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RESEARCH ARTICLE

Characterization, Antibacterial and Antioxidant Potential of Exopolysaccharide Produced by *Limosilactobacillus reuteri* MK1 Isolated from Poultry Intestine

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ABSTRACT

Exopolysaccharides (EPSs) synthesized by lactic acid bacteria (LAB) have garnered significant attention due to their potential health-promoting properties. In this study, we characterized and evaluated the biological activities of EPS produced by *Limosilactobacillus reuteri* MK1, a strain isolated from chicken intestines. The MK1 strain was identified using 16S rRNA gene sequencing. The structural characterization of the EPS was performed through Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy followed by analysis of physicochemical properties. The EPS exhibited strong emulsifying activity, particularly with xylene (72.8%), as well as excellent water and hydrocarbon solubility. Antioxidant potential was assessed via DPPH and H_2O_2 radical scavenging assays, revealing concentration-dependent activities of 64% and 70%, respectively at a concentration of 10 mg/mL. The EPS also demonstrated notable antibacterial activity against tested pathogenic bacterial strains. Optimization of culture conditions enhanced EPS production, achieving a maximum yield of 585 mg/L after 33 hours of incubation. FTIR and NMR analyses confirmed the polysaccharide structure and identified key functional groups of the macromolecule. These results collectively demonstrate that the EPS from *L. reuteri* MK1 is a multifunctional biopolymer with versatile capabilities, including emulsification, radical scavenging, and pathogen inhibition, positioning it as a promising natural additive for food, pharmaceutical, or nutraceutical applications. Future research should focus on scaling production processes and exploring targeted applications in pharmaceutical and nutraceutical industries.

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INTRODUCTION

Lactic acid bacteria (LAB) are widely recognized as safe and beneficial for human health due to their roles in immunomodulation, antianaphylaxis, enhanced nutrient absorption, and cholesterol regulation (Afzal *et al.*, 2020; Aslam *et al.*, 2023; Khurshid *et al.*, 2015; Rashid *et al.*, 2023). Beyond their probiotic effects, LAB synthesize bioactive metabolites such as exopolysaccharides (EPS), organic acids, bacteriocins, and aromatic compounds which hold significant potential for food and pharmaceutical applications (Saleem *et al.*, 2021; Srifani *et al.*, 2023; Susalam *et al.*, 2024). Among these metabolites, EPS has garnered attention for its versatile physicochemical properties, including roles as an emulsifier, stabilizer, gelling agent, and absorbent in food and cosmetics. EPS also exhibits bioactive properties such as antioxidant, antimicrobial, and anticancer activities, positioning it as a promising candidate for nutraceutical and pharmaceutical use (Oleksy & Klewicka, 2018; Wu *et al.*, 2023).

LAB-derived EPS are typically composed of high-molecular-weight, water-soluble heteropolysaccharides with diverse sugar compositions and three-dimensional structures. Studies have demonstrated that LAB metabolites, including EPS, enhance intestinal function, mucosal immunity, and systemic immune responses (Cristofori *et al.*, 2021; Liu *et al.*, 2020). For instance, EPS from LAB strains such as *L. reuteri* L26 have shown immunomodulatory effects comparable to tumor necrosis factor-α (TNF-α) in activating dendritic cells (Kiššová *et al.*, 2024).

Despite considerable research on EPS from LAB species like *L. plantarum*, *L. rhamnosus*, and *L. casei*, the EPS-producing potential of *L. reuteri* remains underexplored. Certain strains, such as *L. reuteri* Lb121 demonstrated high yields of α-glucan and β-fructan (up to 10 g/L), underscoring the industrial relevance of this species (Werning *et al.*, 2022; Zeidan *et al.*, 2017). However, knowledge gaps persist regarding the functional diversity of EPS from native *L. reuteri* strains and their full spectrum of biological activities. To address this knowledge gap, the present study focuses on the isolation of *L. reuteri* MK1 strain from the intestinal tract of healthy poultry followed by purification, characterization and detailed physicochemical profiling. Moreover, the functional potential of the EPS was evaluated by assessing its antimicrobial efficacy against pathogenic bacteria and its antioxidant capacity through radical scavenging assays. These investigations collectively seek to elucidate the bioactive and industrial relevance of *L. reuteri* MK1-derived EPS for its subsequent applicability in biomedical, nutraceutical, and industrial sectors. The emulsifying, stabilizing, and therapeutic properties of MK1-derived EPS could offer sustainable alternatives to synthetic additives.

MATERIALS AND METHODS

Bacterial isolation and cultivation: The bacterial strain was isolated from the intestinal tissue sample of a healthy chicken obtained from the Faisalabad poultry market in Pakistan. Specifically, approximately 0.1 g of intestinal tissue was homogenized in 1 mL of sterile phosphate-buffered saline (PBS, pH 7.0). The mixture was then transferred to an anaerobic environment using a Bactron Anaerobic Chamber (Shel Lab, USA). The serial dilutions were prepared in peptone-yeast extract-glucose (PYG) medium. A 100 µL aliquot of the diluted sample was spread onto PYG agar plates and incubated at 37°C for 72 hours under anaerobic conditions (gas phase: 95% N_2, 5% H_2, and 5% CO_2). Individual colonies were selected, purified, and subsequently identified for functional characterization. The isolated strain was designated as MK1 and was stored in a sterile 20% (v/v) glycerol solution at -80°C for preservation.

Molecular identification: The extraction of genomic DNA was performed using a DNA extraction kit (Favorgen Biotech Corp, Taipei City, Taiwan). Universal forward and reverse primers were used for PCR as previously described (Algarni, 2022). The amplified product was run on 1.5% agarose gel electrophoresis using a 100-bp plus Gene Ruler (DNA Ladder) (Thermo Scientific, USA). The amplicons were sent to Macrogen™ (Republic of Korea) for purification and sequencing. The sequences were aligned using MEGA-11 (Molecular Evolutionary Genetics Analysis) software and the phylogenetic tree was constructed employing the Maximum Likelihood approach. The final sequence was assembled and was submitted to GenBank to obtain an accession number.

Bacterial cell viability and Exopolysaccharide (EPS) quantification: *L. reuteri* MK-1 was cultured in 500 mL of MRS broth supplemented with 4% glucose to promote exopolysaccharide (EPS) production. The culture was incubated anaerobically at 37 °C for 48 hours. At regular intervals (0–48 hours), 1.0 mL aliquots of the bacterial suspension were withdrawn to measure pH levels. Microbial cell viability was assessed using a two-fold serial dilution method, followed by colony counting. Carbohydrate content was then quantified via the phenol-sulfuric acid method with glucose as the standard (Rajoka *et al.*, 2022).

Extraction of MK1-EPS: The EPS from strain MK-1 was extracted following a previously established method (Hu *et al.*, 2021) with minor modifications. Briefly, *L. reuteri* MK-1 was cultured in MRS broth supplemented with 4% glucose under anaerobic conditions at 37 °C for 48 hours. The cell-free culture supernatant (CFCS) was obtained by centrifuging the culture at 8000 × g and 4°C for 10 minutes. Trichloroacetic acid (4% final concentration) was added to the supernatant to precipitate proteins, and the mixture was incubated at 4°C for 6 hours. Following centrifugation (8000 ×g, 4°C, 20 minutes), the supernatant was combined with twice its volume of pre-chilled 95% ethanol and incubated at 4°C for 24 hours. The crude EPS was recovered via centrifugation, dissolved in distilled water, and purified sequentially using dialysis membranes (HR3-344: 6000–8000 Da molecular weight [Mw] cutoff; HR3-346: 12,000–14,000 Da Mw cutoff; Sigma-Aldrich, USA) against distilled water for 48 hours at 4°C. The dialyzed EPS was freeze-dried and further purified via DEAE-52 cellulose column chromatography, using double-distilled water as the eluent (flow rate: 1 mL/min). The purified EPS was freeze-dried again and stored at 4°C for subsequent analysis.

Characterization of EPS

FTIR analysis: Fourier-transform infrared (FTIR) spectroscopy was performed using an FTIR Spectrometer Tensor 27 (Bruker Optik GmbH, Germany) to identify functional groups in the MK-1-EPS. Spectra were recorded in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans per measurement. For analysis, 0.6 mg of the EPS sample was homogenized with 100 mg of potassium bromide (KBr) and pressed into a translucent pellet. The resulting spectra were processed and analyzed using OPUS software (Bruker, Germany).

NMR analysis: To acquire ^{1}H NMR and ^{13}C NMR spectra, the freeze-dried MK-1-EPS (15 mg) was dissolved in 1 mL of D_2O and analyzed at 600 MHz using a Bruker Avance III spectrometer (Bruker BioSpin, Germany).

Molecular weight (Mw) determination: The molecular weight of the EPS was determined using gel permeation chromatography (GPC) (Wyatt Technology, Goleta, CA, USA) equipped with Shodex SB-806 HQ and SB-804 HQ columns (Resonac, Tokyo, Japan). Refractive index (RI) and laser light scattering (LLS) measurements were performed at 25 °C. Briefly, the EPS solution was injected into the GPC system and eluted with ultrapure water at a flow rate of 1 mL/min.

Physicochemical characterization of bacterial EPS

Emulsifying activity (EA): The emulsifying activity (EA) of MK-1-EPS was evaluated following the already published method (Vinothkanna *et al.*, 2021). Briefly, 2 mL of each organic solvent (n-hexadecane, xylene, hexane, and toluene) was added to separate MK-1-EPS samples, vortexed for 2 minutes, and allowed to stabilize. The height of the emulsion layer was then measured. For the positive control, SDS (1% w/v) was used, and emulsifying activity was calculated using the formula:

Emulsifying activity (%) = Emulsion layer (height) / Total height × 100

Water holding ability (WHA): The water-holding ability (WHA) of MK-1-EPS was determined as follows: 0.2 g of MK-1-EPS was vortex-mixed with 10 mL of distilled water for 5 minutes. The mixture was then centrifuged at 12,000 × g for 30 minutes to remove unbound water and facilitate partial drying. For complete drying, the EPS was collected by filtration using pre-weighed filter paper (Shivangi *et al.*, 2020). The EPS was dried to constant weight and re-weighed. The WHA was calculated using the formula:

WHA (%) = weight of sample after absorption (total) / weight of dry residue × 100

Water solubility ability (WSA): The water solubility ability (WSA) of MK-1-EPS was analyzed as follows: MK-1-EPS was reconstituted in distilled water to a concentration of 50 mg/mL and agitated at 35 °C for a defined period. A 0.2 mL aliquot of the supernatant was mixed with sodium acetate solution (concentration/pH specified if available) to precipitate dissolved EPS. The mixture was centrifuged at 8000 × g at 4 °C for 15 minutes. The pellet was vacuum-dried at 50 °C, and the weight difference between the initial dry EPS and the recovered residue was measured (Shivangi *et al*., 2020). WSA was calculated using the formula:

WSA (%) = dry solids in supernatant (weight) / dry sample weight ×100

Biological activities of EPS MK-1

Antimicrobial potential: The antimicrobial activity of MK-1-EPS was evaluated through bacterial growth kinetic analysis following the already described method (Jeong *et al.*, 2017). Four pathogens including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella enterica* serovar Typhi were selected for testing. Bacterial strains were cultured in Luria-Bertani (LB) broth at 37°C for 24 hours. MK-1-EPS was filter-sterilized using 0.22µm pore membranes (Millipore, MA, USA) and added to LB broth. Aliquots (200µL) of the LB broth–EPS mixtures (25, 50, 75 and 100% concentrations) were dispensed into a 96-well microtiter plate. Bacterial suspensions (2µL, ~10^7 CFU/mL) were inoculated into each well. The positive control consisted of LB broth with bacterial suspension, while the negative control contained LB broth alone. The plate was transferred to a microplate spectrophotometer (INNO, LTEK, South Korea), and optical density at 600nm was measured every 2 hours for 16 hours at room temperature. Experiments were performed in triplicate, and mean values were reported.

Antioxidant assay

DPPH free radical scavenging assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of MK-1-EPS was assessed using already described protocol (Srinivash *et al.*, 2023). Briefly, 0.5mL of MK-1-EPS at varying concentrations (20, 40, 60, 80, and 100 µg/mL) was mixed with 0.5 mL of 0.1 mM DPPH solution (prepared in 95% methanol). The reaction mixture was incubated in the dark at 25°C for 30 minutes. Ascorbic acid (1 µg/mL) served as positive control, while 95% methanol acted as the blank. Absorbance was measured at 517 nm using a spectrophotometer, and scavenging activity was calculated as:

Scavenging activity (%) = Abs (control) – Abs (sample) /Abs (control) × 100

Hydrogen peroxide scavenging ability: The hydrogen peroxide (H_2O_2) scavenging activity of MK-1-EPS was evaluated using a previously described method (Srinivash *et al*., 2023). Briefly, five concentrations of MK-1-EPS (20–100µg/mL, in increments of 20µg/mL) were mixed with 2mL of 10mM H_2O_2 solution prepared in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The mixtures were incubated at 37°C for 8 minutes. A negative control (PBS without H_2O_2) and a positive control (H_2O_2 solution without EPS) were included. Absorbance was measured at 230 nm using a spectrophotometer, and scavenging activity was calculated as:

Scavenging activity (%) = Abs (control) – Abs (sample) /Abs (control) × 100

RESULTS

Phylogenetic analysis using the Maximum Likelihood method revealed that *Limosilactobacillus reuteri* MK-1 shares significant similarity with other *Limosilactobacillus* species. Pairwise comparisons of the 16S rRNA gene sequences showed an average similarity of 98%, indicating high genetic relatedness (Fig. 1).

***L. reuteri* growth and EPS yield analysis:** The growth kinetics of *L. reuteri* MK-1, EPS production, and pH changes in the culture medium were analyzed over time (Fig. 2). Viable cell counts increased with incubation time, peaking at 10^7 CFU/mL after 36 hours. Concurrently, the medium pH decreased steadily, reaching a final pH of 2.1. EPS production peaked at 585 mg/L after 33 hours but declined with prolonged incubation.

FTIR analysis: FTIR spectroscopy identified functional groups in MK-1-EPS (Fig. 3). A broad, intense peak at 3402 cm^1 indicated O–H stretching vibrations from hydroxyl groups. Additional peaks at 2933 cm^1 (C–H stretching) and 1652 cm^1 (C=O stretching or N–H bending) were observed.

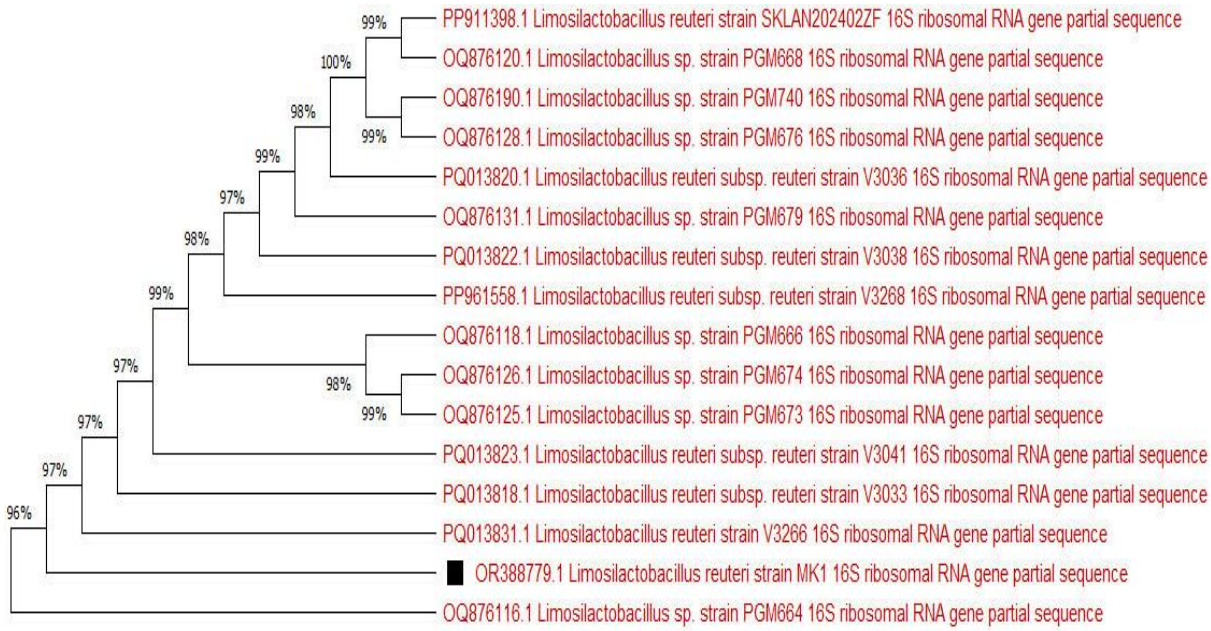

Fig. 1: Phylogenetic analysis based on sequences (16S rRNA) of *L. reuteri* isolates showing the genetic relationship of *L. reuteri* strain MK1.

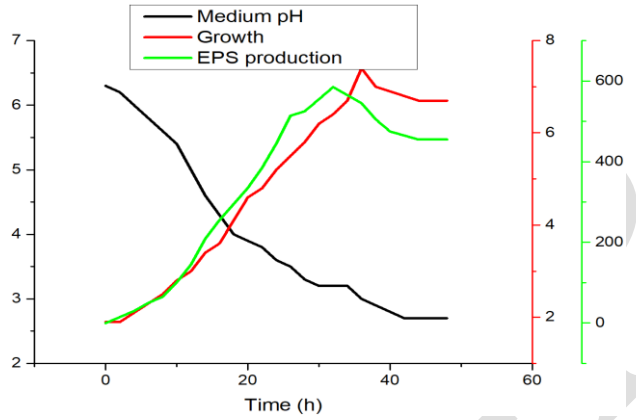

Fig. 2: Kinetic growth curve analysis of *L. reuteri* strain MK-1 in MRS medium over a duration of 48 hours.

NMR analysis: The ^{1}H NMR analysis of MK-1-EPS revealed signals in the δ 3.0–4.5 ppm range (Fig. 4A), characteristic of anomeric protons in polysaccharides, whereas the ^{13}C NMR spectra (Fig. 4B) showed signals between δ 60–90 ppm, corresponding to ring carbons (C2–C5) of monosaccharide units.

Mw determination: Gel permeation chromatography (GPC) determined the molecular weight (Mw) of MK-1-EPS to be 1.36×10^5 Da (136,000 Da), with a polydispersity index (PDI; Mw/Mn= 1.3), indicating a relatively homogeneous high-molecular-weight polymer.

Physiochemical properties of EPS: The emulsifying potential of *L. reuteri* MK-1-derived exopolysaccharide (MK-1-EPS) was systematically evaluated using a panel of hydrophobic substrates, including n-hexadecane, toluene, hexane, and xylene, to assess its surfactant-like properties (Fig. 5). Among these hydrocarbons, MK-1-EPS exhibited the highest emulsification efficiency (72.8%) with xylene at a concentration of 1 mg/mL, indicating strong interfacial activity between the aqueous and nonpolar phases. Notably, the emulsification indices for hexane, n-hexadecane, and toluene were 71.1, 66.3, and 69.8%, respectively, at the same EPS concentration.

Water-Holding and Solubility Capacity: The hydration properties of MK-1-EPS were quantitatively assessed to determine its functional applicability in water-intensive systems. The water-holding capacity (WHC) was measured at 386.8 ± 0.6%, reflecting the ability of MK-1-EPS to bind and retain water molecules within its polysaccharide network. Concurrently, the water solubility index (WSI) of MK-1-EPS was determined to be 28.5 ± 0.5% under standardized conditions, indicating moderate solubility that aligns with typical microbial EPS behavior.

Biological activity of EPS: The antimicrobial efficacy of MK-1-EPS was rigorously tested against Gram-positive i.e. *Staphylococcus aureus*) and Gram-negative i.e. *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica* serovar Typhi pathogens using growth inhibition assays (Fig. 6). MK-1-EPS demonstrated concentration-dependent bactericidal effects, with complete suppression of *E. coli* growth observed at both 75% and 100% (w/v) EPS concentrations. In contrast, the full inhibition of *K. pneumoniae*, *S.* Typhi, and *S. aureus* required the highest tested concentration (100% w/v).

Antioxidative activities: The radical scavenging capacity of MK-1-EPS was evaluated *in vitro* using two assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide (H_2O_2) scavenging (Fig. 7). MK-1-EPS exhibited dose-dependent neutralization of stable DPPH radicals, with scavenging activity increasing from 35% at 0.5 mg/mL to 64% at 10 mg/mL. Similarly, MK-1-EPS effectively quenched reactive oxygen species (ROS) generated by H_2O_2, with scavenging rates rising from 37% (0.5 mg/mL) to 70% (10 mg/mL).

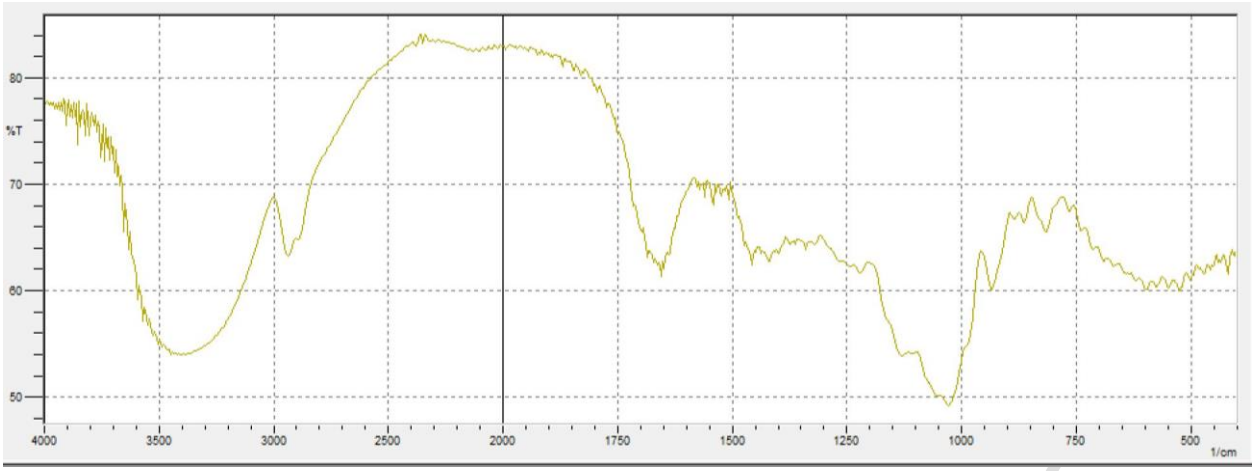

Fig. 3: FTIR spectra of EPS from *L. reuteri* strain MK-1 in the range of 4000–400 cm^{-1}.

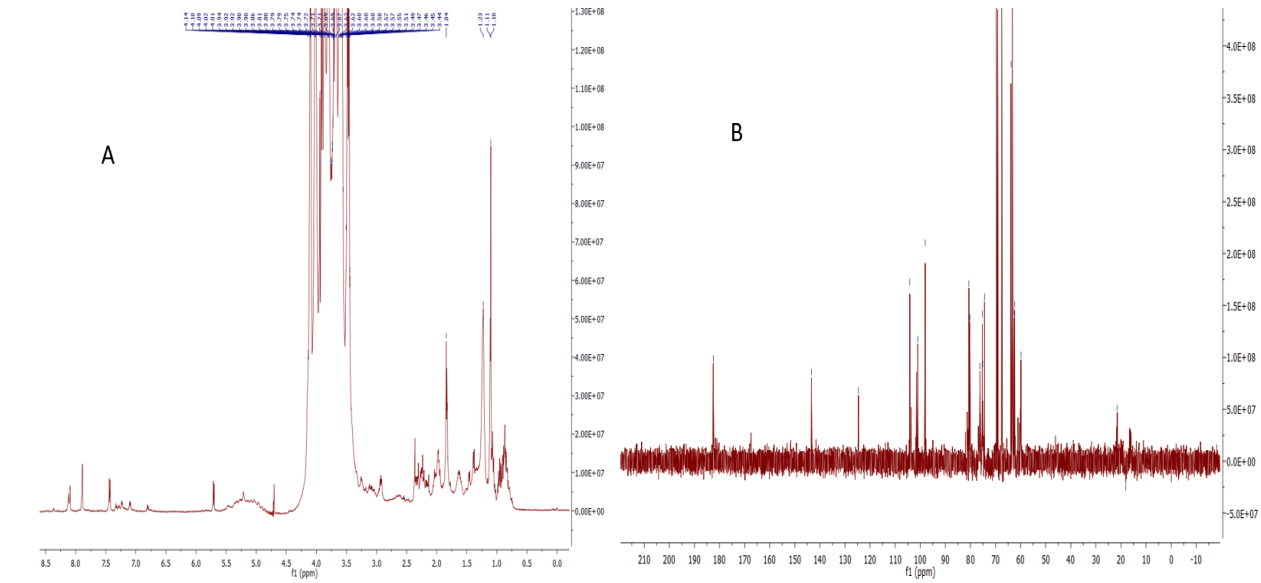

Fig. 4: 1H spectra (A) and 13C spectra (B) of EPS from *L. reuteri.*

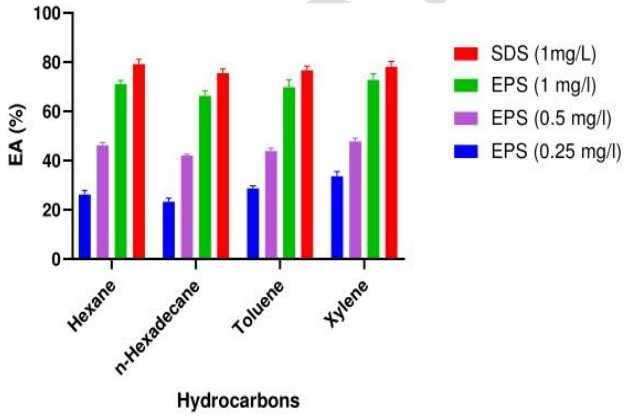

Fig. 5: Emulsification activity of EPS MK-1 with different hydrocarbons. The y-axis represents the emulsification index (% EI), which indicates the % total height occupied by the emulsion layer. The x-axis categorizes the results by hydrocarbon type. The error bars represent the standard deviations calculated from three independent replicates.

DISCUSSION

This study reports the isolation, identification, and characterization of *L. reuteri* MK1 from poultry intestine. Molecular identification involved 16S rRNA gene amplification, sequencing, and phylogenetic analysis to confirm taxonomic placement. Growth kinetics, including incubation conditions, were evaluated. Biochemical and structural properties of the purified exopolysaccharide (EPS) were analyzed via FTIR and NMR spectroscopy. The molecular weight, emulsifying capacity, water-holding ability, and antimicrobial activity of the EPS were assessed to explore its industrial potential.

The growth kinetics of *L. reuteri* MK1, EPS production, and pH changes in the culture media were monitored over various incubation periods (Fig. 2). The maximum viable cell count (10^7 CFU/mL) occurred after 36 hours, while the lowest pH (2.1) coincided with peak EPS yield (585 mg/L) at 33 hours. Prolonged incubation beyond this period reduced EPS yield, consistent with reports attributing this decline to enzymatic degradation (Elmansy *et al.*, 2023). Similar trends were observed in *Bifidobacterium* strains from camel milk (Yasmin *et al.*, 2020). *and L. plantarum* (Silva *et al.*, 2019). Notably, *L. reuteri* MK1 demonstrated higher EPS yields than *L. plantarum* (Abo Saif & Sakr, 2020), underscoring the critical role of incubation time in bacterial growth and

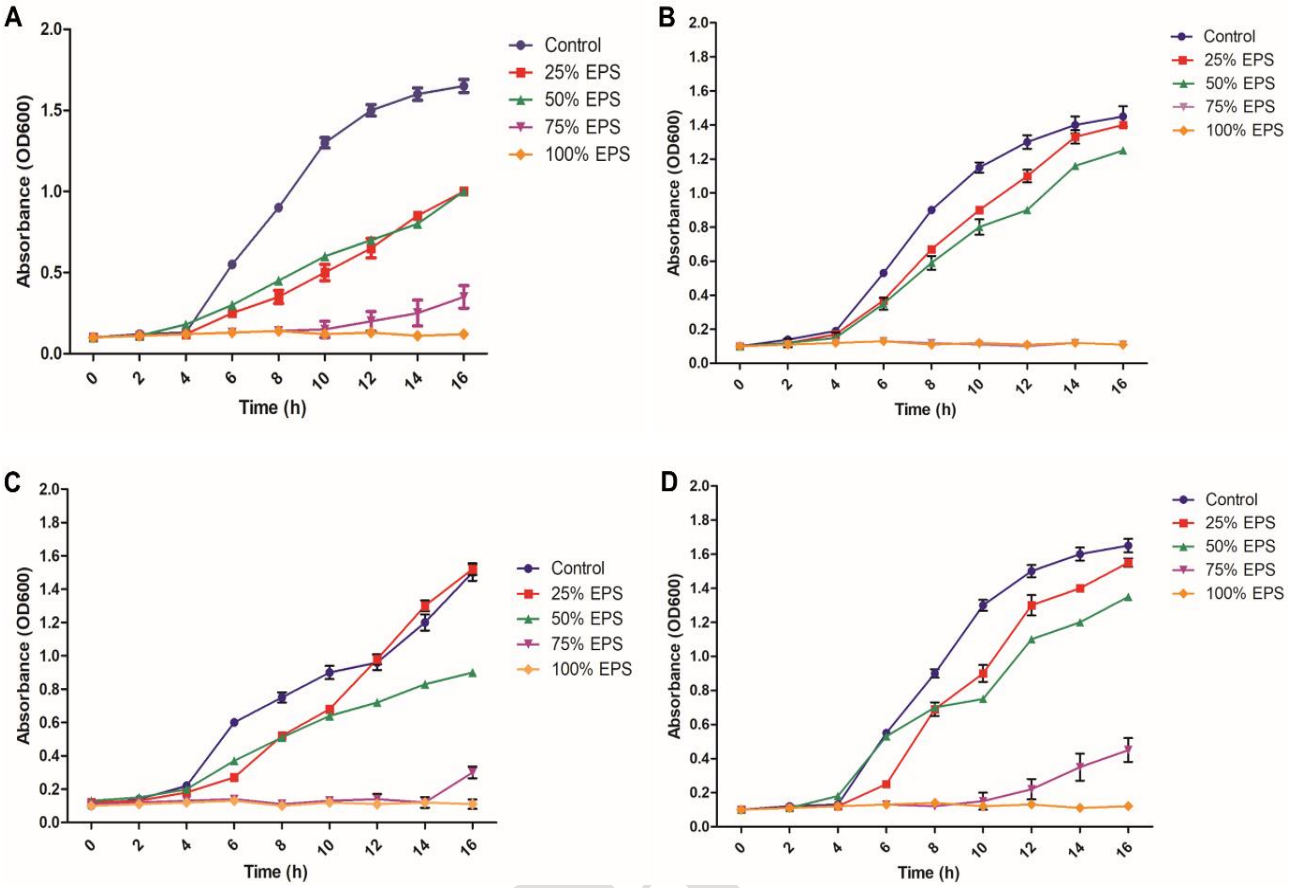

Fig. 6: Antibacterial activity of EPS of *L. reuteri* MK1 against S. aureus (A), E. coli (B), K. pneumoniae (C), *S.* Typhi (D). The error bars represent the standard deviations calculated from three independent replicates.

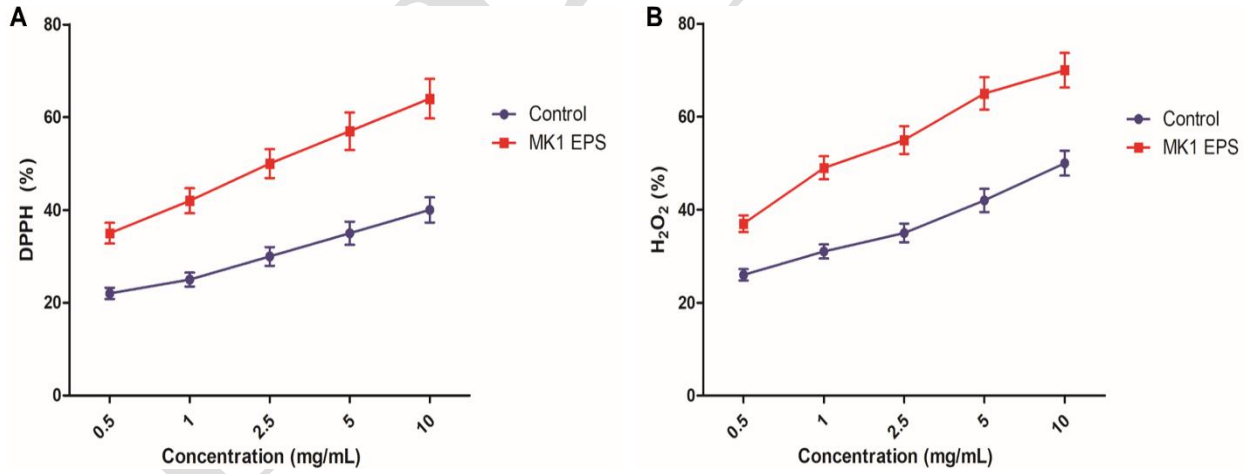

Fig. 7: Analysis of the antioxidant activity of EPS synthesized by *L. reuteri* MK1. (A) DPPH radical scavenging assay; (B) H_2O_2 radical scavenging activity. The error bars represent the standard deviations calculated from three independent replicates.

EPS production. The purified EPS contained 93.27±1.7% carbohydrates, with no detectable proteins, nucleic acids, uronic acid, or sulfuric acid.

FTIR spectroscopy identified functional groups characteristic of polysaccharides. A broad peak at 3402 cm^{-1} corresponded to O–H stretching vibrations, typical of hydroxyl groups in polysaccharides (Zhang *et al.*, 2013). The peak at 2933 cm^{-1} was attributed to C–H stretching in methyl and methylene groups, indicative of aliphatic hydrocarbons (Xu *et al.*, 2019). A distinct absorption band at 1652 cm^{-1} suggested carbonyl (C=O) stretching, potentially from acetyl or uronic acid groups (Asker *et al.*, 2018). Peaks at 1028 cm^{-1} confirmed C–O stretching vibrations associated with glycosidic linkages and the polysaccharide backbone (Zhu *et al.*, 2022).

^{1}H NMR analysis (Fig. 4A) revealed proton signals between 3.0–4.5 ppm, consistent with protons attached to carbons bearing hydroxyl (-OH) or amide (-NH) groups, characteristic of complex polysaccharides. ^{13}C NMR (Fig. 4B) displayed signals in two key regions: δ 60–100 ppm (C2–C5 carbons of monosaccharide rings) and δ 90–110 ppm (anomeric carbons), corroborating the carbohydrate nature of EPS-MK1 (Lin *et al.*, 2022). The absence of signals in the δ 80–95 ppm range suggests a lack of

complex substitutions (e.g., aromatic or alkynyl groups) and minimal contamination, supporting a predominantly unsubstituted polysaccharide structure (Fontana & Widmalm, 2023). This structural simplicity may enhance functional properties such as solubility and interaction with aqueous systems.

The EPS exhibited a molecular weight (Mw) of 1.36 × 10^5 Da with a polydispersity index (PDI) of 1.3, indicating moderate homogeneity. Comparable Mw values have been reported for EPS from *L. plantarum* (2.68 × 10^5 Da) (Ismail & Nampoothiri, 2014), and *Leuconostoc mesenteroides* (1.46 × 10^5) (Kim *et al.*, 2023). High Mw polysaccharides are preferred for their viscosity-enhancing and emulsion-stabilizing properties, making EPS-MK1 a promising candidate for dairy products (e.g., yogurt, cheese), functional foods, and drug delivery systems (Jurášková *et al.*, 2022; Wu *et al.*, 2021).

EPS-MK1 demonstrated robust emulsifying activity against hydrocarbons: xylene (72.8%), hexane (71.1%), toluene (69.8%), and n-hexadecane (66.3%) at 1 mg/mL. This performance is attributed to its amphiphilic structure, which stabilizes oil-water interfaces by forming a mechanical barrier around oil droplets, preventing coalescence (Vandana & Das, 2021). Similar emulsification efficiencies have been observed in EPS from *Bacillus licheniformis* (67.79%) (Vinothkanna *et al*., 2021), and *L. rhamnosus* (23–45%) (Yang *et al.*, 2015).

The results indicated that EPS-MK1 exhibits strong hydration properties, including water solubility (28.5 ± 0.5%) and water-holding capacity (386.8 ± 0.6%). These superior hydration properties suggest that EPS-MK1 could serve as an excellent component in fermented food products (Yang *et al.*, 2022). The findings represent an improvement over previous work reporting a water solubility index of 14.2 ± 0.208% for EPS derived from the *L. lactis* strain (Saravanan & Shetty, 2016). EPS structures are composed of long monosaccharide chains linked by glycosidic bonds. These polymer chains form a semi-permeable network capable of retaining water molecules, with structural variability ranging from simple linear chains to complex branched or cyclic forms. The hydroxyl (-OH) groups in EPS sugar units can form hydrogen bonds with water molecules, enhancing moisture retention in emulsions and thereby improving stability. Furthermore, the strong hydrophilic nature of EPS facilitates interactions with the aqueous phase of emulsions (Jiang *et al.*, 2022; Lotti *et al.*, 2019; Wu *et al.*, 2022). These properties suggest that bacterial EPS, such as those produced by lactic acid bacteria, could be effectively incorporated into foods as hydrocolloids for use as stabilizers or bio-thickeners.

The antibacterial activity of EPS-MK1 was evaluated against *S. aureus*, *E. coli*, *K. pneumoniae*, and *S.* Typhi. As shown in Figure 6, EPS-MK1 inhibited the normal growth rate of these pathogens. Complete growth inhibition of *E. coli* was observed at 75% and 100% EPS concentrations, while *K. pneumoniae*, *S.* Typhi, and *S. aureus* required 100% EPS concentration for total inhibition. These results align with recent studies demonstrating antibacterial effects of EPS from *Lactiplantibacillus plantarum* and other lactic acid bacteria against various pathogens (Li *et al.*, 2014; Trabelsi *et al.*, 2017). Similarly, EPS from *L. kefiranofaciens* and *L. garvieae* strains have shown bactericidal activity (Ayyash *et al.*, 2020; Jeong *et al*., 2017). While research on the antibacterial mechanisms of lactic acid bacteria-derived EPS remains limited, some studies suggest that EPS may disrupt bacterial cell division, metabolism, and membrane integrity. The negatively charged EPS can bind to positively charged bacterial cell surfaces, altering membrane permeability and causing leakage of cellular constituents, ultimately leading to cell death (Abdalla *et al.*, 2021; Salimi & Farrokh, 2023). Additionally, EPS may inhibit pathogen colonization in the intestine by competing for binding sites or modifying bacterial cell surface properties (Yang *et al.*, 2023).

Studies have reported that high molecular weight (Mw) EPS exhibits stronger antimicrobial activity against Gram-positive pathogens compared to low Mw EPS (Wu *et al*., 2023). High Mw EPS often possess complex structures with greater surface area for microbial interactions, facilitating stronger binding and membrane disruption. Moreover, their multiple functional groups (e.g., hydroxyl, carbonyl, and phosphate) may target bacterial membranes, compromising integrity and functionality (Abdalla *et al*., 2021; Wang *et al.*, 2023b). The antibacterial efficacy of EPS-MK1 highlights its potential as a novel strategy to combat foodborne pathogens.

The radical scavenging activity of EPS-MK1 against DPPH and H_2O_2 free radicals was assessed using an in vivo method. EPS-MK1 exhibited DPPH scavenging activity of 35, 42, 50, 57 and 64% at concentrations of 0.5, 1, 2.5, 5, and 10 mg/mL, respectively H_2O_2 scavenging activity was 37, 49, 55, 65 and 70% at the same concentrations. Notably, EPS-MK1 achieved 64% DPPH scavenging at 10 mg/mL, comparable to the 66.36% activity reported for *Pleurotus eryngii* EPS at 400 mg/L (Sun *et al.*, 2013). This indicates competitive antioxidant potential relative to other EPS sources. Consistent with prior studies, higher EPS concentrations correlated with increased scavenging activity, as seen in *Bifidobacterium animalis* EPS (Kim *et al*., 2023; Wang *et al.*, 2023a). Functional groups in EPS, such as hydroxyl and carboxyl moieties, may donate protons to neutralize free radicals, explaining this dose-dependent trend. The observed H_2O_2 scavenging activity of EPS-MK1 aligns with antioxidant properties reported for EPS from other microbial sources (Kim *et al*., 2023; Wang *et al*., 2023a), reinforcing the potential of EPS as natural antioxidants. Thus, EPS-MK1 represents a promising candidate for further exploration in food and healthcare applications as a natural antioxidant.

Conclusions: The *L. reuteri* MK1 strain demonstrates significant potential for industrial applications due to its high EPS yield, structural simplicity, and multifunctional properties. Its emulsifying, water-retaining, antimicrobial, and antioxidant activities align with demands in food, pharmaceutical, and cosmetic sectors. Further investigation should address scalable production methodologies, in vivo bioactivity verification, and optimal delivery system development to facilitate commercial translation of this promising bacterial metabolite.

Conflicts of interest: The authors have no conflicts of interest to declare.

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