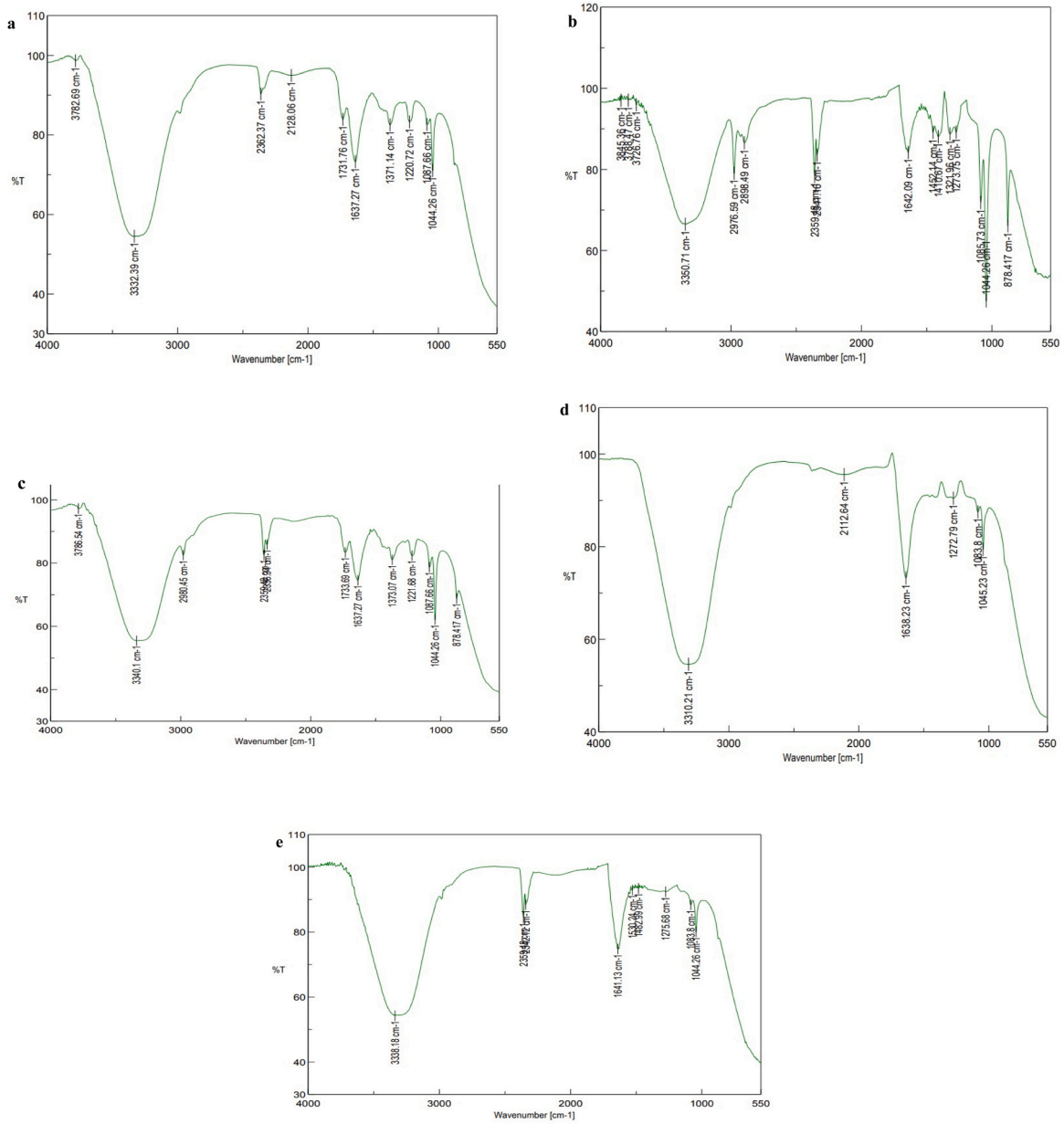


**Fig. 5.** FT-IR spectrum analysis of a. *Limosilactobacillus fermentum* GD29, b. *Lactobacillus helviticus* GD2SC, c. *Limosilactobacillus fermentum* GD6, d, *Lactiplantibacillus plantarum* GD26, e. *Limosilactobacillus fermentum* GDS82.

NMR spectrum, signals were observed at 1.062–1.097 ppm, 1.916 ppm, 3.528–3.581 ppm, and 4.697 ppm. The signals at 1.062–1.097 ppm are characteristic of methyl ($CH_3$) protons in an aliphatic environment, while the peak at 1.916 ppm corresponds to methylene ($CH_2$) protons. The signals in the range of 3.528–3.581 ppm are indicative of protons attached to carbon atoms bonded to electronegative atoms such as oxygen, which is typical of polysaccharide structures. The resonance at 4.697 ppm is associated with anomeric or hydroxyl-associated protons, suggesting the presence of carbohydrate moieties. The $^{1}{}^{3}C$ NMR spectrum displayed peaks at 16.84 ppm and 57.51 ppm. The signal at 16.84 ppm corresponds to aliphatic carbons, while the peak at 57.51 ppm indicates carbon atoms bonded to oxygen, supporting the presence of alcohol or ether functional groups commonly found in polysaccharides. Overall, the combined NMR data indicate the presence of carbohydrate-based structures with aliphatic and oxygenated functional groups, which are characteristic of EPS. These findings support the structural complexity of the EPS and confirm the presence of functional groups and linkages typical of microbial polysaccharides.

### 3.4. Antibacterial activity of EPS molecule

The antibacterial activity of EPS derived from *Limosilactobacillus fermentum* GDS82, *Lactobacillus helveticus* GD2SC, *Lactobacillus plantarum* GD26, and *Limosilactobacillus fermentum* GD29 was evaluated against foodborne pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Vibrio cholerae*. Using the agar well diffusion method, the EPS samples demonstrated notable antibacterial activity. The inhibition zones were measured in millimeters, with *Lactobacillus plantarum* GD26 and *Limosilactobacillus fermentum* GD29 exhibiting inhibition zones of 11 mm and 14 mm, respectively, against *E. coli*. Additionally, EPS from *Limosilactobacillus fermentum* GD29 showed an inhibition zone of 12 mm against *Salmonella* spp. Among the tested strains, *Limosilactobacillus fermentum* GD29 exhibited the strongest antibacterial activity, effectively inhibiting all four tested pathogens.

**Table 1**
FT-IR spectrum frequency with structure and functional group of Limosilactobacillus fermentum GD29, Limosilactobacillus fermentum GD S82, Limosilactobacillus fermentum GD6, Lactobacillus helveticus GD2SC, and Lactobacillus plantarum GD26.

| S. No | Frequency ($cm^{-1}$) | Structure | Functional group |
|---|---|---|---|
| 1 | 3782.69 | O-H stretch | Alcohol |
| 2 | 3332.39 | N-H | Aliphatic primary amine |
| 3 | 2362.37 | O═C═O stretch | Carbon dioxide |
| 4 | 2128.06 | N = N = N stretch | Azide |
| 5 | 1731.76 | C═O | Aldehyde |
| 7 | 1371.14 | S═O | Sulfonate |
| 8 | 1220.72 | C-O | Vinyl ether |
| 9 | 3845.36 | O-H stretch | Alcohol |
| 10 | 3788.47 | O-H stretch | Alcohol |
| 11 | 3726.76 | O-H stretch | Alcohol |
| 12 | 3350.71 | N-H stretch | Aliphatic primary amine |
| 13 | 2976.59 | C-H stretch | Alkane |
| 14 | 2898.49 | C-H stretch | Alkane |
| 15 | 2359.48 | O═C═O | Carbon dioxide |
| 16 | 2341.16 | O═C═O | Carbon dioxide |
| 17 | 1642.09 | C═N stretch | Imine/oxime |
| 18 | 1452.14 | C-H bend | Alkane |
| 19 | 1410.67 | S═O stretch | Sulfonyl chloride |
| 20 | 1321.96 | C-N stretch | Aromatic amine |
| 21 | 1273.75 | C-O stretch | Alkyl aryl ether |
| 22 | 1085.73 | C-O stretch | Primary alcohol |
| 23 | 878.417 | C═C bend | Alkene |
| 24 | 3786.54 | O-H stretch | Alcohol |
| 25 | 3340.1 | O-H stretch | Alcohol |
| 26 | 2980.45 | C-H stretch | Alkane |
| 27 | 2359.48 | O═C═O stretch | Carbon dioxide |
| 28 | 2336.34 | O═C═O stretch | Carbon dioxide |
| 29 | 1733.69 | C═O stretch | Aldehyde |
| 30 | 1637.27 | C═C stretch | Alkene |
| 31 | 1373.07 | O-H bend | Phenol |
| 32 | 1221.68 | C-O stretch | Vinyl ether |
| 33 | 1087.66 | C-O stretch | Secondary alcohol |
| 34 | 3310.21 | N-H stretch | Secondary amine |
| 35 | 2112.64 | N = C═S Stretch | Isothiocyanate |
| 36 | 1638.23 | C═C stretch | Alkene |
| 37 | 1272.79 | C-O stretch | Alkyl aryl ether |
| 38 | 1083.8 | C-O stretch | Primary alcohol |
| 39 | 1045.23 | CO-O-CO | Anhydride |
| 40 | 3338.18 | N-H stretch | Aliphatic primary amine |
| 41 | 2359.48 | O═C═O | Carbon dioxide |
| 42 | 2342.12 | O═C═O | Carbon dioxide |
| 43 | 1641.13 | C═C stretch | Alkene |
| 44 | 1530.24 | N-O stretch | Nitro compound |
| 45 | 1482.99 | C-H bend | Alkane |
| 46 | 1275.68 | C-O stretch | Alkyl aryl ether |
| 47 | 1044.26 | CO-O-CO | Anhydride |

These findings indicate that EPS derived from *Limosilactobacillus fermentum* GD29 and *Lactobacillus plantarum* GD26 possess significant antibacterial potential against foodborne pathogens **(**Fig. 8**).**

## 4. Discussion

The present study aimed to evaluate the ability of eight lactic acid bacteria (LAB) isolates to produce extracellular polymeric substances (EPS) and to explore their potential industrial applications. The findings demonstrated considerable variation in EPS production among the isolates, with *Limosilactobacillus fermentum* GD29 exhibiting the highest yield and desirable properties for potential applications. Lactic acid bacteria are increasingly recognized in the food industry due to their strong fermentative capabilities and their ability to produce valuable metabolites such as EPS, which contribute to the quality and functionality of fermented foods, particularly dairy products. EPS are known to exhibit diverse biological activities, including antibacterial, antioxidant, antihypertensive, and anticancer properties, and they may also enhance the survival of probiotic strains through their role in extracellular matrix formation (Rajoka et al., 2019). Furthermore, EPS molecules often possess charged functional groups that facilitate water retention and ion-binding capacity, thereby contributing to their functional and technological properties (Liu et al., 2017). Previous studies have also reported EPS production from various *Limosilactobacillus fermentum* strains, highlighting the ongoing interest in their characterization and application (Wei et al., 2019).

In the present investigation, both qualitative and quantitative analyses confirmed that all eight LAB isolates were capable of producing EPS, although with varying efficiencies. The formation of mucoid and ropy colonies on culture media further indicated active EPS production. The maximum yield recorded in this study was 0.235 g/mL, suggesting strong biosynthetic potential, particularly in *Limosilactobacillus fermentum* GD29. The observed viscous and gel-like characteristics of the EPS in both liquid and solid media further support its high production capacity.

It is well established that culture conditions, especially medium composition, play a crucial role in influencing EPS production by LAB. Previous reports have emphasized the importance of carbon sources and nutrient composition in enhancing EPS yield (Prete et al., 2021). Various media formulations, including skim milk, whey-based media, semi-defined media, chemically defined media, and basal minimal media, have been employed for optimized EPS production. Among these, milk-based media supplemented with suitable carbon sources have been shown to significantly enhance EPS synthesis, thereby improving both yield and functional properties.

Our findings demonstrate that cold ethanol precipitation is an effective method for EPS purification, yielding approximately 12 g/L. This indicates that prolonged incubation can enhance EPS production, supporting its potential for large-scale industrial applications. Efficient extraction and purification are essential steps for accurately evaluating the physical, chemical, and biological properties of EPS. Techniques such as ion-exchange chromatography and gel filtration are commonly employed to obtain highly purified EPS fractions.

Previous studies have reported *Lactobacillus fermentum* YL-11 as a promising EPS-producing strain isolated from fermented milk. However, it has also been noted that *Lactobacillus* species generally produce lower amounts of EPS compared to other lactic acid bacteria, often synthesizing heteropolysaccharides in relatively limited quantities. This can restrict their economic feasibility unless production conditions are optimized. EPS synthesis typically reaches its maximum during the exponential growth phase and decreases in later stages of fermentation, suggesting that these polymers may also serve as alternative carbon reserves.

Reported EPS yields in earlier studies include 47.4 mg/L for *Lactobacillus fermentum* 139, 55.1 mg/L for strain 263, and 55.6 mg/L for strain 296. In contrast, certain strains such as *Lactobacillus fermentum* Lf2 have demonstrated improved production levels of up to 1 g/L under optimized conditions. Recent investigations have further highlighted the structural diversity of EPS produced by LAB, including α-glucans commonly synthesized by genera such as *Lacticaseibacillus*, *Pediococcus*, and *Weissella*. Despite these advances, most LAB strains typically produce less than 1 g/L of EPS under non-optimized conditions, with *Limosilactobacillus fermentum* generally yielding around 0.75–0.85 g/L.

Fermented milk enriched with EPS may enhance its retention time within the gastrointestinal tract, thereby supporting the temporary colonization of probiotic bacteria. Furthermore, certain EPS can be metabolized in the colon to produce short-chain fatty acids (SCFAs), such as butyric acid, which are associated with various health benefits (Valentino et al., 2024).

The present study demonstrated that EPS-producing strains contain substantial levels of carbohydrates, proteins, and reducing sugars, as determined using the DNS assay. This compositional profiling is essential for evaluating the potential applications of EPS. In particular, a high carbohydrate content is advantageous for applications in the food and pharmaceutical industries, where EPS can function as thickeners, stabilizers, and bioactive agents. EPS derived from *Lactobacillus* species are primarily composed of monosaccharides such as glucose, galactose,

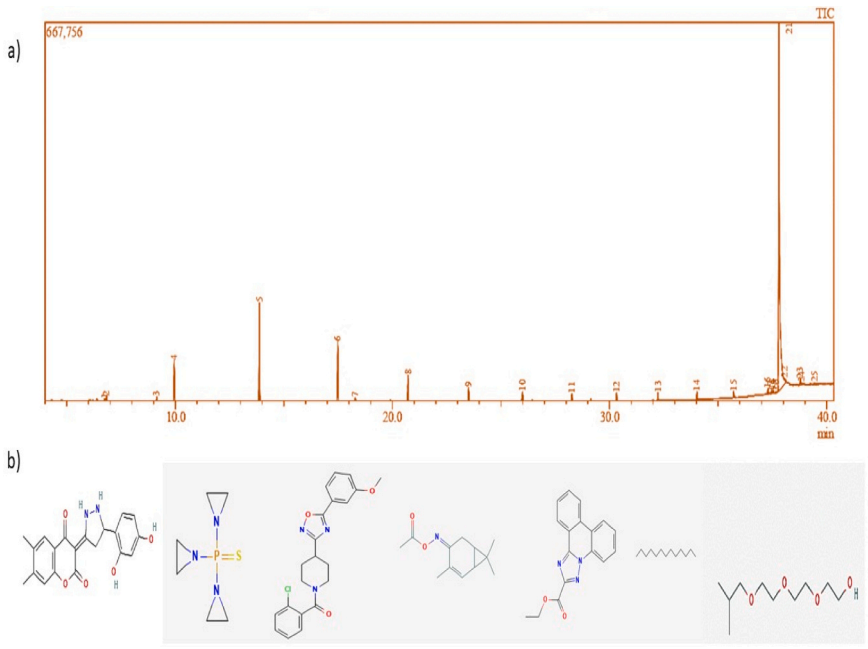


**Fig. 6.** Chemical structure sugar molecules using GC-MS.

mannose, and rhamnose. In addition, components such as N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, and glycerol may also be present in varying proportions within the EPS structure (Shah et al., 2024).

Fourier-transform infrared (FT-IR) spectroscopy further confirmed the presence of diverse functional groups within the EPS, including hydroxyl (O–H), carbonyl (C═O), aldehyde, alkene, sulfonate, vinyl ether, phenolic, and isothiocyanate groups. These functional groups play a significant role in determining the physicochemical and biological properties of EPS, thereby enhancing its functional versatility. The detection of such groups indicates a structurally complex biopolymer with broad potential for industrial applications (Behare et al., 2013; Mekonnen, 2023). Additionally, the FT-IR spectra exhibited prominent absorption bands corresponding to hydroxyl groups, along with less intense peaks associated with C–H and C–O bonds, and characteristic signals indicative of carbonyl functionalities (Wang et al., 2020).

Recent studies by Liu et al. (2024) reported that EPS produced by *Lactobacillus fermentum* V10 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 285 are heteropolysaccharides, with glucose:rhamnose: galactose ratios of 1:13:1.5 and 1:6.8, respectively. These findings highlight common monosaccharide compositions typically observed among lactic acid bacteria (Berthold-Pluta et al., 2019). Similarly, Korcz described α-D-glucan homopolysaccharides produced by *Limosilactobacillus fermentum*, particularly dextran with α-(1→6)-linked D-glucopyranose units, exhibiting molecular weights ranging from $10^3$ to $10^7$ Da.

Such homopolysaccharides are widely utilized in the food industry due to their functional properties, including improving softness, crumb structure, and loaf volume in bakery products, as well as preventing crystallization, enhancing moisture retention, and increasing viscosity in confectionery and ice cream applications (Jurášková et al., 2022). In addition, these polymers can function as emulsifiers, stabilizers, and adjuvants in various formulations. Studies by Daba et al. (2021) and Ale et al. (2020) further emphasized that while repeating units of these polysaccharides are synthesized intracellularly, their polymerization occurs extracellularly.

Nuclear Magnetic Resonance (NMR) spectroscopy plays a crucial role in elucidating the structural characteristics of EPS. Typically, $^1$H NMR spectra display signals in the anomeric proton region (δ 4.5–5.5 ppm), ring proton region (δ 3.1–4.5 ppm), and alkyl region (δ 1.2–2.3 ppm). Correspondingly, $^{1\ 3}$C NMR spectra reveal peaks in the anomeric carbon region (δ 95–110 ppm) and ring carbon region (δ 50–85 ppm). These spectral features are indicative of complex polysaccharide structures, often consisting of multiple repeating units.

Furthermore, FT-IR analysis revealed characteristic absorption peaks at 1226.7 $cm^{-1}$ , 1058.9 $cm^{-1}$ , and 1413.8 $cm^{-1}$ , corresponding to key functional groups such as carbonyl (C═O) and carboxyl groups. An additional peak at 584.4 $cm^{-1}$ suggests the presence of alkyl groups. These observations are consistent with earlier reports describing similar functional group profiles in EPS derived from *Lactobacillus* species, confirming the structural complexity and functional diversity of these biopolymers (Pajaro-Castro et al., 2014; Radhamanalan and Dharumadurai, 2023).

The present study highlights the notable antibacterial activity of EPS-producing strains against common foodborne pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Vibrio cholerae*. Among the isolates, *Limosilactobacillus fermentum* GD29 demonstrated the strongest inhibitory effects, indicating its potential as a promising candidate for the development of natural antimicrobial agents and food preservatives. Such properties may contribute to extending the shelf life of food products while enhancing safety by reducing the risk of microbial contamination (Sørensen et al., 2022; Yadav et al., 2011).

EPS are predominantly produced by lactic acid bacteria, although a limited number of *Bifidobacterium* species have also been reported to synthesize these polymers. Previous studies have shown that *Lactobacillus fermentum* AI2 exhibits antimicrobial activity against clinical pathogens such as *E. coli* NG 502121 and *S. aureus* AY 507047 in co-culture systems, suggesting the involvement of bioactive metabolites beyond organic acids, potentially including EPS. Furthermore, Yu et al. (2023) reported that LAB-derived EPS exhibit inhibitory effects against both Gram-positive and Gram-negative foodborne pathogens, along with the ability to competitively exclude harmful bacteria in the gastrointestinal tract. For example, EPS-Ca6 isolated from *Lactobacillus* sp. Ca6 demonstrated antimicrobial activity against *Salmonella enterica* ATCC 43972 and *Micrococcus luteus*. Similarly, Sharma et al. (2020) observed inhibition zones ranging from 13 to 16 mm for purified EPS against various test organisms. These findings collectively support the role of EPS in suppressing pathogenic microorganisms and promoting host safety by modulating intestinal microflora (Korcz and Varga, 2021).

The EPS produced by *Limosilactobacillus fermentum* GD29 exhibits significant potential for diverse applications. In the food industry, it may

**Table 2**
List of compounds identified by GC-MS and their application.

| S. No | RT (min) | Compound Name | Molecular Formula | Molecular Weight (g/mol) | Application |
|---|---|---|---|---|---|
| 1 | 6.75 | Acetophenone | $C_{11}H_{12}O_2$ | 176 | Pharmaceutical precursor, solvent, fragrance. Used in the synthesis of various pharmaceuticals, and as a flavoring agent or fragrance in cosmetics and personal care products |
| 2 | 6.825 | Dodecane | C12H24F2 | 206 | Solvent, chemical analysis. Commonly used as a solvent or reference material in chromatographic analysis. |
| 3 | 9.95 | Cyclopentasiloxane | C10H30O5Si5 | 370 | Cosmetics, lubricants. Widely used in personal care products for its smooth texture and spreadability, as well as in industrial lubricants. |
| 4 | 13.87 | Cyclohexasiloxane | C12H36O6Si6 | 444 | Industrial applications. Used in manufacturing and chemical processes, particularly in silicone-based products. |
| 5 | 17.48 | Cycloheptasiloxane | C14H42O7Si7 | 518 | Industrial applications. Utilized in the production of silicone-based products and as a lubricant or sealant. |
| 6 | 18.285 | 2-Tert-Butyl-4-(1,1,3,3-Tetramethylbutyl) Phenol | C18H30O | 262 | Antioxidant. Used to stabilize polymers, rubbers, and fuels by preventing oxidative degradation. |
| 7 | 20.715 | Benzoic acid | C16H30O4Si3 | 370 | Food preservative. Commonly used as a preservative in food, cosmetics, and pharmaceutical products due to its antimicrobial properties. |
| 8 | 23.51 | Cyclononasiloxane | C18H54O9Si9 | 666 | Industrial applications. Used in the production of silicone elastomers, resins, and as a lubricant or sealant. |
| 9 | 25.995 | 20-IcosamethylcyclodecasiloxaneI | C20H60O10Si10 | 740 | Industrial applications. Found in the manufacturing of silicone-based materials and products. |
| 10 | 28.265 | Trimethylsilyl ester | C16H30O4Si3 | 370 | Industrial, chemical synthesis. Used in organic synthesis and as a derivatizing agent in analytical chemistry. |
| 11 | 30.325 | Octadecamethyl | C18H54O9Si9 | 666 | Industrial applications. Used in silicone-based products for lubrication and sealing. |
| 12 | 32.225 | Cyclononasiloxane | C18H54O9Si9 | 666 | Industrial applications. Similar to other cyclo-siloxanes in silicone manufacturing and as a lubricant. |
| 13 | 34.025 | 3,4-Dihydroxymandelic acid | C20H40O5Si4 | 472 | Industrial applications. Used in chemical synthesis and potentially in the manufacturing of specialty chemicals. |
| 14 | 35.715 | Cyclononasiloxane | C18H54O9Si9 | 666 | Industrial applications. Repeated identification in the sample indicates its prevalence in silicone manufacturing. |
| 15 | 37.29 | Cyclododecasiloxane | C24H72O12Si12 | 888 | Industrial applications. Used in high-performance silicone materials. |
| 16 | 37.435 | 1H-Indole | C17H18N2O2 | 282 | Pharmaceutical, research. Found in the synthesis of various pharmaceuticals and as a research chemical. |
| 17 | 37.48 | 1-Bromomethyl-3 | C14H23Br | 270 | Chemical synthesis. Used as an intermediate in organic synthesis. |
| 18 | 37.56 | Orotic Acid | C23H46N2O4Si3 | 498 | Pharmaceutical research. Known for its role in the synthesis of nucleotide and nucleic acid derivatives. |
| 19 | 37.685 | 3-Dimethyl | C10H14Cl2O | 220 | Industrial applications. Likely used in chemical synthesis or as an intermediate. |
| 20 | 37.8 | 13-Docosenamide | C22H43NO | 337 | Anti-inflammatory, surfactant. Used in formulations as a surfactant or for its anti-inflammatory properties. |
| 21 | 38.065 | Cyclopenta[*e*]tetrahydropyran-2-one | C18H22O3 | 286 | Industrial applications. Utilized in chemical manufacturing and synthesis. |
| 22 | 38.785 | Octadecamethyl | C18H54O9Si9 | 666 | Industrial applications. Repeated identification, consistent with silicone-based uses. |
| 23 | 38.82 | Pentanentrile | C6H11NO | 113 | Industrial applications. Used in chemical synthesis and as a solvent. |
| 24 | 39.43 | 5-(7′-Chloroquinolin-4′-ylamino) | C20H20ClN3O | 353 | Medicinal chemistry. Used in the development of pharmaceutical agents |

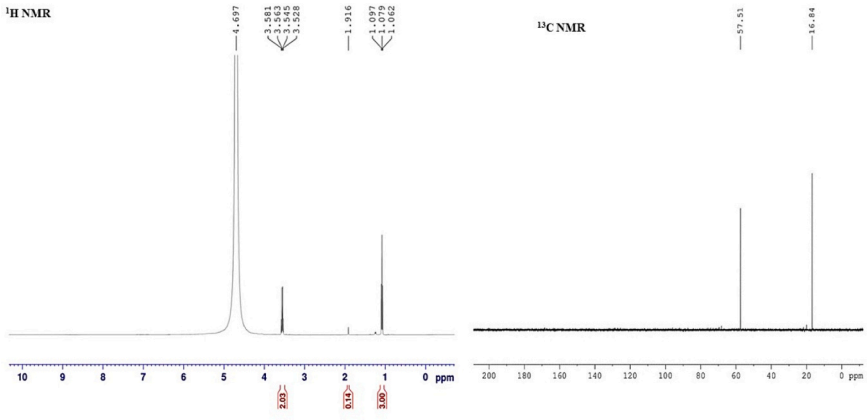


**Fig. 7.** Structural analysis of EPS by $^{1}$H NMR and $^{13}$C NMR Spectrum.

serve as a natural thickener, stabilizer, and texture-enhancing agent due to its gel-like consistency and water solubility (Zubkov and Kouznetsov, 2023). Additionally, the presence of bioactive components suggests its suitability for incorporation into functional foods with health-promoting benefits (Balyan et al., 2025). Its antimicrobial properties further support its application as a natural preservative, aligning with the increasing demand for clean-label and minimally processed food products.

Beyond food applications, the functional groups identified through FT-IR and NMR analyses indicate that this EPS may also be useful in

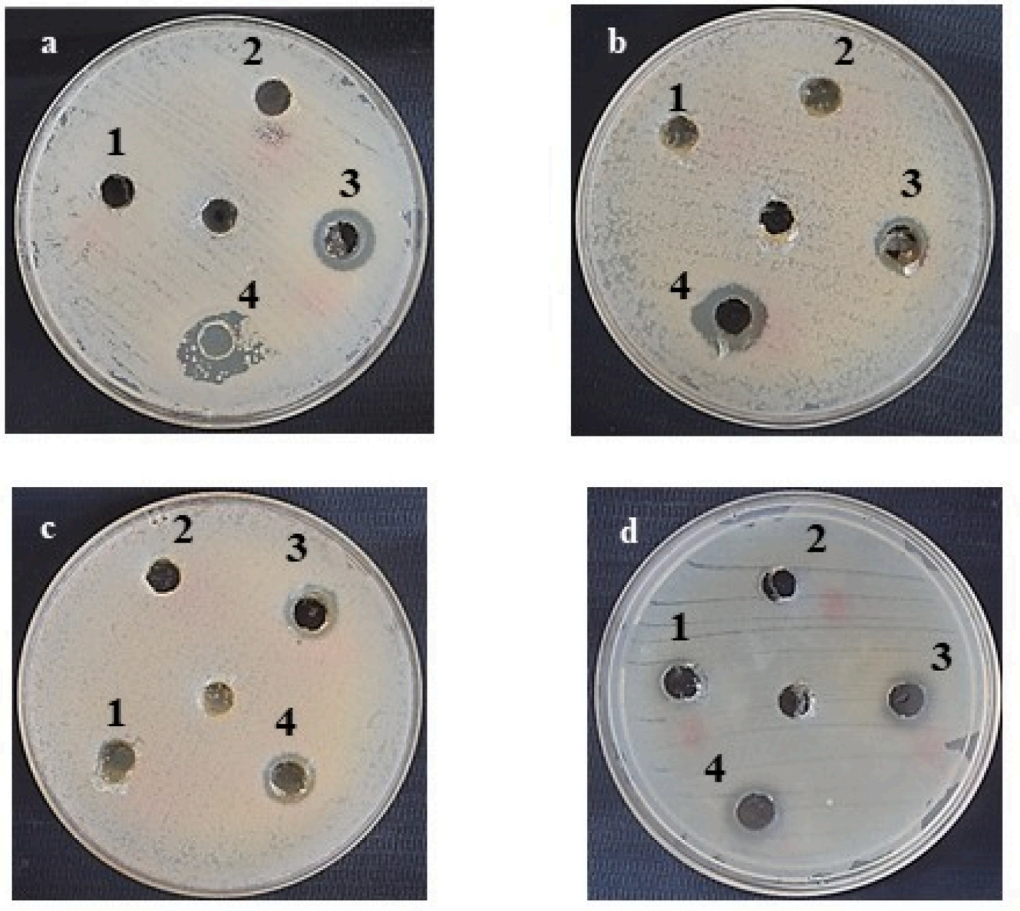


**Fig. 8.** EPS molecules demonstrated antibacterial activity against several bacteria, including a) *E. coli, b) Salmonella, c) S. aureus*, and d) *V. cholerae.* The specific strains tested for this activity were 1. *Limosilactobacillus fermentum* GDS82, 2. *Lactobacillus helveticus* GD2SC, 3. *Lactobacillus plantarum* GD26, 4. *Limosilactobacillus fermentum* GD29 and C -Control.

industrial and biotechnological sectors. These include potential applications in biodegradable materials, biofilm-like matrices, and environmentally sustainable products. The presence of diverse sugar components and monosaccharides further enhances its applicability in pharmaceutical and biotechnological formulations, highlighting its versatility as a multifunctional biopolymer.

## 5. Conclusions

This study identified eight LAB species with high EPS production capacity. Among them, *Lb. fermentum* GD29 emerged as a potent EPS producer with significant potential for industrial and biotechnological applications. The high yield, favorable functional properties, and strong antibacterial activity of the EPS underscore its value in the food, pharmaceutical, and industrial sectors. However, *in vivo* studies remain limited, necessitating further research to increase EPS yield and expand the industrial applications. More *in vivo* studies are required to validate the full potential of EPS as functional adjuncts. Future research should focus on optimizing production conditions and exploring the comprehensive range of applications of EPS from *Lb. fermentum* GD29, ensuring sustainable and efficient use in the food industry. EPS derived from *Lb. fermentum* can used as food preservatives and microbial control agents.

## Author Contributions

GR & DD: Concept, Investigation, Writing and Reviewing, Interpretation. GR & SS: Data collection, Analysis, and Methodology, GR and DD: Final draft and Supervision. All authors have read and approved the final version of the manuscript.

## CRediT authorship contribution statement

**RADHAMANALAN GUHANRAJ:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Dhanasekaran Dharumadurai:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **Sowmiya Soundararajan:** Writing – review & editing, Validation, Supervision, Resources, Methodology.

## Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI tools were used in the preparation of this manuscript.

## Declaration of Competing Interest

None.

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## Data availability

Data will be made available on request.

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