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Research Article

Keywords: Probiotics, Fermented foods, Immunomodulatory activity, Antimicrobial activity

Posted Date: November 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10919668/v1

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Additional Declarations: No competing interests reported.

Probiotic characterization, metabolite profiling, and immunomodulatory activity of heat-killed strains from fermented Manipur bamboo shoots

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Abstract:

This research focused on the identification and analysis of potential probiotic bacteria and their metabolites derived from Manipur's traditional fermented products. The investigation resulted in the successful isolation and rigorous testing of four unique probiotic strains. Safety assessments confirmed their suitability for human consumption, demonstrating their sensitivity to key antibiotics and the absence of hemolytic or gelatinase activities. The identified strains demonstrated a strong functional profile, survived bile exposure and showed excellent adhesion through high hydrophobicity and aggregation capabilities. They also display key enzymatic activities, such as BSH and β-galactosidase production. GC–MS analysis confirmed the secretion of immunomodulatory compounds and essential short-chain fatty acids (SCFAs), such as acetic propionic and butyric acids. Additionally, extracellular polymeric substances (EPSs) display diverse functional groups according to FT-IR, whereas extracellular proteins exhibit antimicrobial activity against pathogenic bacteria. All four heat-killed probiotic strains exhibited dose-dependent cytotoxicity in HT-29 cells, maintaining >70% viability up to 20 µg/mL, with B2 and B7 demonstrating superior biocompatibility. Evaluation of the heat-killed isolates in HT-29 cells revealed significant, strain-dependent immunomodulatory activity characterized by diverse cytokine expression profiles. Specifically, isolates such as B4 strongly upregulated anti-inflammatory and regulatory markers such as IL-10 and IFN-γ, whereas isolates B7 and B2 prominently increased MUC2 mucin gene expression to support mucosal barrier function.

Keywords: Probiotics, Fermented foods, Immunomodulatory activity, Antimicrobial activity

1. Introduction:

Modern medicine is increasingly pivoting toward disease prevention and functional nutrition, with probiotics playing a central role in managing metabolic, immune, and digestive health. Consuming these live microorganisms optimizes gut microbiota and metabolic output, offering therapeutic potential against chronic conditions such as inflammatory bowel disorders (Parlindungan et al. 2021). Lactic acid bacteria (LAB) isolated from traditional fermented foods are particularly valued for synthesizing bioactive metabolites and antimicrobial agents while possessing enhanced ecological resilience compared to synthetic strains. Within this context, fermented bamboo shoots from Northeast India represent an underexplored repository of novel functional microbes (Behera and Balaji 2021; Pyo et al. 2024).

In Northeast India—specifically states like Manipur—indigenous products such as *soibum* and *soidon* are produced via spontaneous anaerobic fermentation. This process fosters a complex microbial landscape dominated by *Lactobacillus*, *Leuconostoc*, and *Pediococcus* species, which produce organic acids and bioactive compounds that extend shelf-life and promote host health (Phùng et al. 2025). Despite their widespread consumption and cultural significance, the probiotic and paraprobiotic potential of bacterial isolates from Manipur's fermented bamboo shoots remains largely uncharacterized scientifically. Transitioning from traditional knowledge to evidence-based functional food applications requires systematic strain validation across safety assessment, gastrointestinal survival, bioactive metabolite profiling, and cellular immunomodulation (Long et al. 2023; Goyal et al. 2024).

Ensuring biosafety is paramount; candidate probiotics must lack virulence factors, hemolytic activity, gelatinase production, and clinical antibiotic resistance. Functional attributes—including tolerance to low pH and bile salts, cell surface hydrophobicity, aggregation dynamics, and epithelial cell adhesion—are essential for gut colonization. Beyond live cell survival, the therapeutic value of probiotics is heavily mediated by their bioactive metabolites and cell wall components. Short-chain fatty acids (SCFAs), quantified via gas chromatography–mass spectrometry (GC–MS), strengthen intestinal barrier integrity and regulate host immunity (Markowiak-Kopeć and Śliżewska 2020), while exopolysaccharides (EPSs) characterized by Fourier-transform infrared spectroscopy (FT-IR) display anti-inflammatory and antimicrobial properties (Pourjafar et al. 2023). Furthermore, heat-killed probiotic strains (paraprobiotics) offer distinct safety and stability advantages. Evaluating their cytotoxicity via MTT assay and their impact on host gene expression—specifically mucosal

barrier integrity (*MUC2*) and cytokine regulation (*IL-10, IL-8, and IFN-γ*) in HT-29 colon cells via qPCR—provides critical insight into their immunomodulatory and therapeutic potential (Karimi Ardestani et al. 2019; Noh et al. 2022).

Given the untapped genetic and functional diversity of Manipur's fermented bamboo shoots, this study was designed to isolate, characterize, and evaluate novel probiotic candidates from this indigenous source. The specific objectives were to:

1. Isolate bacterial strains from traditional fermented bamboo shoots (*soibum* and *soidon*) of Manipur.
2. Perform safety screening via hemolytic activity testing, gelatinase production, and antibiotic susceptibility profiling.
3. Evaluate functional survival, including acid and bile tolerance, cell surface hydrophobicity, aggregation capacity, and intestinal epithelial cell adhesion.
4. Profile bioactive metabolites, including SCFA quantification via GC–MS, EPS functional group characterization via FT-IR, and extracellular antimicrobial protein activity.
5. Assess cell viability and cytotoxicity of the isolates on HT-29 human colon carcinoma cells using the MTT assay.
6. Quantify immunomodulatory gene expression induced by heat-killed probiotic strains in HT-29 cells via qPCR, evaluating mucosal protection (*MUC2*) and anti/pro-inflammatory cytokine responses (*IL-10*, *IL-8*, and *IFN-*γ).

This integrated approach aims to identify promising indigenous probiotic and postbiotic/paraprobiotic candidates for potential application as functional food ingredients with scientifically validated health benefits.

2. Materials and Methods:

2.1 Chemicals and reagents

Bacterial culture media were obtained from HiMedia Laboratories Pvt. Ltd. (India), while other chemicals and consumables were sourced from Sigma–Aldrich unless otherwise stated.

2.1.1 Microorganisms and culture conditions

This study utilized *Salmonella Typhimurium* MTCC 3232, *Pseudomonas aeruginosa* MTCC 3361, *Staphylococcus aureus* MTCC 3160, and *Escherichia coli* MTCC 40 as pathogenic

strains, which were cultured aerobically in Luria–Bertani (LB) broth at 37°C. The probiotic strains *Lacticaseibacillus paracasei* B2, *Lacticaseibacillus rhamnosus* B3, *Pediococcus pentosaceus* B4, and *Limosilactobacillus fermentum* B7 were grown in de Man, Rogosa, and Sharpe (MRS) broth.

2.1.2 Sample collection and isolation

Soibum, a traditional fermented bamboo shoot product from Manipur, India, was sourced from Tamenglong district (24.99° N, 93.5° E) for probiotic strain screening. The samples were aseptically collected in sterile containers and stored at 4°C until processing. Homogenates were prepared in sterile phosphate buffer (pH 7.2) at a 1:10 (w/v) ratio. Aliquots (1 mL) were pour-plated onto MRS agar, and colony-forming units (CFU/mL) were enumerated after incubation. Presumptive isolates were purified by streaking on fresh MRS agar and incubating at 37°C. Colonies with distinct morphologies were selected, subcultured in MRS broth, and screened for gram-positive, catalase-negative characteristics. Pure cultures were preserved as 20% glycerol stocks at −80°C for future use.

2.1.3 Molecular characterization of strains

The probiotic candidate strains B2, B3, B4, and B7, which were selected for their prominent functional traits, were identified through 16S rRNA gene sequencing. Genomic DNA extraction was performed via the QIAamp DNA Mini Kit (Qiagen, Germany), with quality confirmed via electrophoresis on a 1.2% (w/v) agarose gel. The amplification of the 16S rRNA gene was performed with universal primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′; 1492R: 5′-GGTTACCTTGTTACGACTT-3′) in a PCR setup (Thermo Fisher Scientific). The thermal cycling protocol included initial denaturation at 95°C for 5 min; 35 cycles of denaturation (95°C, 30 s), annealing (55°C, 40 s), and extension (72°C, 30 s); and a final extension at 72°C for 5 min. PCR products were visualized on 1.2% agarose gels, purified, and sent for commercial sequencing (1st BASE, Apical Scientific, Singapore; previously Molbiogen). The obtained sequences were submitted to NCBI GenBank for accession numbers. Phylogenetic trees were constructed via the neighbor-joining algorithm in MEGA 12 software.

2.2 Evaluation of Safety and Virulence Traits in Isolated Strains

2.2.1. Multiple antibiotic resistance index (MAR)

The following strains were tested for antibiotic susceptibility (S) or resistance (R) via HiMedia (India) commercial discs: chloramphenicol (30 µg), cotrimoxazole (25 µg), fusidic acid (30 µg), linezolid (30 µg), rifampicin (5 µg), and tetracycline (30 µg). The discs were aseptically placed on even bacterial lawns prepared on MRS agar plates, followed by incubation at 37°C for 24 h. Zones of inhibition (ZOIs) were measured to the nearest mm post-incubation (Ayandele et al., 2020). All the assays were conducted in triplicate. The MAR index was determined as outlined below.

$$\text{MARIndex} = \left(\frac{Number\ of\ antibiotics\ ineffective}{Number\ of\ antibiotics\ used}\right)$$

2.2.2 Hemolytic activity

Hemolytic activity was evaluated according to Kaktcham et al. (2018). Overnight cultures in MRS broth were streaked onto blood agar plates and incubated at 37°C for 24 h. Plates were inspected for hemolytic zones: β-hemolysis (clear halos surrounding colonies), α-hemolysis (greenish discoloration), or γ-hemolysis (no zones).

2.3 In vitro screening of probiotic properties

2.3.1 Screening of cultures for BSH activity

Bile salt deconjugation by LAB strains was assessed following Hernández–Gómez et al. (2022). Sterile 6-mm filter discs saturated with overnight MRS broth cultures were placed on MRS agar plates supplemented with 0.5% (w/v) taurodeoxycholic acid sodium salt (TDCA; Sigma–Aldrich, USA) and 0.37 g/L $CaCl_2$. The plates were incubated at 37°C for 48 h, after which the diameters of the precipitation zones (opaque halos) were measured. Unsupplemented MRS agar served as a negative control.

2.3.2 Survival under simulated human gastrointestinal tract tests

Tolerance to simulated gastrointestinal conditions was assessed via the protocol of Saikia et al. (2018), with modifications. The cells from the 24-h MRS broth cultures were collected via centrifugation (8000 × g, 5 min, 4°C), washed once in PBS (pH 7.4), and resuspended to 10^8CFU/mL in simulated gastric fluid (SGF) or simulated intestinal fluid (SIF). SGF consisted of sterile PBS (pH 2, 3, or 4) supplemented with pepsin (3 g/L). SIF was prepared by adding pancreatin (Sigma–Aldrich; 1 g/L) and bile salts (0.3 g/L) to sterile PBS (pH 6.8 or 8).

The samples were incubated at 37°C for 0, 1, 2, or 3 h, vortexed, serially diluted, spread-plated on MRS agar, and incubated at 37°C for 24 h. Viable cell counts (CFU/mL) were calculated to assess strain survival.

2.3.3 Tolerance to bile salt

Bile tolerance was evaluated via the protocol described by Kassa et al. (2024). Acid-tolerant isolates were grown overnight in MRS broth at 37°C with aeration. Harvested cells (centrifuged at 8000 × g for 10 min at 4°C) were washed twice in sterile PBS (pH 7.2) and resuspended in MRS broth containing 0.3% or 0.6% (w/v) bile salts. The suspensions were then incubated for 24 h at 37°C under aerobic and anaerobic conditions. After incubation, 1 mL samples were serially diluted in 9 mL sterile PBS, plated onto MRS agar, and incubated anaerobically at 37°C for 24 h. Survival rates were calculated as percentage viability: $[(N_1/N_0) \times 100]$, where N_1 is the viable CFU/mL postexposure and N_0 is the initial count at time zero.

2.3.4 Coaggregation

Coaggregation assays were performed following Tarique et al. (2022). Isolates (optical density ≈1.0) were mixed with two pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and incubated at 37°C for 1, 3, and 6 h. The results were quantified as the coaggregation percentage via the established formula.

$$Co - aggregation\ \% = [1 - \frac{A_t}{A_0}] \times 100$$

where A_t denotes the absorbance at time t and A_0 denotes the initial absorbance at time zero.

2.3.5 Cell Surface Hydrophobicity

The surface hydrophobicity of selected LAB strains was determined via the method of Soleimani et al. (2023), with modifications. Overnight MRS broth cultures were centrifuged (10,000 rpm, 10 min, 4°C), washed twice in PBS, and resuspended to an OD_{620} of ≈1.0. Aliquots (3 mL) were vortexed with 1 mL of xylene (Sigma–Aldrich) for 2 min and then incubated undisturbed at 37°C for 1 h to allow phase separation. The OD_{620} of the aqueous phase was measured, and the hydrophobicity was calculated as follows:

Hydrophobicity (%) $= [1 - (A_f/A_0)] \times 100$, where A_0 is the initial absorbance and A_f is the absorbance of the aqueous phase after extraction.

2.3.6 Autoaggregation

Autoaggregation assays were conducted via a modified protocol from Gao et al. (2021). Overnight cultures of *Lactobacillus* strains were diluted to 1.0% (v/v) in MRS broth and incubated statically at 37°C for 12 h. The OD_{600} of the upper suspension was recorded (OD_1), followed by vortexing the full suspension and measuring the total OD_{600} (OD_2). The autoaggregation percentage was calculated as:

$$\text{Auto-aggregation (\%)} = (1 - \frac{OD_1}{OD_2}) \times 100$$

2.3.7 DPPH-Free Radical Scavenging Activity

DPPH radical scavenging capacity of bacterial suspensions ([1ml, 10^8CFU/mL]) was evaluated following Son et al. (2017). Samples were reacted with 0.05 mM DPPH (1:1 v/v) for 30 min at 37°C in the dark, centrifuged (14,000×g, 10 min, 4°C), and analyzed at 517 nm. Scavenging activity (%) was calculated as

$$\text{Activity (\%)} = [1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}}] \times 100$$

where A_{sample} is the absorbance of the cell–DPPH mixture, A_{blank} is the cell-only absorbance, and A_{control} is the DPPH-only absorbance in PBS.

2.3.8 Adhesion to HT-29 Intestinal Epithelial Cells

Adhesion of LAB strains to HT-29 cells was determined following Soleimani et al. (2023) with modifications. Monolayers cultured in DMEM with 10% FBS and 1% antibiotics (24-well plates, 24 h) were washed with PBS and incubated with LAB suspensions (10^8 cells/mL in DMEM; 1 mL/well) for 2 h at 37°C. Non-adherent bacteria were removed by washing twice with PBS. Adherent cells were detached using 0.25% trypsin-EDTA for 30–60 s and lysed with 0.2% Triton X-100 for 10 min. Viable adherent bacteria were enumerated on MRS agar (37°C, 24 h). Adhesion percentage was calculated as:

$$\text{Adhesion (\%)} = (\frac{B_1}{B_0}) \times 100$$

where B_1 is the CFU/mL of recovered adherent bacteria and B_0 is the CFU/mL of initial inoculum.

2.4 Isolation and FT-IR characterization of crude-purified EPS

LAB strains were cultured in MRS broth, and supernatants were obtained by centrifugation (5,000 × g, 10 min). Three volumes of 99% ethanol were added to the supernatant, and the mixture was stored at 4°C for 24 h. Precipitated EPS was recovered by centrifugation (12,000 × g, 5 min, 4°C), washed with 70% ethanol, and dried at room temperature using a vacuum evaporator to remove residual ethanol.

For FT-IR analysis, 10 mg of homogenized crude-purified EPS was examined via a PerkinElmer Spectrum 100/Frontier IR spectrometer over a frequency range of 4,000–650 cm^{-1}.

2.4.1 Secreted protein extraction and antimicrobial activity

Secreted proteins were extracted, and their antimicrobial activity was assessed via a modified protocol from Lei et al. (2020). Stationary-phase cultures (5 mL) were centrifuged (10,000 × g, 10 min, 4°C), and the supernatants were sterile-filtered (0.22 μm). Proteins from microbial culture supernatants and microbe-free MRS media were precipitated with 80% saturated ammonium sulfate overnight at 4°C, recovered by centrifugation (10,000 × g, 20 min, 4°C), and resuspended in minimal acetate buffer (10 mmol/L, pH 4.5). Protein concentrations were quantified via the Bradford assay.

Antimicrobial activity was evaluated by well diffusion on Mueller–Hinton agar (HiMedia). Pathogens (*Salmonella typhimurium* and *Escherichia coli*) were grown for 17 h, and 100 μL was spread onto the plates. The wells (7 mm) received 100 μL of secreted protein extracts, which were preincubated at 4°C for 2 h to facilitate diffusion and then incubated at 37°C for 24 h. Gentamicin served as the positive control, and proteins from microbe-free MRS served as the negative control.

2.5 Derivatized Short-Chain Fatty Acid (SCFA) Analysis

Cell-free supernatants (1 mL, 0.22 μm filtered) were acidified with 100 μL conc. HCl and extracted with 1 mL diethyl ether (1:1 v/v). Extract aliquots (100 μL) were derivatized with 5 μL BSTFA. Samples (1 μL) were analyzed on an Agilent 8890 GC coupled to an Agilent 7010B TQ mass detector using an HP-5MS UI column (30 m×0.25 mm, 0.25 μm). Carrier gas (helium) flow was 1.0 mL/min with a 50:1 split injection at 250°C. The oven program ramped from 90°C to 250°C (20 min total run). Spectra (70 eV) were acquired in full-scan and SIM modes (targeting m/z 74, 87, and 143) for SCFA identification and quantification against NIST libraries and authentic standards. Uninoculated MRS broth served as the control.

2.5.1 Underivatized Volatile Metabolite Profiling

Underivatized extracellular metabolites were extracted following García-Villalba et al. (2012) with minor modifications. Filtered supernatants (1 mL) were acidified with 40 µL conc. HCl, extracted with 1 mL diethyl ether, and centrifuged (16,000×g, 10 min). Aliquots (1 µL) were injected (10:1 split, 250°C) into a PerkinElmer Clarus GC-MS equipped with an Elite-5MS column (22 m×0.25 mm, 0.25 µm). The oven temperature was ramped from 90°C to 250°C (14 min total run). EI mass spectra (70 eV, m/z 30–250) were matched against NIST 08 and Wiley 7N spectral libraries to identify volatile organic compounds

2.6 Culture and maintenance of the HT-29 cell line

The HT-29 cell line was used as an intestinal cell model. The cell line was purchased from the National Centre for Cell Science (NCCS), Pune India. HT-29 (human colorectal adenocarcinoma) cells were propagated and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% inactivated FBS (Gibco, Inc., USA) and 1% Penicillin-Streptomycin (Thermo Fisher Scientific). The flasks were then incubated at 37 °C, 5% CO_2, and 95% humidity to reach confluency. At approximately >80% confluency, the cells were trypsinized. The cells were again seeded in 24-well cluster under the same culture conditions and used for real-time PCR analysis of the expression of different cytokine genes.

2.6.1 Preparation of heat-inactivated probiotics

To prepare the heat-inactivated probiotics, the 24-h cultured probiotics were centrifuged at 4°C and washed twice with PBS. The pellet was resuspended in PBS (10^9 CFU per ml) and placed in a water bath at 80°C for 30 min. To ensure that the bacteria were inactivated, they were cultured in MRS media and incubated at 37°C for 72 h (Pradhan et al. 2025).

2.6.2 MTT assay

Cytotoxicity of heat-killed probiotic strains on HT-29 cells was evaluated via the MTT assay following Karimi et al. (2019) with modifications. Briefly, cells seeded in 24-well plates (500 µL/well) were treated with heat-killed strains at 10, 20, 40, 60, and 100 µg/mL (5×10^6–5×10^7 CFU/well) and incubated for 24 h (37°C, 5% CO_2). Next, 50 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. Formazan crystals were solubilized in 500 µL DMSO for 20 min after aspirating the medium, and absorbance was measured at 570 nm. Cell viability (%) was calculated relative to untreated control cells.

2.6.3 Heat-killed probiotic treatment, RNA isolation and cDNA synthesis

The HT-29 cell line was coincubated with four heat-killed isolates at a 1:50 ratio in 4 wells, and one well without heat-killed isolates was used as a negative control in 6-well plates. Total RNA was isolated from HT-29 cells via TRIzol reagent. The RNA concentrations were determined with a NanoDrop Lite Spectrophotometer (Thermo Scientific, Wiltham, USA) and adjusted to working concentrations with nuclease-free water. A Verso cDNA Synthesis Kit (Thermo Scientific) was then used for cDNA synthesis with oligo dT primers according to the manufacturer's instructions.

2.6.4 Real-time PCR for cytokine gene expression

Cytokine gene expression in treated cells was analyzed by real-time quantitative PCR (qRT–PCR) via Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific) in a volume of 20 µl. Thus, 10 µl of iTaq Universal SYBR Green Supermix (Bio-Rad), 0.6 µl of each specific primer (Table 1), 1 µl of cDNA, and 8.4 µl of nuclease-free water were used to prepare each sample. The *GAPDH* gene was used as an internal control. The PCR program was set to the following specifications: initial denaturation at 95°C for 10 min, followed by 40 cycles (95 °C for 15 s, 60°C for 40 s, and 72°C for 20 s), and 1 cycle at 72°C for 5 min as the final extension. The gene expression ratio was analyzed according to the Ct values of the target gene and an endogenous control (GAPDH) for each experiment and was finally calculated via the following equation: Ratio = $2^{-\Delta\Delta Ct}$.

2.7 Statistical analysis

All the test measurements were performed in triplicate, and the results are expressed as the means ± standard deviations (SDs), percentages and figures. The results were subjected to ANOVA via GraphPad Prism software (GraphPad Software, version 5.0, Inc., CA, USA).

3. Results and discussion

3.1 Isolation and Molecular Characterization of Bacterial Strains

Despite the historical importance of fermented bamboo shoots in Manipur, India, comprehensive reports regarding their resident probiotic microbiota remain limited. Consequently, the present study sought to isolate and characterize novel probiotic strains, with a specific focus on their potential for bioactive metabolite production. Following initial selection on the basis of distinct colony morphology, gram-positive, catalase-negative isolates were prioritized for safety/virulence assessments and in vitro probiotic property screening. 16S rRNA gene sequencing revealed that strain B2 shares 96.82% identity with *L.*

paracasei PX149206.1 (GenBank), B3 shares 99.71% identity with *L. rhamnosus* PQ662311.1 (GenBank), B4 shares 94.77% identity with *P. pentosaceus* KY883563.1 (GenBank), and B7 shares 96.52% identity with *L. fermentum* MF354552.1 (GenBank). These strains were designated *Lacticaseibacillus paracasei* (GenBank: PX830583), *Lacticaseibacillus rhamnosus* (GenBank: PX705822), *Pediococcus pentosaceus* (GenBank: PV855993), and *Limosilactobacillus fermentum* (GenBank: PV936928), respectively. The phylogenetic relationships among these strains are depicted in Figure 1A. Certain strains have previously been identified as promising probiotics from fermented bamboo shoots (Kabui et al., 2025).

3.2 Evaluation of Safety and Virulence Traits in Isolated Strains

According to the FAO/WHO guidelines, the designation of a strain as a probiotic necessitates a thorough assessment of its safety for human use and its technological/functional properties. Safety evaluations of the isolates were thus initiated by calculating the multiple antibiotic resistance (MAR) index and screening for key virulence factors, including hemolytic activity.

3.2.1 Multiple antibiotic resistance index (MAR) index

The multiple antibiotic resistance (MAR) index is a vital diagnostic tool used to quantify the multidrug resistance potential of bacterial isolates and assess the associated environmental health risks. All strains were sensitive to most of the tested antibiotics, including linezolid (LZ, 30 μg), fusidic acid (FC, 30 μg), rifampicin (RIF, 5 μg), chloramphenicol (C, 30 μg), cotrimoxazole (COT, 25 μg), and tetracycline (TE, 30 μg), with an MAR index of 0.125 (Table 2A). When the MAR index was less than 0.2, the isolates were deemed nondrug resistant (Das et al., 2022).

3.2.2 Hemolytic activity

The absence of virulence factors, particularly hemolytic activity (beta [β]- and alpha [α]-hemolysis), is a primary safety criterion for potential probiotics per human health standards. All four isolates exhibited gamma (γ)-hemolysis or no hemolytic activity (Figure 1B). This profile aligns with prior probiotic strains deemed safe for application (Lad et al., 2022).

3.3 In vitro screening of probiotic properties

Strains passing safety thresholds (MAR index <0.2; nonhemolytic) were evaluated for core probiotic traits, such as bile salt hydrolase activity, gastric acid and bile salt tolerance, coaggregation, cell surface hydrophobicity, autoaggregation, β-galactosidase production, free-radical-scavenging activity, antimicrobial activity, and adhesion activity toward epithelial cells.

3.3.1 Screening of cultures for BSH activity

BSH activity was assessed for four strains via the plate method (Liang et al., 2020). As shown in Figure 1C, all four strains exhibited bile salt hydrolysis via the formation of distinct white precipitate halos on MRS agar plates supplemented with 0.5% TDCA, a primary human bile salt. Each strain hydrolyzed TDCA exclusively, exhibiting varying levels of activity, likely attributable to the strain-specific substrate specificity of their BSH enzymes.

3.3.2 Assessment of Probiotic Survival Under Simulated Gastrointestinal Conditions

Survival under simulated gastric conditions—low pH (2.0–4.0) and pepsin activity—serves as a critical selection criterion for probiotics navigating stomach barriers. Strain B2 showed near-complete loss of viability at pH 2.0 for 2 h, a sharp decline at pH 3.0/4.0, but stability at pH 6.8/8.0; by 3 h, survival reached 86 ± 1.4% (pH 3.0) and 54 ± 1.5% (pH 4.0). B3 viability decreased to zero at pH 2.0 after 3 h (87 ± 1.4% at pH 3.0; 45 ± 1.5% at pH 4.0), with minimal changes at neutral/alkaline pH. B4 exhibited zero survival at pH 2.0, 79 ± 1.5% (pH 3.0), and 18 ± 1.5% (pH 4.0). B7 did not survive at pH 2.0, 52 ± 1.5% at pH 3.0, and 31 ± 1.5% at pH 4.0. Pepsin exposure did not fully eliminate viable cells across strains. The isolated strains exhibited high tolerance to simulated intestinal fluid (SIF; pancreatin, pH 6.8–8.0), maintaining a viability of >90%. These findings suggest that the intestinal environment had a negligible effect on the survival of the probiotic candidates (Figure 2A). These profiles differ notably from the superior gastric tolerance reported for *Lactobacillus fermentum* TIU19 from Haria (Das et al., 2022).

3.3.3 Tolerance to bile salt

Bile salts, which act as detergents that disrupt bacterial lipid membranes, pose a key intestinal barrier; tolerance—often linked to cell surface polysaccharides—determines probiotic viability and efficacy (Tarique et al., 2022). All four isolates tolerated 0.3% bile salts with minimal viability changes, but the reductions increased at higher concentrations: at 0.5%, B2 lost 22 ±

1.2%, B3 13 ± 1.23%, B4 16 ± 1.32%, and B7 21 ± 1.25%; at 0.7%, the losses reached 36 ± 1.12% (B2), 20 ± 1.04% (B3), 34 ± 1.05% (B4), and 38 ± 1.11% (B7) (Figure 2B). Strain-specific responses suggest adaptation to bamboo fermentation niches, with potential recovery postexposure; these profiles diverge from those of fermented milk LAB (Kathiriya et al., 2018).

3.3.4 Coaggregation

"In preliminary probiotic screening, coaggregation is a vital trait that facilitates the recognition of pathogens and the formation of a physical barrier, thereby preventing pathogenic adhesion to the intestinal epithelium. In the present study, the coaggregation capabilities of the isolated strains were evaluated against two reference pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 2B).

3.3.5 Cell Surface Hydrophobicity

The cell surface hydrophobicity (CSH) of the *Lactobacillus* isolates was evaluated using xylene as the hydrocarbon phase. The tested strains presented hydrophobicity values ranging from 30% to 59%, demonstrating varying degrees of surface affinity (Table 2C).

3.3.6 Autoaggregation

Autoaggregation is a key trait evaluated during preliminary screening of potential probiotic bacteria. This property allows probiotics to form substantial biomass, which is essential for conferring health benefits. In this study, autoaggregation was quantified for four isolated strains, with percentages ranging from 36.98% to 49% (Table 2C).

3.3.7 DPPH-Free Radical Scavenging Activity

The ability of lactic acid bacteria (LAB) to colonize the intestinal tract and neutralize free radicals is essential for host protection against oxidative stress. In this study, the antioxidant capacity of four live LAB isolates was evaluated via a DPPH radical scavenging assay. The results revealed significant interstrain variability in scavenging efficiency. Among the tested isolates shown in Figure 2C, strain B2 presented the highest DPPH scavenging activity (87.91±0.89%), followed by B3 (79.11±1.21%), B4 (72.17±1.04%), and B7 (68.22±1.12%). These findings indicate that live cultures typically demonstrate superior antioxidant activity, necessitating further investigation (Ampemohotti et al. 2025).

3.3.8 Adhesion assay

The ability of the bacterial isolates (B2, B3, B4, and B7) to adhere to the human colon carcinoma cell line HT-29 was evaluated to determine their potential for intestinal colonization. All four isolates exhibited robust adhesive capabilities, with adhesion percentages exceeding 68% (Figure 2D). Isolate B4 had the highest adhesive affinity, reaching an adhesion percentage of 75.1% ± 2.1%. Isolate B2 had an adhesion rate of 72.4% ± 1.8%. Isolates B7 and B3 had slightly lower but significant adhesion values of 70.6% ± 1.2% and 68.9% ± 1.5%, respectively. Adhesion to the intestinal epithelium is widely recognized as one of the most critical selection criteria for probiotics. It is a prerequisite for transient colonization, which allows bacteria to exert beneficial effects such as the exclusion of pathogens, the modulation of the immune system, and the strengthening of the mucosal barrier (Rohith et al. 2021).

3.4 Isolation and FT-IR characterization of crude-purified EPS

FT-IR spectroscopy confirmed the carbohydrate nature of exopolysaccharides (EPS) extracted from isolates B2, B3, B4, and B7 (Figure 3). All spectra displayed characteristic absorption bands: broad O–H stretching at 3403–3428 cm^{-1} (hydrogen bonding), aliphatic C–H stretching at 2925–2933 cm^{-1} (hexose sugars, well-defined in B4 and B7), and amide I C=O stretching at 1632–1647 cm^{-1}. Distinct amide II bands at 1547–1548 cm^{-1} (N–H bending) in B4 and B7 indicated higher concentrations of amino sugars or proteins. Peaks at 1410–1415 cm^{-1} (symmetric COO^- stretching), 1053–1076 cm^{-1} (C–O–C and C–O–H stretching, confirming pyranose rings), and 929–931 cm^{-1} (glycosidic linkages) verified complex heteropolysaccharide structures.

Despite core structural homology, the unique amide II signals in B4 and B7 reflect greater monosaccharide and side-chain complexity. The abundance of hydroxyl and carboxyl groups supports essential functional properties, including antioxidant activity and heavy metal sequestration (Soliemani et al. 2022). Furthermore, complex EPS matrices facilitate intestinal epithelial adherence and inhibit pathogen biofilm formation (Srinivash et al. 2023). The distinct structural features of EPS from B4 and B7 suggest superior colonization ability and techno-functional potential for synbiotic food applications.

3.4.1 Extracellular protein quantification and antimicrobial activity

The extracellular protein (ECP) content of the probiotic isolates and their subsequent inhibitory effects on two major enteric pathogens, *Salmonella* and *Escherichia coli*, were evaluated (Table 2D). The concentration of secreted proteins varied across the four isolates. B2 produced the greatest amount of extracellular protein at 193.77 µg/ml, while B4 had the lowest yield at 118.30 µg/ml. Isolates B7 and B3 had intermediate concentrations of 170.51 µg/ml and 166.21 µg/ml, respectively. All the tested isolates demonstrated clear zones of inhibition (ZOIs) against both pathogens, confirming the antimicrobial nature of their secreted proteins. Isolate B7 exhibited exceptional potency, with a ZOI of 27 mm against Salmonella, significantly surpassing that of the positive control (24 mm). Isolates B4, B3, and B2 followed zones of 23 mm, 22 mm, and 21.5 mm, respectively. The inhibitory effect on *E. coli* was less pronounced than that on *Salmonella*. B2 was the most effective against *E. coli* (16 mm), followed by B7 (15 mm). B4 had the lowest activity at 10 mm. None of the isolates exceeded the *E. coli* positive control (26 mm). The secretome of probiotic bacteria—comprising organic acids, hydrogen peroxide, and antimicrobial peptides (bacteriocins)—plays a pivotal role in maintaining gut homeostasis by suppressing pathogen growth (Anjana et al. 2022).

3.4.2 SCFA extraction from probiotic supernatants and derivatization

SCFAs, including acetate, propionate, and butyrate, represent the principal metabolites through which probiotics exert their health-promoting effects. Consequently, the analysis of these volatile fatty acids in the culture supernatant is vital for characterizing the ability of isolates to regulate host metabolic and immunological functions. This process characterizes the metabolic capabilities of probiotic strains, identifying high SCFA producers essential for therapeutic applications. Research, such as studies on fermented foods, has quantified SCFA yields to validate probiotic efficacy and support claims for health benefits, such as reducing inflammation or improving barrier function (Calvigioni et al. 2023).

Short-chain fatty acids (SCFAs) were recovered from the sample matrix via solvent extraction via diethyl ether (DE). The extraction yields of SCFAs obtained with DE were significantly greater than those obtained with other solvents, such as dichloromethane, hexane, cyclohexane, ethyl acetate, and petroleum ether (Zhang et al., 2019). Although SCFAs are inherently volatile, underivatized SCFAs possess high polarity, which frequently leads to poor gas chromatography (GC) performance, including severe peak tailing and low sensitivity. Derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylates the polar carboxylic group, generating volatile, thermally stable trimethylsilyl (TMS) esters that

markedly enhance chromatographic peak shape, resolution, and detectability during GC–MS analysis (Fig S1). Among the four evaluated samples, the relative abundance of SCFAs was highest in the B7 samples and lowest in the B2 samples (Table 3A). In addition to the primary SCFAs, other organic acids—including valeric, 4-methylvaleric, hexanoic, and heptanoic acids—were identified within the probiotic supernatants. Conversely, the uninoculated MRS media used as a control contained more than 60% hydrocarbons and more than 30% aromatic compounds.

3.4.3 Other important bioactive compounds found via GC–MS.

Gas chromatography–mass spectrometry (GC–MS) analysis revealed a diverse array of bioactive constituents in addition to the identified short-chain fatty acids (SCFAs). The presence of these metabolites suggests a broad spectrum of potential therapeutic activities. As shown in Table 3B, several bioactive compounds, many of which are known to possess significant immunomodulatory properties, were common to all the tested probiotic strains. There are many compounds found in B2 and B3, but the compounds could not be identified properly. Some important immunomodulatory and antimicrobial compounds have been previously identified by Dawwam et al. 2022.

3.4.4 GC–MS analysis of probiotic metabolites without derivation

GC-MS profiling of underivatized culture supernatants revealed distinct, strain-specific volatile organic compound (VOC) profiles (Fig S2, Table 3C). Isolates B2 and B7 each yielded 10 organic compounds, while B3 and B4 produced seven, consisting primarily of esters, acids, and pyrrole derivatives. While partially aligning with findings by Geng et al. (2025), the isolates demonstrated unique metabolic capabilities, consistently producing immunomodulatory catecholamines, antimicrobial sulfones, pyridines, and long-chain alkanes. The presence of these bioactive metabolites beyond standard short-chain fatty acids underscores the functional potential of these supernatants for nutraceutical applications, particularly in immune modulation and gut-brain axis regulation (Chang et al. 2021).

3.5 Assessment of the Cytotoxicity of Heat-Killed Probiotic Strains on RAW264.7 Cells

The cytotoxic profile of four heat-killed probiotic strains (B2, B3, B4, and B7) against the HT-29 cell line was evaluated following 24 h of exposure via the MTT assay across a range of

concentrations (10--100 µg/mL, corresponding to MOIs of 1:50--500). All four treatments resulted in a clear, concentration-dependent decrease in cell viability and a corresponding increase in cytotoxicity (Figure 4A). At the lowest concentration tested (10 µg/mL/MOI 1:50), all heat-killed strains demonstrated low cytotoxicity and maintained high cell viability. Strain B2 showed the highest cell viability at 89.84% (10.16% cytotoxicity), followed by B3 at 88.37% (11.63% cytotoxicity), B7 at 88.15% (11.85% cytotoxicity), and B4 at 85.84% (14.16% cytotoxicity).

3.5.1 Immune-Stimulating Activity of Heat-Killed Isolates on HT-29 Cells

Heat-killed isolates (B2, B3, B4, and B7) differentially modulated MUC2, IFN-γ, IL-10, and IL-8 gene expression in uninduced HT-29 cells ($2^{-\Delta\Delta Ct}$, Figure 4B). Isolate B4 exhibited the strongest immunoregulatory profile, driving maximum IL-10 induction (10.63-fold) and high IFN-γ expression (8.48-fold) alongside moderate MUC2 elevation (4.0-fold) and suppressed baseline IL-8. Isolate B7 yielded the highest MUC2 upregulation (4.99-fold) accompanied by robust IFN-γ (7.19-fold). Isolate B2 moderately induced MUC2 (3.74-fold) and IFN-γ (4.7-fold), whereas B3 showed minimal MUC2 induction (0.91-fold). Conversely, the untreated control (C1) exhibited elevated pro-inflammatory IL-8 expression (7.21-fold) and suppressed MUC2 and IL-10 levels.

These results demonstrate that non-viable paraprobiotics can reinforce mucosal barrier dynamics and immune homeostasis independently of live colonization. Upregulation of MUC2 by B7 and B2 highlights their capacity to strengthen the protective mucus layer and prevent pathogen translocation (Ren et al. 2018). Furthermore, the exceptional IL-10 induction by B4 aligns with TLR-mediated anti-inflammatory signaling pathways (Noh et al. 2022). Overall, the isolates effectively suppressed baseline pro-inflammatory signaling (IL-8) while enhancing epithelial barrier and regulatory cytokine markers.

4. Conclusion:

In summary, this study demonstrated that fermented bamboo shoots from Manipur represent a prolific reservoir of metabolically diverse and functionally robust probiotic strains. In addition to survival, the identified isolates—specifically *L. paracasei*, *L. rhamnosus*, *P. pentosaceus*, and *L. fermentum*—actively enhance host health via the production of antimicrobial proteins, SCFAs, and bioactive VOCs. These findings position traditional fermented North East Indian

staples as sustainable sources for bioprospecting, offering novel solutions to gut dysbiosis and antibiotic resistance. While in vitro models have confirmed high adhesion and survival, transitioning to murine models and subsequent human clinical trials is critical to validate their immunomodulatory effects and therapeutic efficacy against conditions such as IBS and metabolic syndrome. Given their inherent stability derived from a competitive fermentation environment, these indigenous strains hold significant promise as high-quality starter cultures and resilient ingredients for functional foods and synbiotic formulations. The data highlight strain-dependent variations among heat-killed isolates. While B4 acts as a powerful inducer of regulatory/anti-inflammatory pathways (IL-10/IFN-γ), B7 and B2 excel in barrier fortification via MUC2 upregulation, making them promising candidates for functional food or therapeutic applications targeting gut health.

Funding

Author N G acknowledges the financial support received in the form of a scholarship under the Maulana Azad National Fellowship (MANF) scheme, funded by the University Grants Commission (UGC), Government of India [Award No. MN01000888, Ref. No. F. No. PLNG/13/2023-PLANNING-NMDFC]. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

Not applicable. This study was conducted exclusively *in vitro* using the established HT-29 cell line and did not involve any human participants or live animal subjects.

Consent to Participate

Not applicable. This study did not involve human participants, animal subjects, or primary personal data collection.

Data Availability Statement

The 16S rRNA gene sequence data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) database, and the corresponding accession numbers are provided in the manuscript. All other datasets supporting the findings of this study are available within the article and its supplementary files.

Author contributions

N G Ibamlung Azor Newme: Conceptualization, Data curation, Resources, Visualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, **Manabendra Mandal:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing - Review & Editing, Validation.

Acknowledgment:

We would like to thank Maulana Azad National Fellowship (MANF) for providing fellowship to the Author (Beneficiary code: BMNTA00007498). We would also like to thank our colleagues, Suraj Phukan, Shubham Roy Choudhury, Rahul Kumar Das, Dhirangkana Bora, Mayur Jyoti Nath, Anupama Sarkar, Munmi Borah for their helpful discussions and feedbacks.

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Figure 1A. A, B, C and D represent phylogenetic tree of strain B2, B3, B4 and B7 respectively.

Figure 1B. A represents hemolytic activity of samples B4 and B2 whereas B represents B3 and B7.

Figure 1C. Bile salt hydrolase activity of isolates

Figure 2A. B2, B3, B4 and B7 strains survivability under simulated human gastrointestinal tract at different period of time.

Figure 2B. Tolerability of B2, B3, B4 and B7 to different concentration of bile salt at different incubation period.

Figure 2C. DPPH radical scavenging activity of bacterial isolates. Antioxidant capacity (%) of probiotic isolates (B2, B3, B4, and B7) evaluated via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Data are presented as mean ± SD ($n = 3$).

Figure 2D. Comparative adherence of probiotic isolates B2, B3, B4, and B7 to HT-29 human intestinal cells.

Figure 3. Comparative Fourier-transform infrared (FT-IR) spectroscopic analysis of EPS produced by probiotics B2, B3, B4, and B7.

Figure 4A. Dose-dependent cytotoxic effect of probiotic isolates on HT-29 cells. Cell viability (%) of HT-29 colon cancer cells following treatment with probiotic isolates (B2, B3, B4, and B7) at concentrations ranging from 10µg/mL to 100µg/mL, evaluated via MTT assay. Bars represent mean ± SD. Statistical comparisons are shown relative to isolate B2 at each corresponding concentration (∗p<0.05, ∗∗p<0.0, ∗∗∗p<0.001, ∗∗∗∗p<0.0001, ns=non-significant).

690 Figure 4B. qPCR analysis of gene expression ($2^{-\Delta\Delta Ct}$) induced by heat-killed
691 probiotics. Expression profile of *MUC2*, *IFN-γ*, *IL-10*, and *IL-8* across probiotic strains (B2,
692 B3, B4, B7, and C1). Values represent mean ± SD. Asterisks denote statistical significance
693 relative to *MUC2* within each isolate group (∗p<0.05, ∗∗p<0.01, ∗∗∗p<0.001, ∗∗∗∗p<0.0001;
694 ns = not significant).
695 Figure S1: GC-MS chromatograms of short-chain fatty acids (SCFAs) extracted from probiotic
696 isolates (B2, B3, B4, and B7) and uninoculated MRS control media after BSTFA+TMS
697 derivatization.
698 Figure S2: GC-MS chromatograms of volatile metabolites extracted with diethyl ether (without
699 derivatization) from probiotic isolates B2, B3, B4, and B7.

Table 1: Primers of four cytokine genes and GAPDH genes.

Sl no	Gene	Primer (5′-3′)
1	MUC2 F	CAGCACCGATTGCTGAGTTG
2	MUC2 R	GCTGGTCATCTCAATGGCAG
3	IL-10 F	AAAGAAGGCATGCACAGCTC
4	IL-10 R	AAGCATGTTAGGCAGGTTGC
5	IL-8 F	TGGCTCTCTTGGCAGCCTTC
6	IL-8 R	TGCACCCAGTTTTCCTTGGG
7	IFN-γ F	ATCCAGTTACTGCCGGTTTG
8	IFN-γ R	GAAGCACCAGGCATGAAATC
9	GADPH F	TGCACCACCAACTGCTTAGC
10	GADPH R	GGCATGGACTGTGGTCATGAG

Table 2A. Assessment of Multiple Antibiotic Resistance (MAR) Indices for the Selected Isolates

Sl no	**Isolated strain**	**Sensitivity**	**Resistance**	**MAR Index**
1	B2	LZ, FC, RIF, C, COT, TE	Nil	0.00
2	B3	LZ, FC, COT, TE, C	RIF	0.167
3	B4	LZ, FC, RIF, C, TE	COT	0.167
4	B7	FC, RIF, C, COT, TE	LZ	0.167

Table 2B. Percentage of co-aggregation of isolates with two pathogenic strains at different period of time (All data are the mean of three independent replications (mean ± SD).

Sl no	**Isolates**	***Pseudomonas aeruginosa***			***Streptococcus aurues***		
		1 hr	3 hr	6 hr	1 hr	3 hr	6 hr
1	B2	11.13±1.21	19.17±1.02	28.51±1.11	11.03±1.05	20.93±0.63	26.86±1.09
2	B3	11.41±1.03	24.67±1.23	34.91±1.17	8.33±1.32	16.11±1.04	25.70±1.21
3	B4	12.74±1.14	25.44±1.26	38.04±1.67	10.46±1.43	15.96±1.04	25.47±1.08
4	B7	15.94±1.06	29.43±1.03	42.47±1.05	13.27±1.35	22.43±1.03	32.66±1.23

Table 2C. Percentage of cell surface hydrophobicity (CSH) and Auto-aggregation

Sl no	**Isolates**	**% CSH**	**% Auto-aggregation**
1	B2	34.81±1.02	36.98±1.23
2	B3	57.72±1.3	49.85±1.2
3	B4	59.62±1.05	42.99±1.09
4	B7	30.68±1.08	48.98±1.04

Table 2D. Antagonistic potential of probiotic-derived extracellular proteins against *Salmonella* and *E. coli*

Sample	**Amount of Extracellular proteins (µg/ml)**	**Against *Salmonella***	**Positive Control**	**Against *E.coli***	**Positive Control**	**Negative Control**
B2	193.77	21.5	24	16	26	0
B3	166.21	22	24	13	26	0
B4	118.30	23	24	10	26	0
B7	170.51	27	24	15	26	0

Table 3A. Short chain fatty acids produce by four isolates

SCFAs	B2	B3	B4	B7
Acetic Acid	28.00% (RT: 3.425)	44.70% (RT: 3.416)	47.16% (RT: 3.405)	59.00% (RT: 3.399)
Propionic Acid	Not Detected	2.28% (RT: 3.578)	2.45% (RT: 3.571)	3.11% (RT: 3.559)
Isobutyric Acid	Not Detected	Not Detected	1.68% (RT: 3.798)	3.18% (RT: 3.782)
Butyric Acid	10.15% (RT: 4.395)	12.85% (RT: 4.385)	9.74% (RT: 4.366)	10.55% (RT: 4.367)

Table 3B. Other important immunomodulatory compounds found from metabolites of four isolates

Metabolite name	Metabolite	Immunomodulatory properties	B2	B3	B4	B7	Con
Benzeneacetic acid (Phenylacetic acid)	Aromatic carboxylic acid	Anti-inflammatory, immune regulation, antimicrobial	–	–	✓	✓	–
Propanedioic acid, phenyl (Phenylmalonic acid)	Aromatic dicarboxylic acid	Anti-inflammatory, immunoregulatory	–	–	✓	✓	–
n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	TLR4 signaling, immune activation, anti-inflammatory	–	–	✓	–	–
Dodecanoic acid (Lauric acid)	Saturated fatty acid	Antimicrobial, immune modulation, anti-inflammatory	–	–	✓	–	–

Octadecanoic acid (Stearic acid)	Saturated fatty acid	Immune regulation, cellular signaling	–	–	✓	–	–
cis-2,3-Epoxyoctane	Epoxide	Potential prebiotic, immunomodulatory, gut barrier function	–	–	✓	✓	–
Oxirane, 2-methyl-3-propyl-, cis-	Epoxide	Potential prebiotic activity, gut health	–	–	✓	✓	–
Oxirane, 2-methylbutvl-	Epoxide	Potential prebiotic effects	–	–	–	✓	–
Hydroperoxide, 1-methylpentyl	Hydroperoxide	Oxidative stress signaling, immune activation	–	✓	✓	✓	–
Hydroperoxide, 1-methylhexyl	Hydroperoxide	Oxidative stress signaling, immune response	–	✓	✓	–	–
Hydroxylamine, O-decvl-	Hydroxylamine derivative	Antimicrobial, potential immunomodulator	–	–	✓	✓	–
1,2,6-Hexanetriol	Polyol	Prebiotic effect, immune tolerance, barrier function	–	–	✓	–	–
Pentyl phenylacetate	Aromatic ester	Potential anti-inflammatory effects	–	–	✓	✓	–

Table 3C. GC-MS identification of volatile compound and predicted biological activities of metabolites produced by four strains.

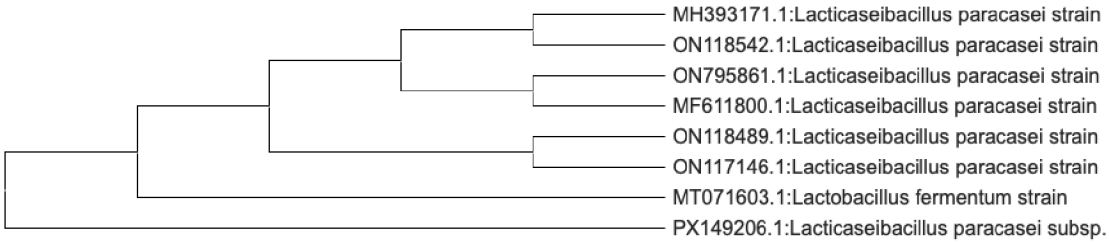
B2 postbiotics with predicted biological activities					
RT (min)	Area %	Compound Name	Molecular Formula	MW (g/mol)	Reported Biological Activity

5.534	1.327	E-11(13-Methyl)tetradecen-1-ol	C15H30O	226	Antimicrobial, membrane-active fatty alcohol
6.668	1.567	Di-n-decyl sulfone	C20H42O2S	346	Antimicrobial lipid derivative
7.585	1.532	Phloroglucitol	C6H12O3	132	Antioxidant, anti-inflammatory
8.036	1.572	1-Methyl-4-(3,4-dihydroxyphenyl)hexahydropyridine	C12H17NO2	207	Antioxidant, neuroprotective potential
10.487	1.741	Phloroglucitol	C6H12O3	132	Radical scavenger, gut-protective
12.589	1.180	Octanal	C8H16O	128	Antibacterial volatile metabolite
12.589	1.180	Dodecanal	C12H24O	184	Broad-spectrum antimicrobial
12.589	1.180	1-Hexadecanamine, N-methyl-	C17H37N	255	Membrane-disrupting antibacterial amine
12.589	1.180	1-Octadecanamine, N-methyl-	C19H41N	283	Antibacterial, antifungal lipid amine
12.589	1.180	2-Heptanol, 6-amino-2-methyl-	C8H19NO	145	Antimicrobial alcohol-amine
B3 postbiotics with predicted biological activities					

RT (min)	Area %	Compound Name	Molecular Formula	MW (g/mol)	Reported Biological Activity
5.117	1.50	3-Ethyl-5-(2-ethylbutyl) octadecane	C26H54	366	Antimicrobial (reported in microbial extracts)
6.660	1.70	4-Aminopentanol	C5H13NO	103	Antimicrobial, precursor of bioactive amines
7.586	3.08	1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanone (TMS derivative)	C9H11NO3 (underivatized core)	181 (core)	Antioxidant, neurotransmitter-like compound
10.487	2.07	Paredrine (TMS derivative)	C9H13NO	151 (core)	Antimicrobial, bioactive amine
11.521	1.53	Di-n-decyl sulfone	C20H42O2S	346	Antimicrobial, antifungal
12.405	2.05	1,2,4-Benzenetricarboxylic acid dimethyl ester	C11H10O6	238	Antioxidant, antimicrobial
13.539	1.54	4-[4-Chlorophenyl]-1-methyl-4-(tetrahydro-2-furyl) piperidine	C16H22ClNO	279	Antimicrobial (reported synthetic bioactive class)
B4 postbiotics with predicted biological activities					
RT (min)	Area %	Compound Name	Molecular Formula	MW (g/mol)	Biological Activity
4.367	2.576	Di-n-decyl sulfone	C20H42O2S	346	Antimicrobial, surfactant-like compound

5.117	2.603	Octadecane derivative	~C26H54	366	Antimicrobial, membrane-active lipid
5.292	**2.723**	1,1-Dodecanediol diacetate	C16H30O4	286	Antimicrobial, antifungal
5.459	1.514	Hexadecanal (Palmitaldehyde)	C16H32O	240	Antibacterial, anti-inflammatory
6.668	2.605	Di-n-decyl sulfone	C20H42O2S	346	Antimicrobial
9.979	1.759	2-Heptanol, 6-amino-2-methyl	C8H19NO	145	Antimicrobial potential
13.474	2.443	Hexadecanal	C16H32O	240	Antimicrobial lipid metabolite
B7 postbiotics with predicted biological activities					
RT (min)	Area %	Compound Name	Molecular Formula	MW (g/mol)	Biological Activity
3.724	2.27	(R)-(-)-Phenylephrine (TMS derivative)	C18H33NO3Si2	311	Antioxidant, antimicrobial
4.366	1.64	Di-n-decyl sulfone	C20H42O2S	346	Antimicrobial lipid
5.117	2.21	1-Pentanol, 4-amino-	C5H13NO	103	Amino alcohol; antimicrobial
5.292	3.03	Hexahydropyridine derivative	C12H17NO2	207	Antioxidant, anti-inflammatory
6.668	2.17	1-Pentanol, 4-amino-	C5H13NO	103	Bioactive amine metabolite

7.593	2.29	Hexadecanal	C16H32O	240	Membrane-active antimicrobial
8.294	1.57	Carbamic acid derivative	—	353	Enzyme inhibition
9.979	1.47	1,1-Dodecanediol diacetate	C16H30O4	286	Antimicrobial ester
10.496	1.85	Paredrine (TMS derivative)	C9H13NO	223	Amino alcohol
13.631	1.43	2-(Methylamino)ethanol (TMS derivative)	C3H9NO	147	Metabolic intermediate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ON795861.1:Lacticaseibacillus paracasei strain
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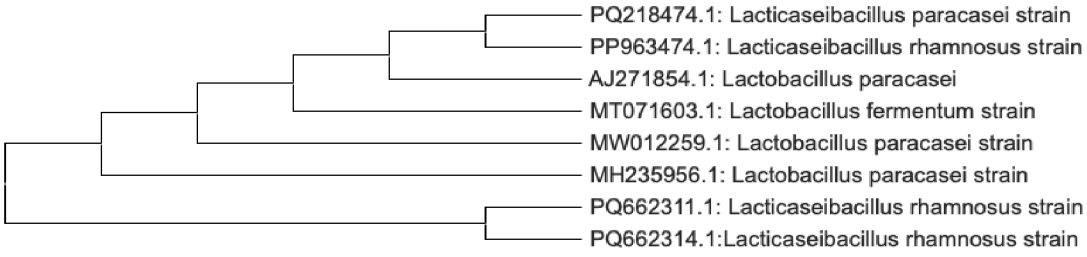
ON118489.1:Lacticaseibacillus paracasei strain
ON117146.1:Lacticaseibacillus paracasei strain
MT071603.1:Lactobacillus fermentum strain
PX149206.1:Lacticaseibacillus paracasei subsp.

A


PQ218474.1: Lacticaseibacillus paracasei strain
PP963474.1: Lacticaseibacillus rhamnosus strain
AJ271854.1: Lactobacillus paracasei
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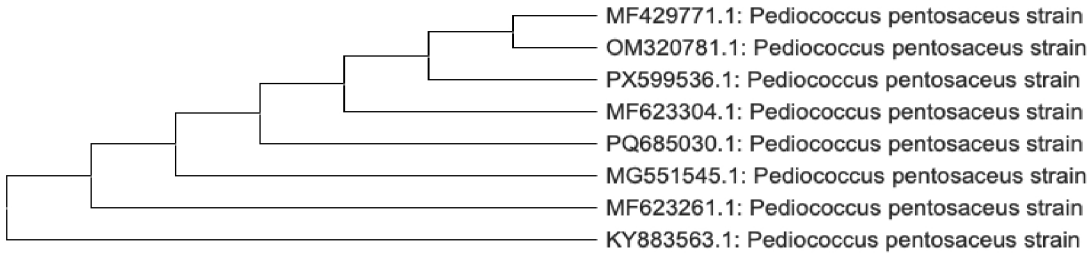
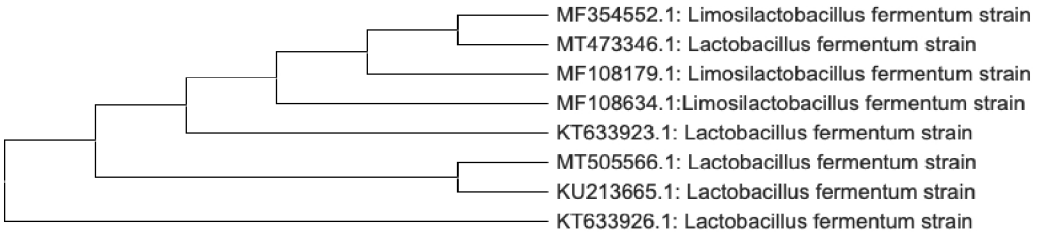
MW012259.1: Lactobacillus paracasei strain
MH235956.1: Lactobacillus paracasei strain
PQ662311.1: Lacticaseibacillus rhamnosus strain
PQ662314.1:Lacticaseibacillus rhamnosus strain

B


MF429771.1: Pediococcus pentosaceus strain
OM320781.1: Pediococcus pentosaceus strain
PX599536.1: Pediococcus pentosaceus strain
MF623304.1: Pediococcus pentosaceus strain
PQ685030.1: Pediococcus pentosaceus strain
MG551545.1: Pediococcus pentosaceus strain
MF623261.1: Pediococcus pentosaceus strain
KY883563.1: Pediococcus pentosaceus strain

C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MF108179.1: Limosilactobacillus fermentum strain
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KT633923.1: Lactobacillus fermentum strain
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D



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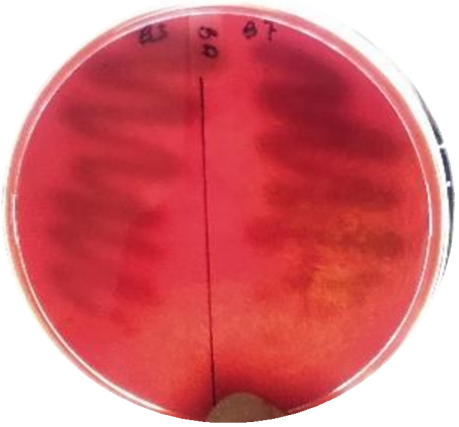


B



B2



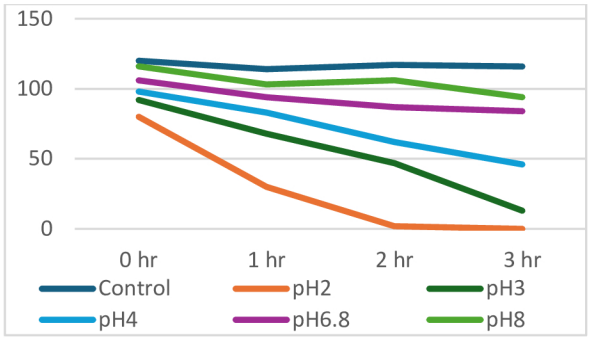
B3



B4



B7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Control
pH2
pH3
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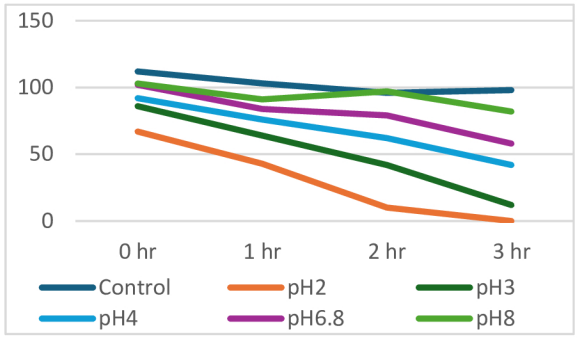
pH6.8
pH8

B2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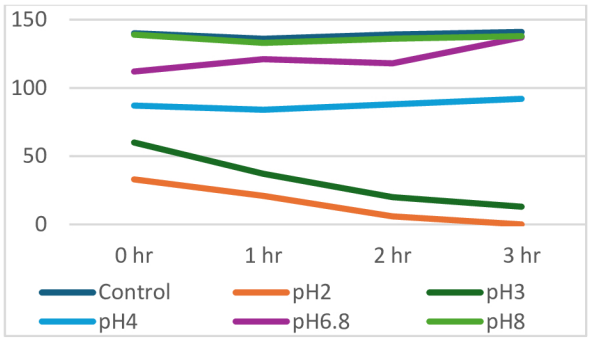
B3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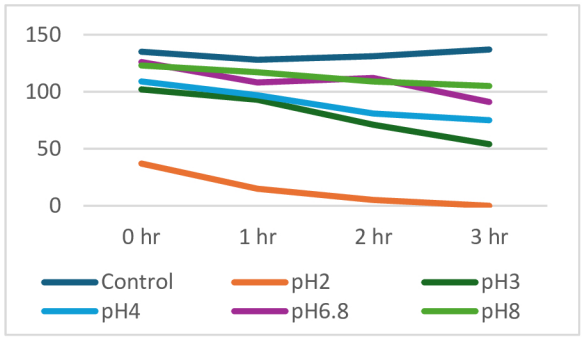
pH8

B4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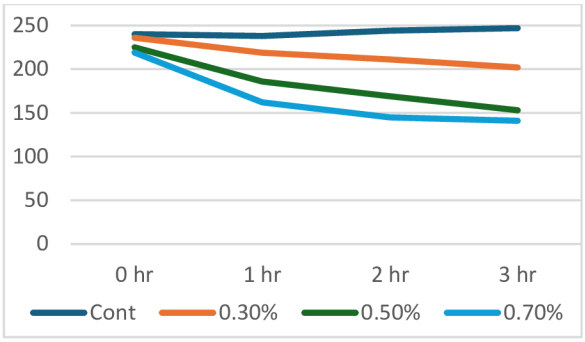
pH8

B7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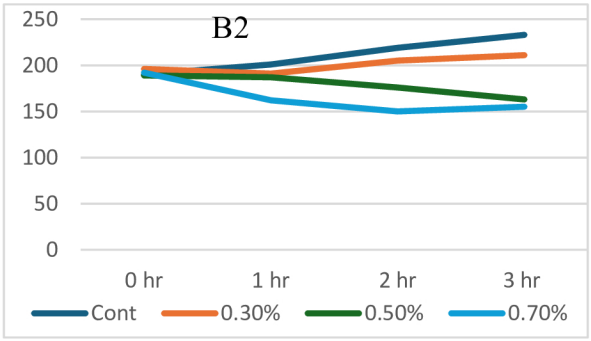
B2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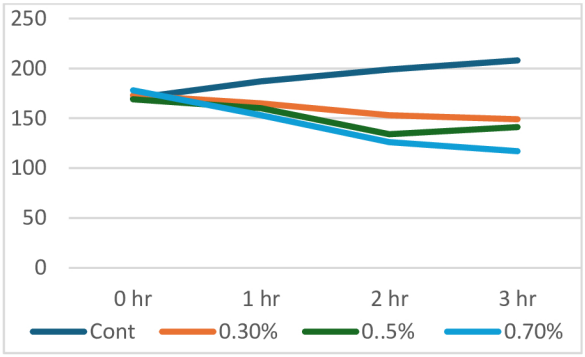
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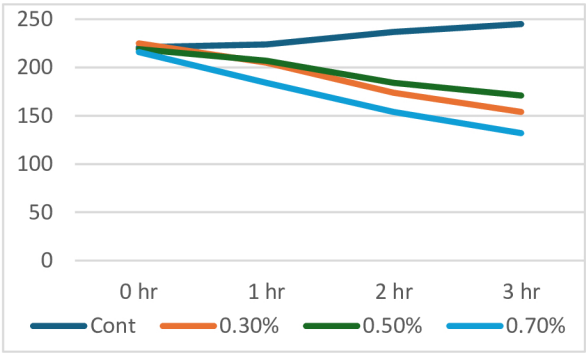
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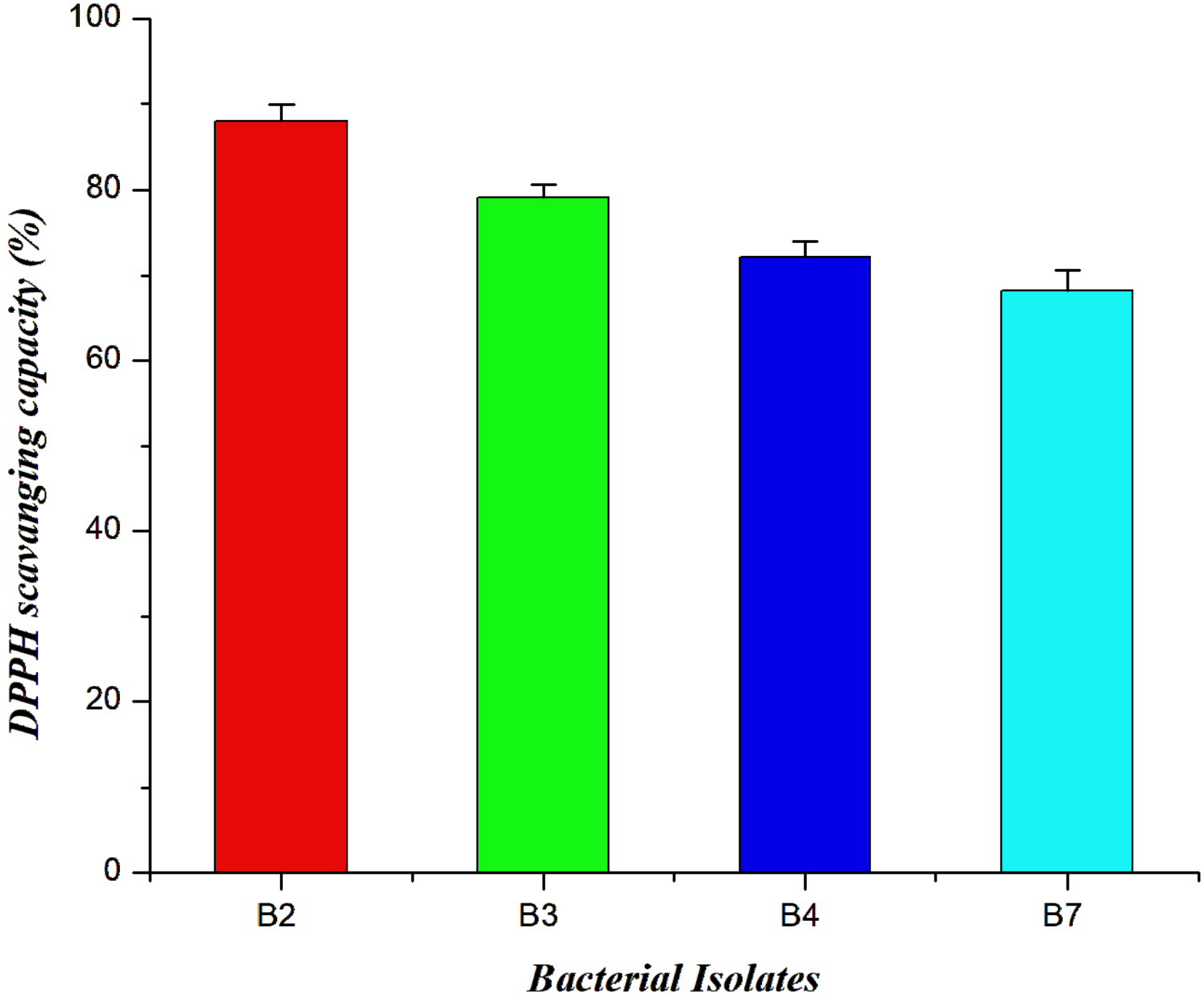
B7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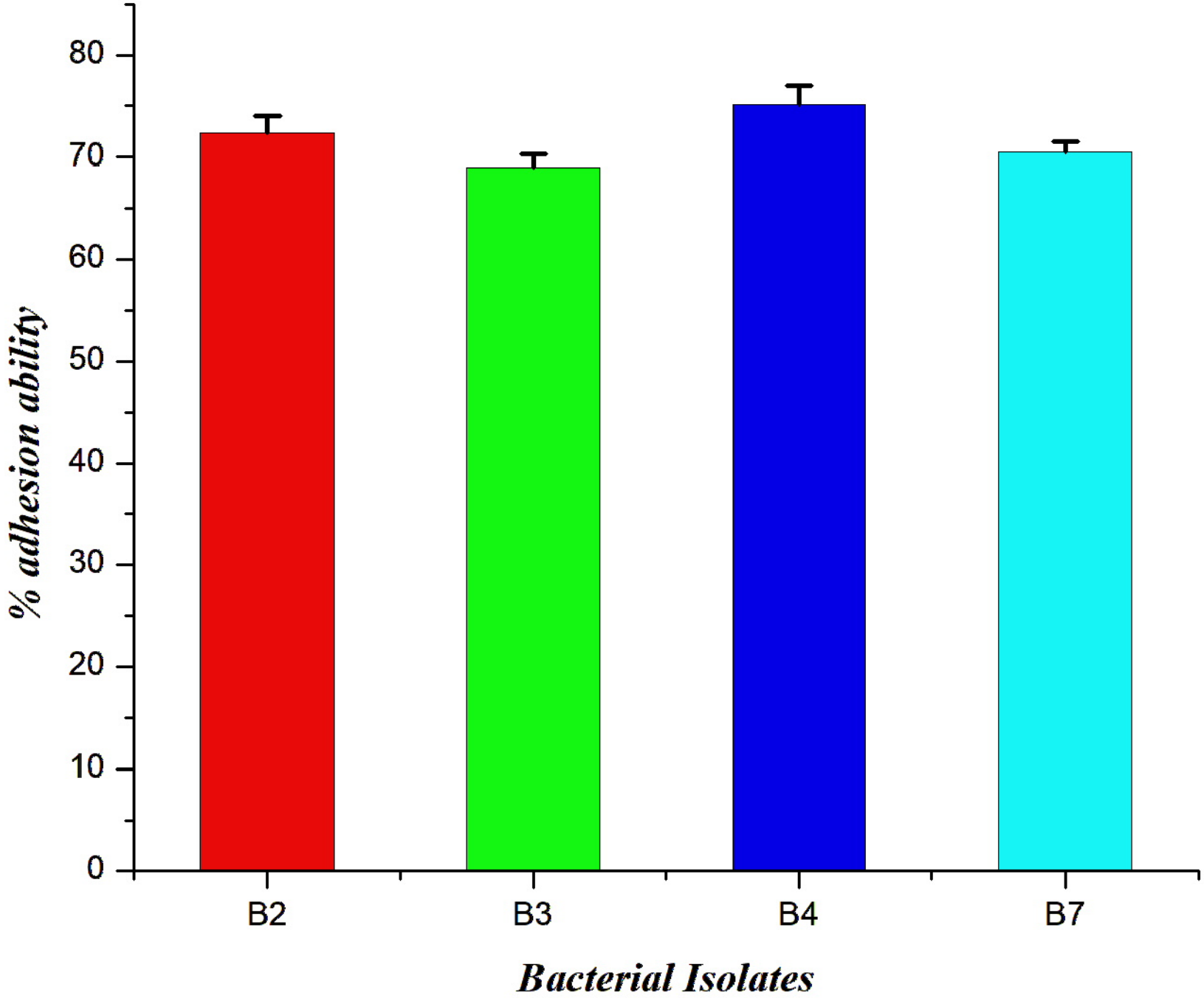
20
0
DPPH scavanging capacity (%)
B2
B3
B4
B7
Bacterial Isolates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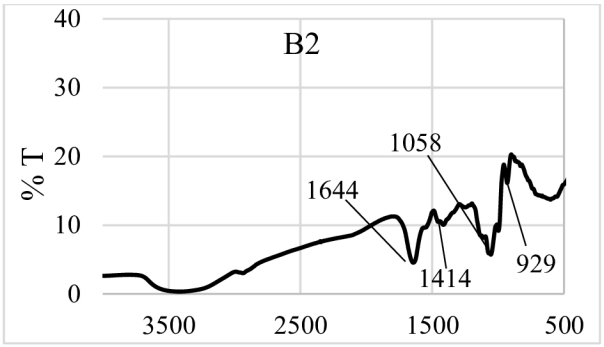
10
0
% adhesion ability
B2
B3
B4
B7
Bacterial Isolates


B2
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1644
1414
929
3500
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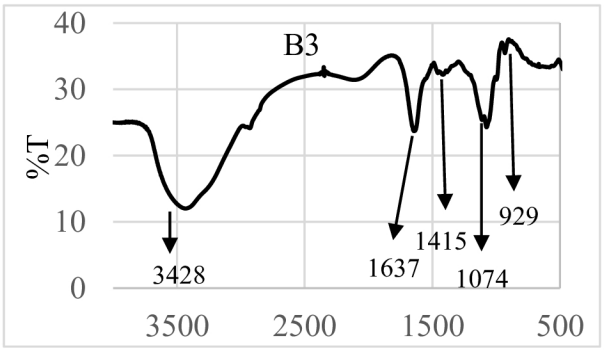
1500
500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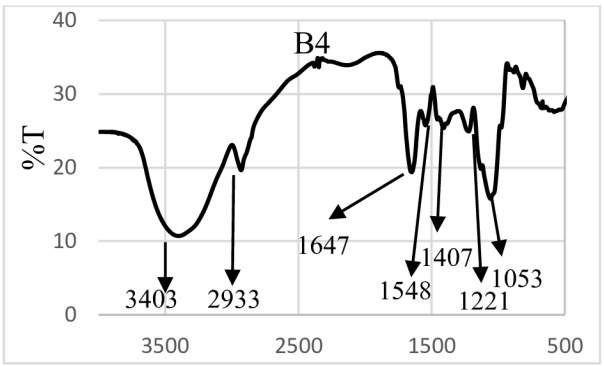
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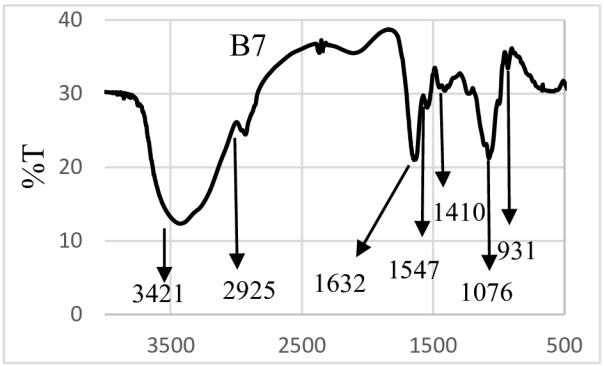
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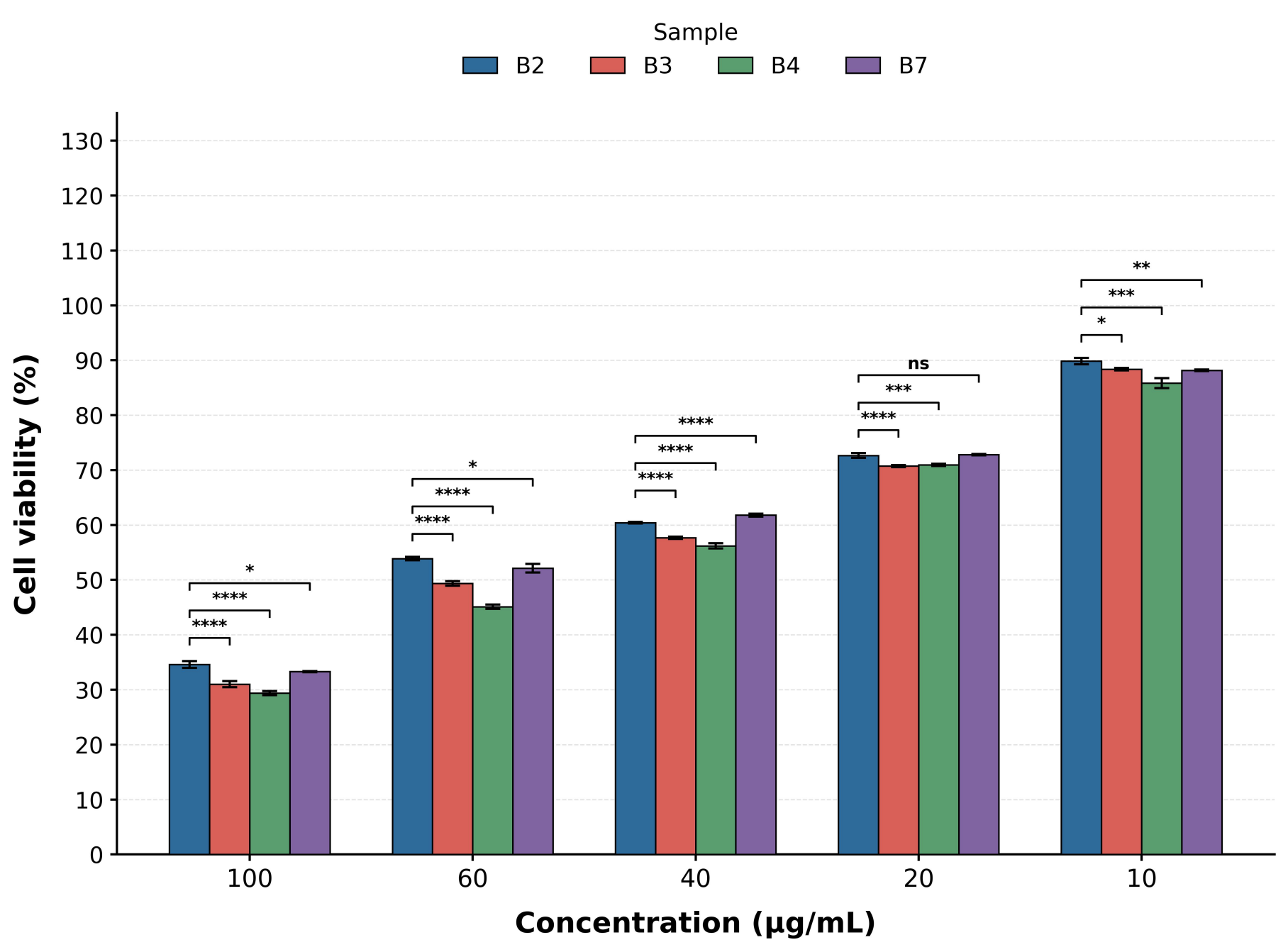
Sample
B2
B3
B4
B7
Cell viability (%)
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120
110
100
90
80
70
60
50
40
30
20
10
0
*
****
****
*
****
****
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ns
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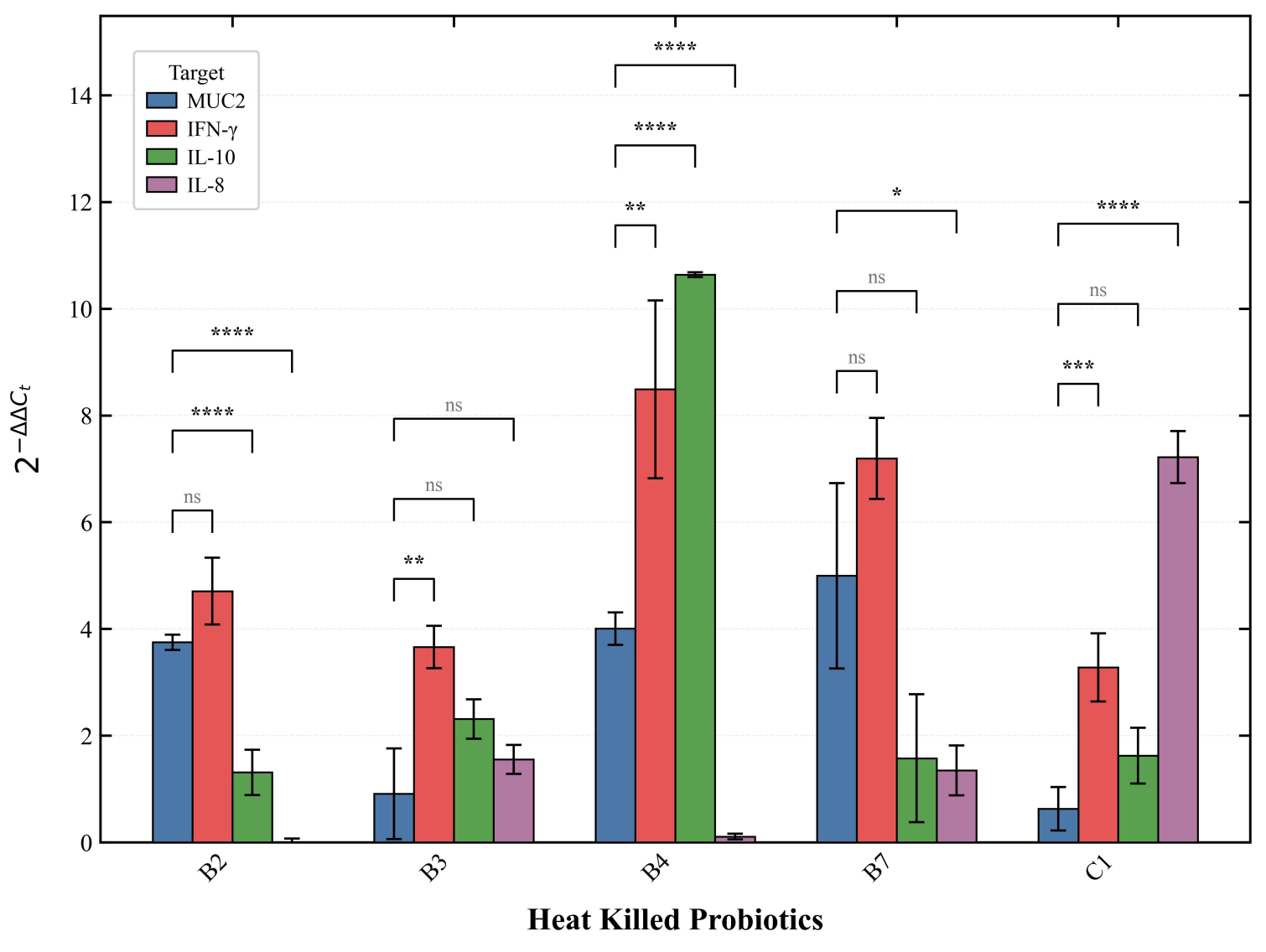
**
***
*
100
60
40
20
10
Concentration (µg/mL)



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MUC2
IFN-γ
IL-10
IL-8
$2^{-\Delta\Delta C_t}$
0
2
4
6
8
10
12
14
****
****
ns
ns
ns
**
****
****
**
*
ns
ns
****
ns
***
B2
B3
B4
B7
C1
Heat Killed Probiotics

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS2.jpg
- FigureS1.jpg