



OPEN ACCESS

EDITED BY

Kumaragurubaran Karthik,
Tamil Nadu Veterinary and Animal Sciences
University, India

REVIEWED BY

Salome Dini,
University of Otago, New Zealand
Ben Ammar Ameni,
National Engineering School of Sfax, Tunisia

*CORRESPONDENCE

Barkha Singhal

✉ barkha@gbu.ac.in;

✉ gupta.barkha@gmail.com

RECEIVED 15 October 2025

REVISED 04 December 2025

ACCEPTED 31 December 2025

PUBLISHED 23 January 2026

CITATION

Pandey K, Kumari M, Mehtab S and Singhal B
(2026) Isolation, purification, and structural
characterization of biosurfactants derived
from indigenous probiotics *Lactobacillus*
helveticus MTCC5463 and *Lactobacillus*
rhamnosus MTCC5462.
Front. Bacteriol. 4:1726048.
doi: 10.3389/fbri.2025.1726048

COPYRIGHT

© 2026 Pandey, Kumari, Mehtab and Singhal.
This is an open-access article distributed under
the terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Isolation, purification, and structural characterization of biosurfactants derived from indigenous probiotics *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462

Kamini Pandey¹, Madhu Kumari¹, Sameena Mehtab²
and Barkha Singhal ^{1*}

¹School of Biotechnology, Gautam Buddha University, Greater Noida, Uttar Pradesh, India,

²Department of Chemistry, G.B.Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

The present study investigates the extraction and characterization of biosurfactants from two Indian originated *Lactobacillus* probiotics. Biosurfactants derived through *Lactobacillus* species have potential applications in food, pharmaceutical, and biomedical sectors. In the current study, *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462 species were aimed to extract, purify, and characterize surface-active properties of produced biosurfactants. The species were cultured in their selective medium and biosurfactants derived are extracted and purified through acid precipitation and solvent extraction. The characterization was performed through measurement of surface tension reduction emulsification index (E24), Fourier transform infrared spectroscopy (FTIR), gas chromatography–mass spectrometry (GC-MS), and ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy. The biosurfactants reduced surface tension from 72 mN/m (distilled water baseline) to 38.73 (± 0.05) mN/m and 41.30 (± 0.04) mN/m for *L. helveticus* MTCC5463 and *L. rhamnosus* MTCC5462, respectively. The emulsification activity was high (E24 > 70%) for various hydrophobic substrates such as petrol, diesel, mustard, and refined oil. FTIR and GC-MS revealed a glycolipid nature, with major fatty acid components such as butanoic acid. The ¹H NMR data support an amphiphilic glycolipid structure characterized by long aliphatic fatty acid chains [δ 0.90–1.50 parts per million (ppm)] and esterified (or hydroxyl-adjacent) methylene groups. The ¹³C NMR data are consistent with a biosurfactant structure comprising long aliphatic chains (δ 14.11; 29.35–31.92 ppm), methylene carbons adjacent to ester or hydroxyl groups (δ 40.95 ppm),

and oxygenated carbons (δ 76.70–77.34 ppm) indicative of glycerol or sugar headgroup backbone. These findings highlighted that *Lactobacillus*-derived biosurfactants have strong surface-active properties that can be explored for various biomedical applications in future studies.

KEYWORDS

biosurfactants, emulsification index, *Lactobacillus* species, physico-chemical characterization, probiotics

1 Introduction

Biosurfactants have become a transformative class of surface-active compounds, often called the “green molecules of the century” because of their remarkable physicochemical properties such as high surface and interfacial activity, low toxicity, biodegradability, and effectiveness under extreme temperatures, pH levels, and salinity (Faccioli et al., 2024; Singh et al., 2024). Conventionally, *Bacillus* and *Pseudomonas* species have been extensively investigated for biosurfactant production, but their commercial production and applications in the food and biomedical sectors have lagged due to their potential pathogenicity. In this context, *Lactobacillus* species, widely recognized as safe (GRAS), have emerged as promising candidates for producing biosurfactants with broad industrial relevance. Several studies have reported biosurfactant production and characterization from different *Lactobacillus* strains, revealing substantial structural diversity. Approximately half of these investigations describe the isolated compounds as glycolipopeptide biosurfactant from *Lactobacillus casei* (Mouafo et al., 2021), while *Lactobacillus jensenii* and *Lactobacillus gasserii* were reported to synthesize glycolipid-type biosurfactants (Morais et al., 2017). *Lactiplantibacillus plantarum* produced a proteinaceous/glycoprotein biosurfactant (Behzadnia et al., 2020), *Lactobacillus helveticus* MRTL9 generated a glycolipopeptide biosurfactant (Sharma et al., 2014), and *Lacticaseibacillus paracasei* A20 was found to produce a glycoprotein/glycolipopeptide biosurfactant (Gudiña et al., 2011), highlighting the heterogeneous molecular nature of *Lactobacillus*-derived biosurfactants (Mouafo et al., 2022). Several representative studies have contributed significantly to understanding these molecules, and more recent works combined biochemical assays, Fourier transform infrared spectroscopy (FTIR), and chromatographic methods to determine detailed structural characteristics (Sakr et al., 2021). Collectively, these studies demonstrate that *Lactobacillus* biosurfactants not only reduce surface tension but also exhibit antimicrobial, anti-adhesive, and emulsifying properties, making them promising candidates for natural and industrial applications (Nataraj et al., 2021; Mouafo et al., 2022). Despite these promising studies, there are considerable gaps in scaling up and achieving cost-effectiveness in their

production (Mouafo et al., 2022). The majority of the studies are focused on well-characterized strains, with little focus placed on indigenous *Lactobacillus* species (Morais et al., 2017). Additionally, there is also a dearth of studies pertaining to structural and compositional characterization of *Lactobacillus* biosurfactants, limiting the researchers from exploring their applications (Satpute et al., 2016). Therefore, the present study proposes to explore biosurfactant production from two indigenous *Lactobacillus* species. By focusing on indigenous strains, this study not only expands the diversity of biosurfactant-producing *Lactobacillus* but also contributes to the rational selection of safe and effective candidates for potential applications in food, biomedical, and environmental sectors. In this context, the present study investigates the screening, extraction, purification, and detailed characterization of biosurfactants produced by *Lactobacillus* spp., employing a comprehensive suite of analytical tools including FTIR, gas chromatography–mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy. By elucidating the structural and functional features of these molecules, the study aims to contribute to the rational design and scalable application of probiotic-derived biosurfactants in biotechnology and healthcare.

2 Materials and chemicals

MRS broth and MRS (de Man, Rogosa, and Sharpe) agar were procured from HiMedia Laboratories (Mumbai, India). Surfactin standard (98% purity) was obtained from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). *L. helveticus* MTCC 5463 and *Lactobacillus rhamnosus* MTCC 5462 were acquired from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Hydrochloric acid (HCl), chloroform, and methanol (analytical grade) were purchased from Merck (Mumbai, India). Mustard oil and refined oil were sourced from a local market. Petrol and diesel were obtained from a local fuel station. TLC plates (silica gel 60 F254, aluminum-backed) were purchased from Merck (Darmstadt, Germany). Deuterated chloroform (CDCl_3 ; $\geq 99.8\%$ D, containing 0.03% TMS as internal standard) was obtained from Sigma-Aldrich for NMR spectroscopy. Distilled water was used for all experimental procedures.

3 Materials and methodology

3.1 Screening for biosurfactant production

L. helveticus MTCC5463 (strain I) strain was originally isolated from the vaginal tract of a healthy adult female, and *L. rhamnosus* MTCC5462 strain (strain V) was originally a gastrointestinal isolate from an infant fecal sample in India at Anand Agriculture University (Prajapati et al., 2011). It was screened for its ability to produce biosurfactant (BS) by five different methods. Strain I and strain V were stored at -80°C in de Man, Rogosa, and Sharpe (MRS) broth (Rogosa et al., 1951; Axelsson, 2004). Biosurfactant production is strongly influenced by the carbon and nitrogen sources provided in the MRS medium (De Man et al., 1960). Components such as maltose (carbon source), peptone, and yeast extract (nitrogen sources) significantly enhance biosurfactant synthesis due to their supportive role in microbial metabolism and surface-active molecule formation (Koim-Puchowska et al., 2021).

The bacterial culture was initially streaked onto MRS agar plates using the quadrant streaking technique to obtain isolated colonies. The plates were incubated at 37°C for 48 h under static conditions. Following incubation, the cultures were maintained at 4°C for up to 2 weeks to preserve viability for short-term use. For subculturing, a single well-isolated colony was aseptically transferred into 10 mL of sterile MRS broth and incubated at 37°C overnight (≈ 16 – 18 h) to obtain an actively growing culture in the logarithmic phase. Selected *Lactobacillus* strains were subsequently subjected to growth curve analysis, where aliquots from the overnight culture were inoculated into fresh MRS broth and monitored at regular intervals (every 2 h) by measuring optical density at 600 nm (OD_{600}) to determine lag, exponential, and stationary phases of growth (Presti et al., 2015) at 37°C for 1 week.

Based on the observed exponential and stationary growth phases of the culture, incubation intervals of 8 h, 16 h, 24 h, 48 h, 72 h, and 1 week were selected for the screening for biosurfactant production. The flasks were harvested by centrifuging the culture at $12,000 \times g$ at 4°C for 20 min. The pellet was collected and washed twice with PBS (phosphate-buffered saline), then pellet was mixed with PBS at a ratio of 1:5. Mixture was kept for gentle stirring at room temperature for 4 h (Sharma et al., 2018). The supernatant (cell-free culture) was collected and used for performing biosurfactant screening assays and surface tension reduction measurement. Assays performed to determine biosurfactant production include drop collapse assay, oil spreading assay, BATH (bacterial adhesion to hydrocarbons) assay, emulsion index assay, and surface tension measurements. The experiment was repeated twice in triplicates (Sharma et al., 2022).

3.2 Qualitative and quantitative screening of biosurfactant production from selected *Lactobacillus* strains

3.2.1 Drop collapse test

Screening of biosurfactant production was performed using the qualitative drop-collapse test. Glass slides were coated by the

addition of 20 μL of various crude oils in the test, such as mustard oil, refined oil, petrol, and diesel followed by 10 μL of culture supernatant (cell-free extract) or controls added to the center of the oil (Bodour and Miller-Maier, 1998). Different oils were used in the drop-collapse assay to evaluate biosurfactant activity across substrates with varying viscosity, polarity, and hydrocarbon composition. Vegetable oils (mustard and refined) provide high-viscosity, long-chain fatty matrices, while petroleum-based substrates (petrol and diesel) represent light and heavy hydrocarbon fractions, respectively. Evaluating biosurfactant behavior across these oils enables comprehensive characterization of surface-active properties and potential application range (Youssef et al., 2004; Morikawa et al., 2000). Surfactin (1%) was taken as a positive control, and water was taken as a negative control. Tests were conducted in triplicate.

3.2.2 Oil spreading test

Biosurfactant production was preliminarily screened using the qualitative oil-spreading method (Shah et al., 2016). Briefly, 25 mL of distilled water was added to a Petri dish, followed by 100 μL of test oils (mustard oil, refined oil, petrol, and diesel) on the surface. A 10- μL aliquot of the cell-free supernatant was then gently placed at the center, and the diameter of the oil-displacement (clear) zone was measured. The oil-displacement area is directly proportional to the concentration and activity of biosurfactants present (Li et al., 2023). All assays were performed in triplicate.

3.2.3 Bacterial adhesion to hydrocarbon assay

Cell hydrophobicity of selected strains was measured by the BATH assay (Thavasi et al., 2013). The quantitative depiction of bacterial adhesion was obtained by a series of adhesion values through varying the volume of used crude oil. To determine the BATH assay, extracted cell-free BS and selected oil (mustard oil, refined oil, petrol, and diesel) were vortexed in a 3:3 (v/v) ratio and kept for a 4-h incubation. After shaking crude oil and BS, the OD of the aqueous phase was then measured at 600 nm in a spectrophotometer. From the OD values, percentage of cells attached to crude oil was calculated using the following formula:

$$\begin{aligned} & \% \text{ of bacterial cell adherence} \\ & = (1 - (\text{OD shaken with oil} / \text{OD original})) \times 100 \end{aligned}$$

where

OD shaken with oil—OD of the mixture containing cells and crude oil.

OD original—OD of the cell suspension in the buffer solution (before mixing with crude oil).

3.2.4 Emulsification index (E24)

Emulsification assay is an indirect method that was used to screen biosurfactant production. An equal volume (3 mL) of cell-free supernatant and petrol was added to a test tube (Tavares et al., 2021). The resulting mixture was vortexed vigorously for 3 min and left undisturbed overnight. After 24 h, the stable emulsion index (E24) was determined as the percentage of height of the emulsified

layer (cm) divided by the height of the entire liquid column (cm). Approximately 10% SDS was taken as positive control and water was taken as negative control. Tests were performed in triplicate.

Emulsification index

$$= (\text{Height of the emulsion layer} / \text{Total height}) * 100$$

3.2.5 Surface tension analysis

Surface tension measurement of cell-free culture broth from selected strains was determined by a tensiometer using the pendant drop method (KRÜSS ADVANCE 1.14.1.16701). Surface tension (dyne/cm) of BS aqueous solutions was measured at room temperature (Makkar and Cameotra, 1999). A decrease in surface tension of the cell-free supernatant due to the growth of microorganisms would confirm the production of biosurfactant. The relationship between the biosurfactant concentration and the surface tension was determined, and to increase the accuracy of the surface tension measurements, more purified determinations were calculated. Surfactin solution prepared at 1 mg/mL concentration was used as a standard.

3.3 Biosurfactant extraction and purification by solvent extraction

The various polar and non-polar solvents like chloroform, ethyl acetate, and methanol were chosen for the study (Medhi et al., 2023). The ratio of solvents was determined by varying the volume of used solvents. For biosurfactant extraction, the pH of the cell-free broth was adjusted to 2 using 6N HCl solution and was stored overnight at 4°C. Surfactant was extracted by adding an equal amount of chloroform and methanol at a ratio of 2:1 and mixed vigorously for 10 min. The mixture was then left undisturbed overnight and allowed to settle, after which the upper phase (aqueous phase/methanol phase) was pipetted into a fresh beaker. The lower phase containing the surfactant was left to evaporate at room temperature inside a fume hood and further stored at 4°C.

3.4 Characterization of produced biosurfactants in indigenous *Lactobacillus* sp.

3.4.1 Characterization of produced biosurfactants by TLC

To determine the nature of the biosurfactant, approximately 4 µL of extracted biosurfactant was loaded onto a TLC plate. After completion of the run, TLC plates were analyzed using UV and developed using iodine and ninhydrin. The plates were taken out, and R_f values were calculated. The preliminary characterization of BS was performed by TLC analysis (Singh and Tiwary, 2016). BS was separated on a silica gel 60 F₂₅₄ aluminum plate using chloroform:methanol:water (65:15:4, v/v/v) as mobile phase. Surfactin solution prepared at 1 mg/mL concentration was used

as a standard.

$$R_f = \frac{\text{distance traveled by component}}{\text{distance traveled by solvent}}$$

3.4.2 Functional group elucidation through Fourier transform infrared spectroscopy

The functional groups of produced biosurfactant were characterized by FTIR analysis using a scanning range of 400–4000 cm⁻¹ at a 4 cm⁻¹ resolution followed by interpretation of spectra through IRPal (Version 2.0) software. FTIR spectroscopy was used for analyzing the surface compositions and for identifying different types of chemical bonds (functional groups), and it can therefore be used to elucidate some components of an unknown mixture (Iqtiaar Md Siddique, 2024). These infrared absorption bands identify specific molecular components and structure.

3.4.3 Molecular weight determination and molecular profiling through GC-MS

The fatty acid profiling of produced biosurfactant was carried out by GC-MS analysis (Smyth et al., 2010). Fatty acids present in biosurfactant was converted into fatty acid methyl esters (FAME) as per the standard method. Approximately 5–10 mg of compound was hydrolyzed with HCl (2 mol/L) at a ratio of 1:10 (w/v) at 100°C for 2 h in a sealed tube followed by recovery and methylation of free fatty acids in 5 mL of n-hexane and 14% boron fluoride-methanol (CH₄BF₃O) reagent, respectively. Helium was used as carrier gas at a suitable flow rate and column pressure. Electron spectra will be generated and analyzed with the standard library of chemical compounds [National Institute of Standards and Technology (NIST)] database.

3.4.4 Nuclear magnetic resonance

To further characterize and confirm the structure of the extracted biosurfactant samples, purified by extraction, ¹H and ¹³C NMR was performed using a Bruker Avance neo spectrometer (Tiwary and Dubey, 2018). For ¹H NMR, 2 mg of extracted biosurfactant was dissolved in 1 mL of CDCl₃, while in ¹³C, 6 mg of the sample was dissolved in 1 mL of CDCl₃. The spectrum was recorded at 298.7 K at a frequency of 400 MHz for both strains.

4 Results

4.1 Screening of selected *Lactobacillus* strains for biosurfactant production

In this study, two lactobacilli strains were screened for cell-bound biosurfactant production. Growth curves were obtained for both strains to establish the relationship between cell growth and biosurfactant production. Growth curve was measured at 8 h, 16 h, 24 h, 48 h, 72 h, and 1 week cultivation; the production of biosurfactants by *L. helveticus* MTCC 5463 (strain I) and *L. rhamnosus* MTCC 5462 (strain V) during growth in MRS

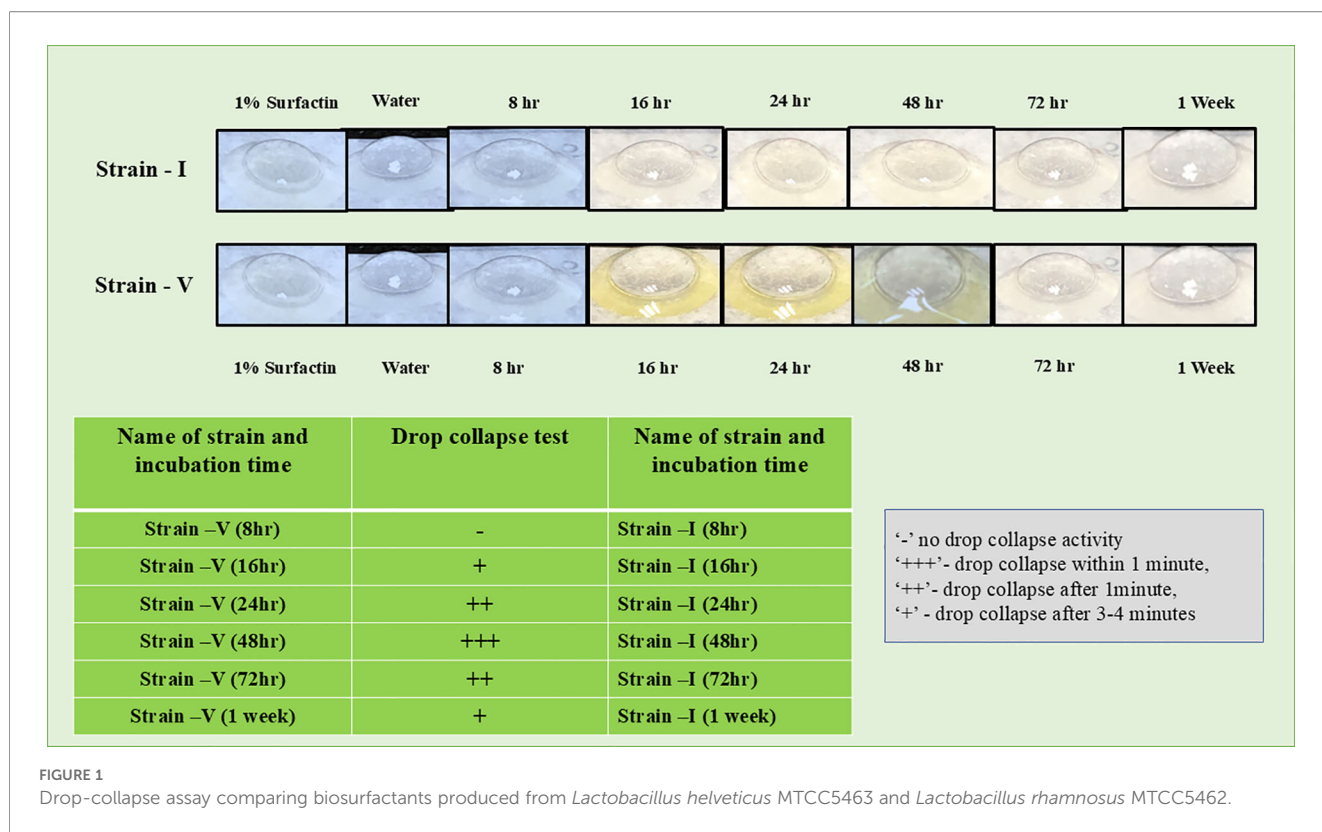


FIGURE 1 Drop-collapse assay comparing biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

broth was monitored by verifying the drop collapse assay, oil spreading assay, BATH assay, emulsion index assay, and surface tension measurements using various oils as organic phase; and the surfactant activity of crude biosurfactant was measured. Qualitative analysis showed biosurfactant activity at 24, 48, and 72 h cultivation in selected strains. For both strains, the cell-bound biosurfactant production increased significantly after 24 h of cultivation, with the highest values at 48 h of cultivation. These three conditions were further selected for purification and quantitative analysis.

4.2 Drop collapse assay

The drop added was left undisturbed for 2 to 3 min and its collapse was monitored. The result was observed as positive (Figure 1) for biosurfactant production when the drop was flat, and those cultures that gave rounded drops were observed as negative, indicative of the lack of biosurfactant production. Strains I and V showed positive results with a flat droplet. *L. helveticus* MTCC 5463 collapsed in 1 min 56 s, and the *L. rhamnosus* MTCC 5462 drop collapsed in 58 s. To further confirm the biosurfactant production, the cell-free culture broths were subjected to other qualitative experiments.

4.3 Oil spreading assay

Oil spreading assay results showed corroboration with the drop collapse assay results, in which selected organisms were found positive for biosurfactant production. Both selected strains were

shown to have the capacity to disperse the selected oil (Figure 2). Oil was displaced with the oil-free clearing zone, and the diameter of this clearing zone indicated the surfactant activity. Strains I and V gave positive results with a clearance zone of 2.5 and 1.8 mm, respectively. The oil displacement test is an indirect measurement of surface activity of biosurfactants: a larger clear area is correlated with higher surface activity.

4.4 BATH assay

Strains I and V indicate the affinity of bacterial cells towards hydrophobic substrates. It was determined that all selected oils showed hydrophobicity efficiently by both crude biosurfactants. The cell surface hydrophobicity with the vegetable oils showed lower bacterial cell adherence than with petrol and diesel. The highest cell adherence was observed at 48 h incubation (Figure 3) in both strains with petrol, *L. helveticus* MTCC 5463 (67.09%) and *L. rhamnosus* MTCC 5462 (66.67%). Visualization of bacterial cells adhered to crude oil confirmed the affinity of cells towards crude oil droplets.

4.5 Emulsification index (E24)

The emulsification activity of BS was determined against mustard oil, refined oil, diesel, and petrol. The maximum emulsification activity of BS was observed at 48 h incubation culture with *L. helveticus* MTCC 5463, 71.43% with petrol, followed by diesel, refined oil, and mustard oil (Figure 4). In all

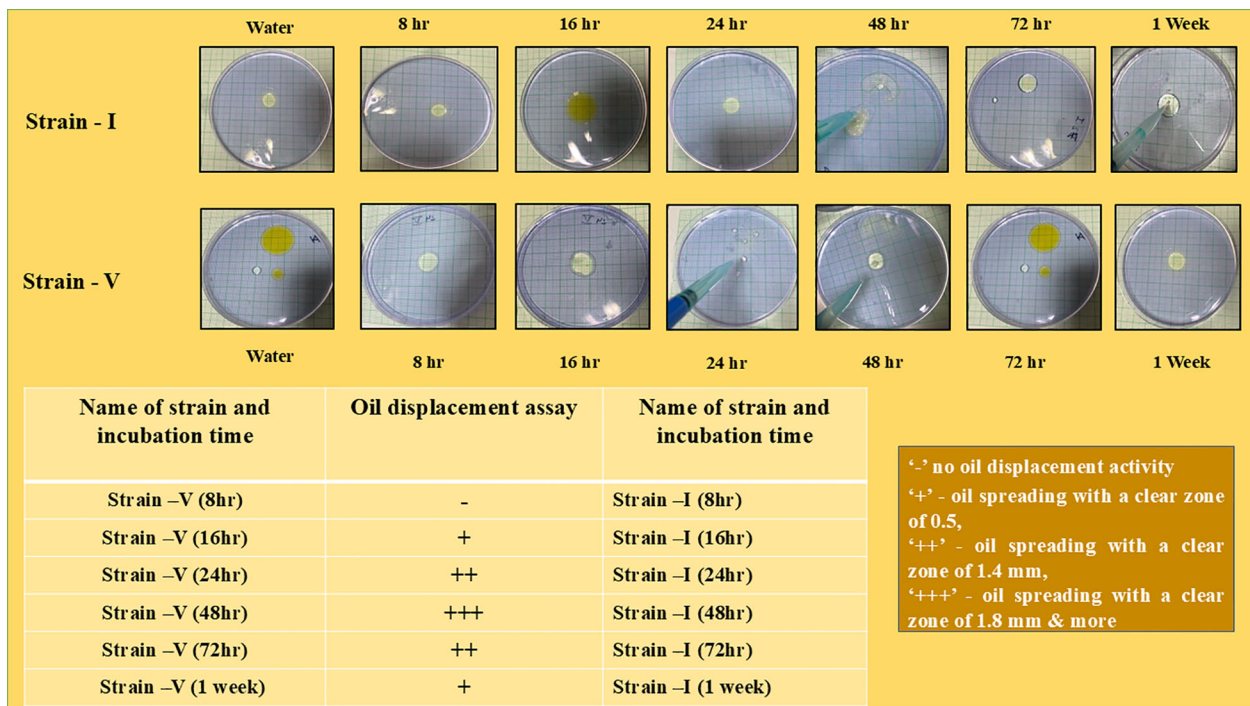


FIGURE 2 Oil-displacement assay comparing biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

cases, E24% was higher than 50% but lower than that obtained with 1% surfactin (used as positive control), whereas the emulsification activity of BS at 48 h of cultivation showed with *L. rhamnosus* MTCC 5462, 70.00% with diesel, followed by mustard oil, refined oil, and petrol.

4.6 Surface tension measurement

Surface tension measurements of crude biosurfactant demonstrated a clear reduction in surface tension, indicating the presence of surface-active compounds. For strain I, crude

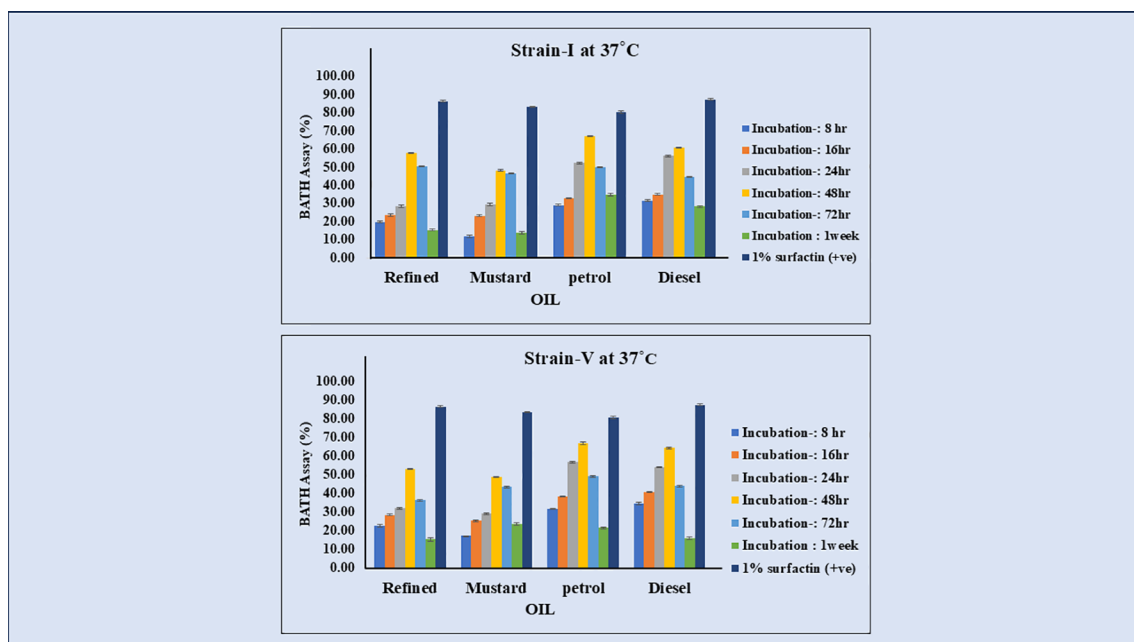


FIGURE 3 BATH assay comparing biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

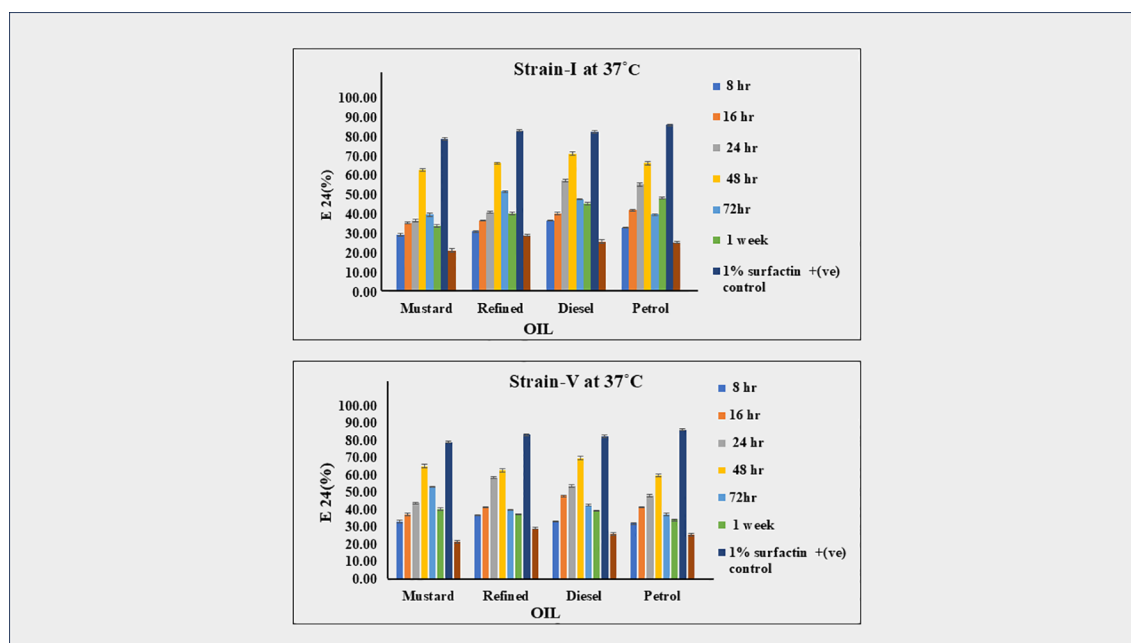


FIGURE 4

E24 index comparing biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

biosurfactant extracts collected at 24, 48, and 72 h of cultivation reduced surface tension at 55.78 ± 0.53 mN/m, 52.25 ± 0.21 mN/m, and 58.12 ± 0.49 mN/m, respectively. In the case of strain V, the crude biosurfactant exhibited surface tension values of 60.86 ± 0.24 mN/m at 24 h, 61.06 ± 0.22 mN/m at 48 h, and 68.12 ± 0.49 mN/m at 72 h. The observed reductions from the typical surface tension of water (72 mN/m) confirm that both strains produce biosurfactant; however, strain I shows comparatively greater surface tension-lowering efficiency across the cultivation period. Both strains gave better results at 48 h with the extracted biosurfactant and were selected for purification. *L. helveticus* MTCC 5463 and *L. rhamnosus* MTCC 5462 at 48 h incubation showed reduction in surface tension to as low as $38.73 (\pm 0.05)$ mN/m and $41.30 (\pm 0.04)$ mN/m, respectively (Figures 5, 6).

4.7 Characterization of produced biosurfactants in indigenous *Lactobacillus* sp.

4.7.1 Thin layer chromatography

The resulting spots were visualized by spraying different-colored developing reagents. The appearance of yellow-brown spots due to the reversible reaction of iodine vapors with lipids was monitored, whereas iodine crystals were used to detect the lipid fraction of BS, and anisaldehyde reagent (2%) was used to detect the carbohydrate group of BS. Anisaldehyde-sulfuric acid reagent was used in thin layer chromatography (TLC) for the detection of glycolipid-type biosurfactants, as it reacts strongly with sugar moieties and lipid chains, producing characteristic color development upon heating (Tuleva et al., 2002; Ammar et al.,

2024). After heating the plates at 110°C for 10 min, separated components appeared as spots on the plate, and the retention factor (R_f) of each component was assessed. TLC analysis of purified BS showed a single spot in replica plates with an R_f value of 0.6–0.9 in both strains (Figure 7). The BS fraction showed a positive reaction with the anisaldehyde reagent and iodine vapor, indicating the presence of carbohydrate and lipid. The above result of TLC analysis demonstrated the glycolipid nature of BS. Similar reports of the production of glycolipids BS by *Pseudomonas* (R_f value 0.7–0.9) are found in the literature (Sim et al., 1997).

$$R_f = \frac{\text{Distance traveled by component}}{\text{Distance traveled by solvent}}$$

4.7.2 Functional group elucidation through Fourier transform infrared spectroscopy

The FTIR spectrum of the biosurfactant I produced by *L. helveticus* MTCC 5463 provided valuable insights into its characteristic functional groups (Figure 8). A prominent broad absorption band centered at $3,275\text{ cm}^{-1}$ indicated the presence of O–H stretching vibrations, consistent with hydroxyl groups from carbohydrate moieties (Okorie and Ogunjobi, 2024). Strong absorption bands observed in the region of $2,955\text{--}2,850\text{ cm}^{-1}$ were attributed to aliphatic C–H stretching, confirming the presence of long hydrocarbon chains within the biosurfactant's structure. An intense absorption band at $1,622\text{ cm}^{-1}$ corresponded to the $>\text{C}=\text{O}$ stretching of ester carbonyl groups, indicative of lipid ester linkages (Okorie and Ogunjobi, 2024). Further supporting the presence of ester bonds, C–O stretching vibrations were observed at $1,274$ and $1,534\text{ cm}^{-1}$. Additional peaks at $1,452\text{ cm}^{-1}$ and $1,412\text{ cm}^{-1}$ were assigned to the bending vibrations of CH^2 and CH_3 groups,

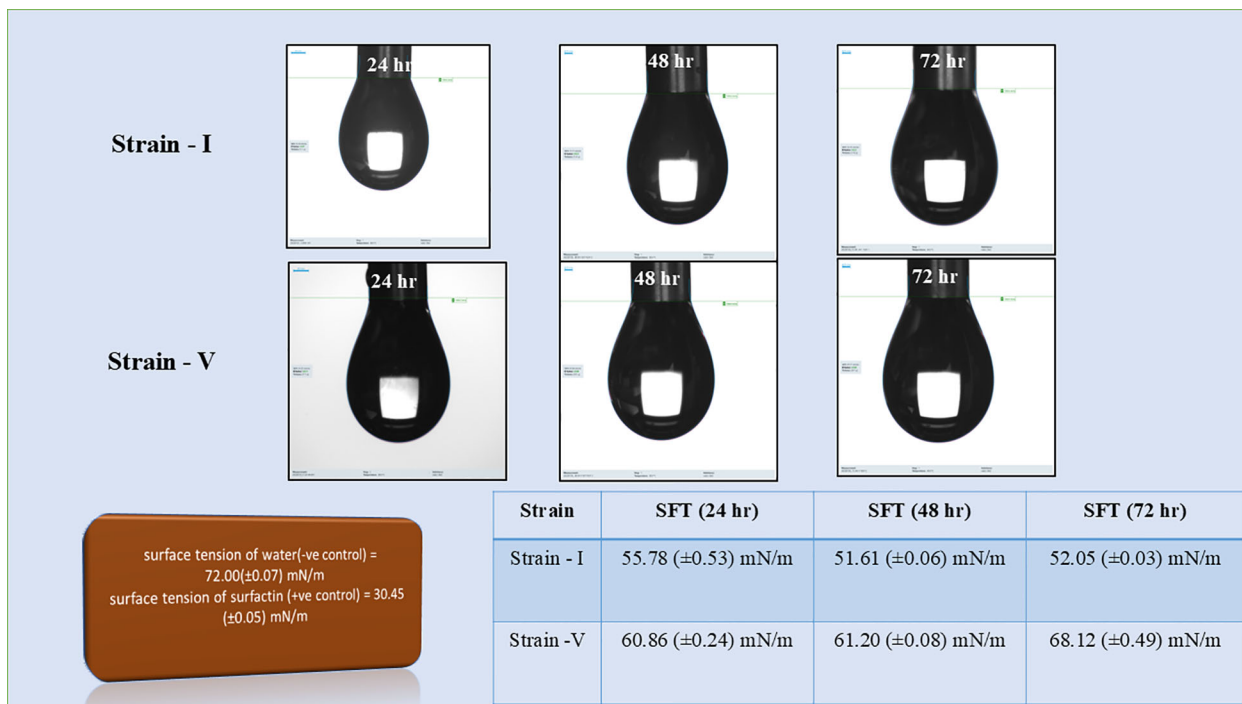


FIGURE 5 Surface tension reduction measurement comparing crude biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.



FIGURE 6 Surface tension reduction measurement comparing purified biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

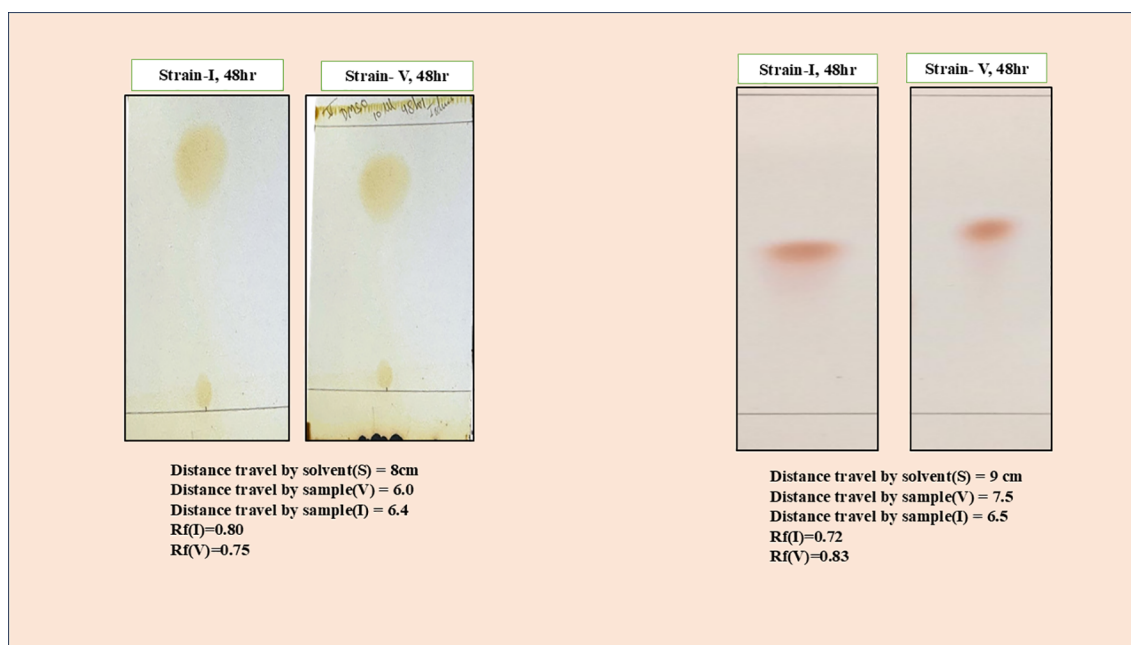


FIGURE 7 Analysis of TLC plates for biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

respectively, further corroborating the aliphatic nature of the compound. Absorption bands in the lower-frequency region, specifically from 1,063 to 1,089 cm^{-1} , were assigned to O–C–O stretching vibrations, typically associated with carboxylic acids in polysaccharides. Furthermore, peaks in the region of 964 to 752 cm^{-1} are characteristic of out-of-plane bending modes of CH^2 groups

within sugar structures. Collectively, these spectral features strongly suggest that the isolated biosurfactant primarily consists of glycolipid-like components. The evident presence of carboxylic acid groups as a functional moiety further indicates the anionic character of the biosurfactant, which is crucial for its surface-active properties.

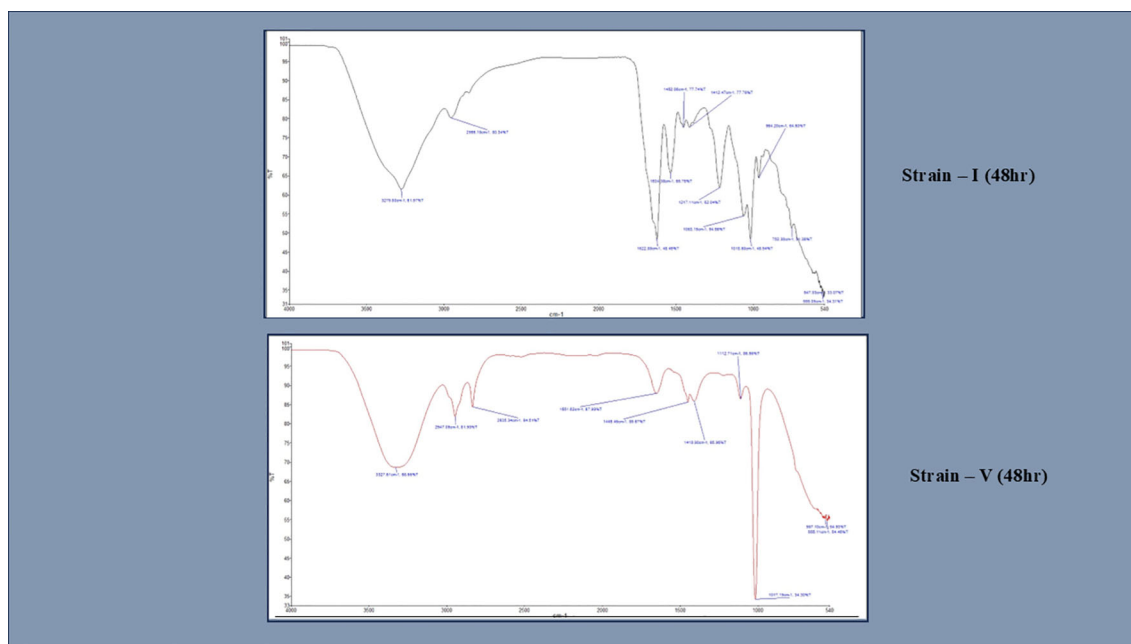


FIGURE 8 Analysis of FTIR spectrum for biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

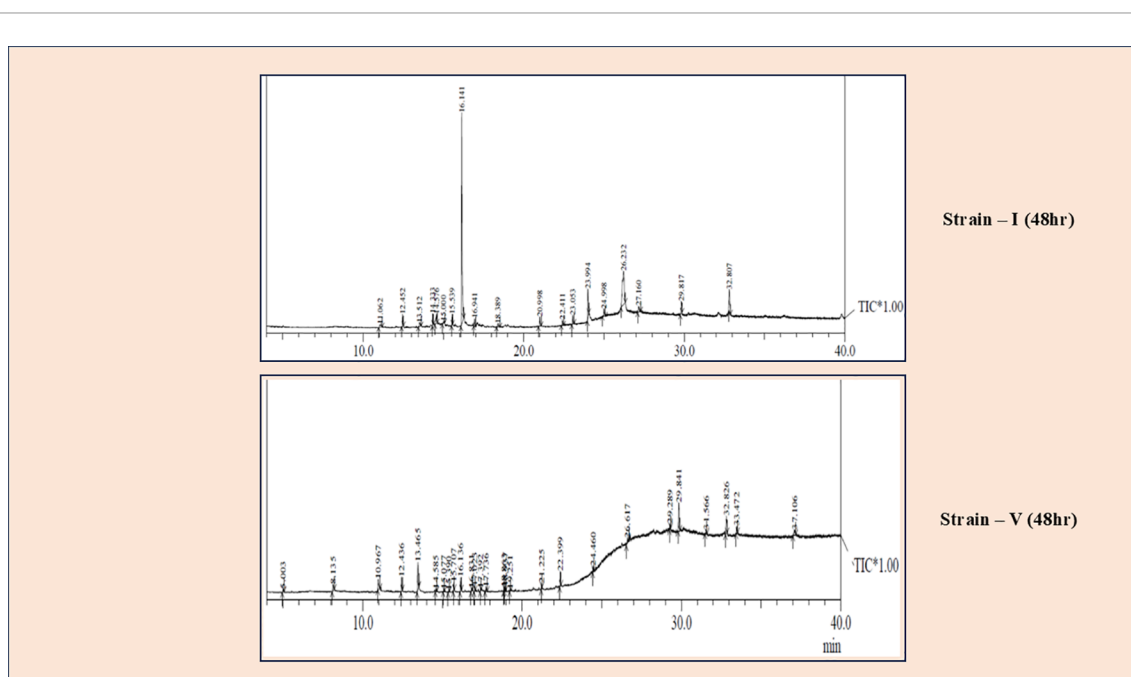
The FTIR spectrum of the biosurfactant V derived from *L. rhamnosus* MTCC5462 revealed distinct absorption bands that reflect the presence of key functional groups (Figure 8). A broad and intense peak of approximately 3,327 cm⁻¹ corresponds to O–H stretching vibrations, indicating hydroxyl functionalities commonly found in carbohydrate structures (Okorie and Ogunjobi, 2024). The strong absorptions within the 2,947 to 2,835 cm⁻¹ range are characteristic of C–H stretching in aliphatic chains, confirming the hydrocarbon backbone typically present in biosurfactants. An absorption band at approximately 1,651 cm⁻¹ is indicative of carbonyl (C=O) stretching, suggestive of ester linkages, which are often present in lipid components. The presence of bending vibrations for methylene (CH²) and methyl (CH₃) groups is evident from peaks near 1,449 and 1,410 cm⁻¹, reflecting the aliphatic character of the molecule. Strong bands in the 1,112 to 1,017 cm⁻¹ region are consistent with C–O–C and O–C–O stretching modes, typically associated with saccharide or glycolipid structures. Furthermore, weak peaks in the 560–550 cm⁻¹ range may correspond to out-of-plane bending of CH² groups in sugar units. Altogether, the spectral features point toward a glycolipid nature of the biosurfactant, combining both lipid and carbohydrate components. The presence of hydroxyl, ester, and carboxyl groups suggests that the molecule possesses amphiphilic properties, essential for its activity as a surface-active agent.

4.7.3 Molecular weight determination and molecular profiling through GC-MS

The GC-MS analysis of strain I, supported by spectral library matching, identified multiple long-chain fatty acid esters and aromatic derivatives (Figure 9). The total ion chromatogram notably highlighted butanoic acid, 1,2,3-propanetriyl ester at a retention time (R. Time) of

16.141 min, constituting a dominant 43.32% of the total area. This significant presence indicates a core triglyceride-like structure, with glycerol esterified by short-chain butanoic acid. Furthermore, the identification of 9-octadecenoic acid (Z)-, 2-(9-octadecen-1-yloxy) ethyl ester at R. Time 26.232 min, contributing 21.00% of the total area, points to the inclusion of unsaturated long-chain fatty acid ether esters within the biosurfactant, crucial for its amphiphilic properties. Minor constituents, such as 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (5.87% at 23.994 min), and a silicon-containing compound, 4-(1-trichlorosilyl-3,3-dimethylbutyl)cyclopentene (6.84% at 32.807 min), were also detected, suggesting additional structural complexities or co-extracted components. These GC-MS findings support the presence of a glycolipid composed of a glycerol backbone esterified with short- and long-chain fatty acids, forming distinct hydrophilic and lipophilic domains vital for surface activity. The results suggest that the biosurfactant is mainly a glycolipid that contains glycerol or sugar units esterified with long-chain fatty acids. This structure creates both hydrophilic and lipophilic regions, giving it amphiphilic properties. The absence of nitrogen-containing compounds indicates that it is not a lipopeptide. Overall, the biosurfactant from *L. helveticus* MTCC 5463 appears to have a triglyceride-based glycolipid structure, suitable for emulsification, reducing surface tension, and possibly antimicrobial use.

The GC-MS analysis of the biosurfactant V extracted from *L. rhamnosus* MTCC 5462 revealed a composition rich in fatty acid esters, hydrocarbons, and phenolic derivatives, indicating its complex amphiphilic nature (Figure 9). A major peak was observed at a retention time of 29.841 min, corresponding to phenol and phosphite (3:1), contributing 16.97% of the total area, suggesting its potential role as a stabilizing or antioxidant component. Another abundant compound was 1,2-benzenedicarboxylic acid, diisodecyl ester



(14.56%) at 13.465 min, indicating the presence of long-chain phthalate-type esters, which are commonly associated with the hydrophobic domains of biosurfactants. Additionally, short-chain components such as butanoic acid, anhydride (4.90% at 16.136 min) further support the amphiphilic structure of the molecule. Hydrocarbons like docosane were consistently detected at various retention times, contributing to the lipophilic character (Okorie and Ogunjobi, 2024). The presence of 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol (11.65% at 32.826 min) and squalene (1.92%) indicates structural diversity and possibly bioactive potential. Minor constituents such as ursolic acid methyl ester (1.21% at 31.566 min) suggest biologically derived triterpenoid components. Collectively, these findings confirm the biosurfactant's glycolipid-like composition, characterized by both polar and nonpolar moieties, essential for its surface activity and potential industrial applications.

4.7.4 ^1H NMR and ^{13}C NMR spectroscopic analysis

The ^1H NMR spectrum of the biosurfactant produced by *L. helveticus* MTCC 5463, recorded in CDCl_3 at 400 MHz, exhibits resonances characteristic of an amphiphilic compound. A multiplet of approximately δ 0.90–1.50 parts per million (ppm) is attributed to terminal methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$) protons, suggesting the presence of extended aliphatic chains. A signal at δ 2.51 ppm corresponds to methylene groups adjacent to electronegative groups, such as carbonyls in ester functionalities. A downfield multiplet at δ 4.67–4.90 ppm likely arises from protons attached to carbons bonded to oxygen atoms, typical of $-\text{CH}-\text{O}$ groups found in sugar alcohols or glycerol backbones. A sharp peak at δ 7.28 ppm is indicative of aromatic protons, suggesting minor aromatic constituents or esters within the structure. Collectively, these chemical shifts support the existence of a glycolipid-like architecture, comprising hydrophobic fatty acid tails and a hydrophilic headgroup.

The ^{13}C NMR spectrum corroborates these findings by revealing signals at δ 14.11 and δ 29.35–31.92 ppm, corresponding to terminal methyl and internal methylene carbons within long aliphatic chains, respectively. A resonance at δ 40.95 ppm may be assigned to methylene carbons adjacent to electron-withdrawing ester or hydroxyl groups. Prominent signals at δ 76.70–77.34 ppm are characteristic of oxygenated carbons, confirming the presence of C–O and C–OH functionalities typical of glycerol or sugar-derived headgroups. The overall pattern of chemical shifts aligns with a glycolipid structure featuring ester linkages between long-chain fatty acids and a polar moiety, likely contributing to the compound's surfactant properties. Collectively, the NMR data of both biosurfactants complement the FTIR and GC-MS findings and confirm that these are glycolipids, likely comprising a glycerol or sugar-based polar headgroup esterified with one or more long-chain fatty acids and minor aromatic components, thus imparting amphiphilic behavior.

The ^1H NMR spectrum of the biosurfactant produced by *L. rhamnosus* MTCC 5462, recorded in CDCl_3 at 400 MHz, revealed distinct peaks corresponding to its amphiphilic molecular structure. Multiplets observed in the range of δ 0.90–1.50 ppm was attributed to terminal methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$)

protons, indicative of long aliphatic fatty acid chains (Figure 10). Signals between δ 2.50 and 3.30 ppm were assigned to methylene groups adjacent to ester carbonyls or hydroxyl functionalities, commonly found in esterified lipid moieties. A prominent peak of approximately δ 4.70–4.90 ppm was ascribed to a proton on a carbon bonded to an oxygen atom, such as the $-\text{CH}-\text{O}$ group of a glycerol or sugar alcohol backbone, supporting the presence of a polar headgroup. Additionally, aromatic protons were detected as a multiplet of approximately δ 7.19–7.28 ppm, consistent with aromatic esters or substituted phenols identified in the GC-MS analysis. These proton signals confirm the presence of long-chain fatty acid esters, a hydrophilic backbone, and minor aromatic constituents, reinforcing the glycolipid nature of the biosurfactant. The ^{13}C NMR spectrum of the biosurfactant provided further confirmation of its glycolipid nature through well-resolved chemical shifts corresponding to key structural motifs. A signal observed at δ 14.12 ppm was assigned to terminal methyl ($-\text{CH}_3$) carbons, while a prominent resonance at δ 29.70 ppm corresponded to methylene ($-\text{CH}_2-$) groups within long aliphatic fatty acid chains. The peak at δ 40.93 ppm indicated a methylene carbon adjacent to an electron-withdrawing group, likely an ester carbonyl. Signals in the region of δ 50.85 was attributed to oxygenated carbon atoms, such as those found in glycerol or sugar-based headgroups esterified with fatty acids. Multiple closely spaced peaks of approximately δ 76.70–77.34 ppm supported the presence of C–O and C–OH functionalities, typical of glycosidic or ester linkages. The overall pattern of chemical shifts is consistent with a structure composed of a hydrophilic glycerol or sugar moiety linked via ester bonds to hydrophobic fatty acid chains.

5 Discussion

In this study, the biosurfactant production by *L. helveticus* MTCC 5463 and *L. rhamnosus* MTCC 5462 was successfully screened by qualitative and quantitative methods (such as drop collapse, oil displacement, BATH assay, E24 test, and surface tension measurement). Qualitative screening assays at different incubation times were performed to determine optimum conditions for biosurfactant action. The highest biosurfactant activity was observed at 48 h of incubation, which corresponds to the late exponential or early stationary phase of *Lactobacillus* growth. Biosurfactant production is linked to secondary metabolism, potentially regulated by quorum sensing and triggered by nutrient depletion. Beyond 48 h, the decline in activity was observed, which may be due to biosurfactant degradation, metabolic by-product accumulation, or reduced viability of the culture. The positive results in all screening methods indicated the presence of surface-active compounds in both indigenous strains, consistent with reports by Madhu and Prapulla (2014) and Adnan et al. (2023), who also observed high biosurfactant activity in LAB strains, particularly *L. plantarum* and *L. acidophilus*. At 48 h of incubation, the surface tension of strain I and strain V was reduced from the initial 72 mN/m (distilled water baseline) to 38.73 (\pm 0.05) mN/m, indicating strong biosurfactant activity and 41.30 (\pm 0.04) mN/m, indicating moderate biosurfactant activity, respectively. This level of reduction is

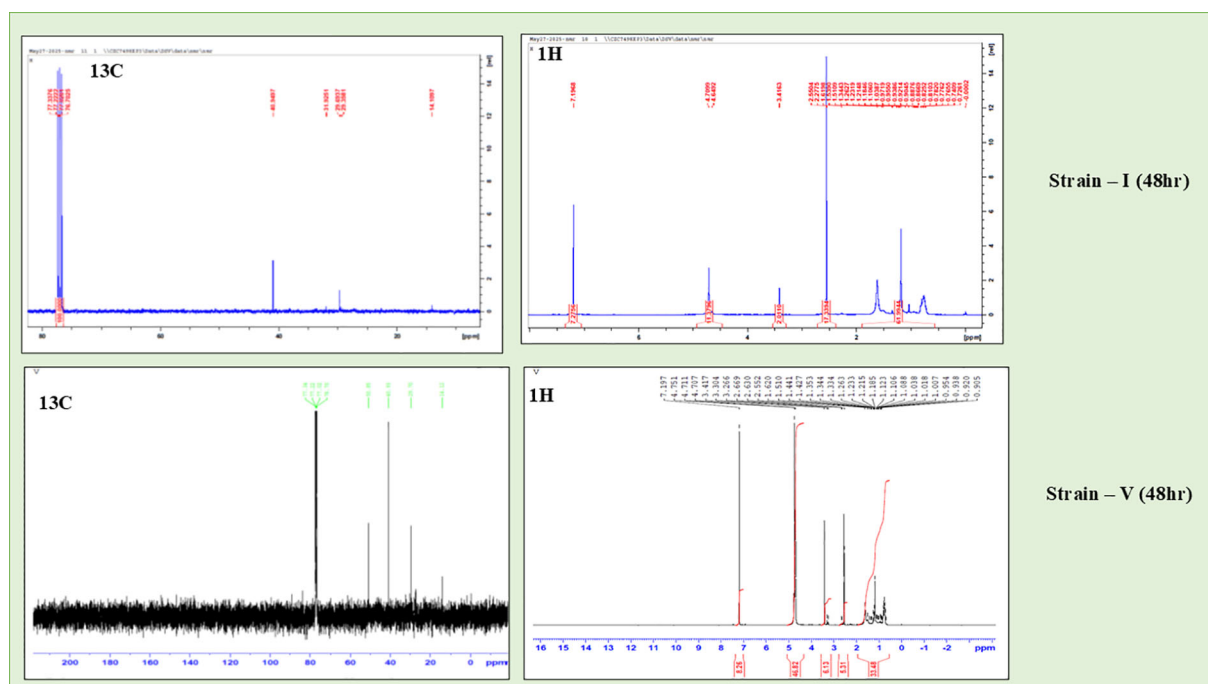


FIGURE 10
Analysis of ^1H and ^{13}C NMR spectrum for biosurfactants produced from *Lactobacillus helveticus* MTCC5463 and *Lactobacillus rhamnosus* MTCC5462.

characteristic of effective microbial biosurfactants, as values below 40 mN/m are generally considered strong surface-active agents. This reduction is comparable to values reported for potent biosurfactants produced by other *Lactobacillus* strains. The significant drop in surface tension confirms the presence of extracellular biosurfactant molecules and correlates with the highest emulsification index and drop-collapse activity observed at the same incubation time. *L. helveticus* MTCC 5463 and *L. rhamnosus* MTCC 5462 produced a surface-active agent that gave positive results when the TLC plate was stained with anisaldehyde reagent and iodine vapor, which confirms the presence of carbohydrates and lipids in the biosurfactant. It gave negative results when stained with ninhydrin, which shows the absence of an amino group. The biosurfactant produced by *L. helveticus* MTCC 5463 and *L. rhamnosus* MTCC 5462 was subjected to FTIR, GC-MS, and NMR analyses to elucidate its structural features and chemical nature. The FTIR spectrum of strain I revealed a broad absorption band at 3,275 cm^{-1} , characteristic of O–H stretching vibrations, suggesting that the presence of hydroxyl groups likely associated with sugar residues or glycerol moieties (Cameotra, 2002). Strong aliphatic C–H stretching bands at 2,955–2,850 cm^{-1} confirmed the presence of long-chain fatty acids. A sharp peak at 1,622 cm^{-1} indicated C=O stretching, suggestive of ester or amide linkages (Satpute et al., 2010). The region between 1,063 and 1,089 cm^{-1} exhibited O–C–O stretching typical of sugar derivatives, and absorptions from 964 to 752 cm^{-1} were associated with CH_2 wagging vibrations. These findings collectively support the presence of an amphiphilic molecule comprising both hydrophobic lipid chains and polar sugar or glycerol-based headgroups (Desai and Banat, 1997). GC-MS analysis of strain I corroborated the FTIR data by identifying several major fatty acid ester derivatives. The dominant compound, butanoic acid, 1,2,3-propanetriyl ester, 9-octadecenoic acid

(Z)-, 2-(9-octadecen-1-yloxy)ethyl ester, indicates the presence of unsaturated long-chain fatty acid esters. These compounds are typical of lipid-based biosurfactants and are responsible for the molecule's emulsifying and surface-active behavior (Joshi et al., 2008). ^1H NMR analysis further substantiated these findings, with a multiplet in the δ 0.90–1.50 ppm range attributed to terminal methyl and methylene protons in aliphatic chains. The ^{13}C NMR spectrum supported these assignments, with signals at δ 14.11 and δ 29.35–31.92 ppm corresponding to terminal methyl and internal methylene carbons. A resonance at δ 40.95 ppm suggested methylene carbons adjacent to electron-withdrawing groups, while strong signals at δ 76.70–77.34 ppm confirmed oxygenated carbons typical of sugar or glycerol-based polar headgroups (Sonoki et al., 2011) in strain I.

The spectral and chromatographic data consistently demonstrate that the biosurfactant is predominantly a glycolipid, likely composed of one or more long-chain fatty acids esterified to a glycerol or sugar-derived headgroup, with minor aromatic components. This structure imparts the molecule with amphiphilic properties, aligning with its observed surface activity and potential for application in biotechnological and industrial formulations (Rodrigues et al., 2006). The FTIR spectrum of strain V exhibited characteristic absorption bands indicative of a glycolipid structure. A broad peak at 3,327 cm^{-1} corresponded to O–H stretching vibrations, suggesting the presence of hydroxyl groups from sugar moieties or glycerol units. Strong bands in the 2,947–2,835 cm^{-1} range were attributed to C–H stretching of methylene and methyl groups in long aliphatic chains. Weak signals in the 560–550 cm^{-1} range were due to CH_2 out-of-plane bending, consistent with saturated hydrocarbon chains (Desai and Banat, 1997; Satpute et al., 2010). These features collectively support the presence of an amphiphilic molecule with both hydrophilic and hydrophobic

domains. GC-MS profiling of strain V confirmed the presence of structurally diverse lipid and aromatic compounds. A major compound identified at 29.841 min was a phenol and phosphite complex (3:1), comprising 16.97% of the total area, which may contribute antioxidant functionality or result from extraction processes. Other prominent constituents included 1,2-benzenedicarboxylic acid, diisodecyl ester (14.56% at 13.465 min), an aromatic ester potentially arising from environmental exposure or laboratory plastics, and 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol (11.65% at 32.826 min), a known antioxidant. Bioactive triterpenoids such as squalene (1.92%) and ursolic acid methyl ester (1.21% at 31.566 min) were also identified, and the extract revealed a dominant ester, butanoic acid, 1,2,3-propanetriyl ester (43.32% at 16.141 min), and 9-octadecenoic acid (Z)-, 2-(9-octadecen-1-yloxy)ethyl ester (21.00% at 26.232 min), indicating the presence of long-chain fatty acid esters that form the hydrophobic tail of glycolipid biosurfactants (Makkar and Cameotra, 2002). These esters enhance emulsification and surface activity, further confirming the amphiphilic nature of the compound. Similarly, the ^1H NMR spectrum showed multiplets in the δ 0.90–1.50 ppm region, assigned to terminal methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$) protons, indicative of long-chain fatty acids. The ^{13}C NMR spectrum supported these findings, showing a signal at δ 14.12 ppm for terminal methyl carbons and a broad resonance at δ 29.70 ppm for internal methylene groups of long aliphatic chains. A peak at δ 40.93 ppm indicated methylene carbons adjacent to ester carbonyls. A signal at δ 50.85 ppm and multiple peaks between δ 76.70 and 77.34 ppm confirmed the presence of oxygenated carbons (C–O and C–OH), typical of sugar or glycerol-based headgroups (Banat et al., 2010; Thavasi et al., 2013). The integration of FTIR, GC-MS, and NMR data establishes the biosurfactant as a glycolipid in both strains, consisting of long-chain fatty acid esters linked via glycosidic or ether bonds to polar headgroups such as glycerol or sugars. The presence of antioxidant phenols, triterpenoids, and aromatic esters adds to the functional complexity of the extract, potentially enhancing its stability and biological activity. This amphiphilic architecture supports its observed surfactant properties and suggests potential application in food, pharmaceuticals, cosmetics, and environmental bioremediation.

6 Conclusion

The current work aimed to isolate, purify, and characterize the biosurfactant of two indigenous *Lactobacillus* species, i.e., *L. helveticus* MTCC5463 and *L. rhamnosus* MTCC5462. The isolated biosurfactants from both species were characterized as glycolipids by using various physicochemical techniques such as TLC, FTIR, GC-MS, ^1H NMR, and ^{13}C NMR. The surface tension was found to be similar to previous reports of biosurfactant produced by other *Lactobacillus* species. This is the first comprehensive report on the structural characterization of indigenous *Lactobacillus* species-derived biosurfactants. The study opens a new avenue for the evaluation of these biosurfactants for futuristic pharmaceutical and other biomedical applications at the commercial level.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KP: Data curation, Formal Analysis, Investigation, Writing – original draft, Methodology, Project administration, Software. MK: Methodology, Visualization, Writing – review & editing. SM: Data curation, Formal Analysis, Supervision, Visualization, Writing – review & editing. BS: Data curation, Validation, Visualization, Writing – review & editing, Conceptualization, Formal Analysis, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This research was supported by the Council of Science and Technology, Uttar Pradesh, India (Grant number: CSTUP-BTBR/4/2024/D-2084).

Acknowledgments

The authors greatly acknowledge the support of Gautam Buddha University (Greater Noida), and G.B. Pant University, Pantnagar in writing this manuscript. The authors acknowledge the support of the DST-FIST grant and UP-CST grant for writing this manuscript. The authors greatly acknowledge the support of Anand Agricultural University, Gujarat, for providing the *Lactobacillus* strains for the research purpose.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Adnan, M., Siddiqui, A. J., Noumi, E., Ashraf, S. A., Awadelkareem, A. M., Hadi, S., et al. (2023). Biosurfactant derived from probiotic *Lactobacillus acidophilus* exhibits broad-spectrum antibiofilm activity and inhibits the quorum sensing-regulated virulence. *Biomol BioMed*. 23, 1051. doi: 10.17305/bb.2023.9324
- Ammar, A. B., Bouassida, M., Bouallegue, A., Fourati, N., Gerardi, G., Muñoz, P., et al. (2024). Isolation and characterization of two glycolipopeptid biosurfactants produced by a *Lactiplantibacillus plantarum* OL5 strain isolated from green olive curing water. *World J. Microbiol. Biotechnol.* 39, 308.
- Axelsson, L. (2004). "Lactic acid bacteria: Classification and physiology," in *Lactic Acid Bacteria: Microbiological and Functional Aspects*, 3rd ed. Eds. S. Salminen, A. von Wright and A. Ouwehand (Food Science and Technology-New York: Marcel Dekker).
- Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., et al. (2010). Microbial biosurfactants production, applications and future potential. *Appl. Microbiol. Biotechnol.* 87, 427–444. doi: 10.1007/s00253-010-2589-0
- Behzadnia, A., Moosavi-Nasab, M., Tiwari, B. K., and Setoodeh, P. (2020). *Lactobacillus plantarum*-derived biosurfactant: Ultrasound-induced production and characterization. *Ultrasonics Sonochemistry* 65, 105037. doi: 10.1016/j.ultrsonch.2020.105037
- Bodour, A. A., and Miller-Maier, R. M. (1998). Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J. Microbiological Methods* 32, 273–280. doi: 10.1016/S0167-7012(98)00031-1
- Cameotra, R. M. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. *Appl. Microbiol. Biotechnol.* 58, 428–434. doi: 10.1007/s00253-001-0924-1
- De Man, J. C., Rogosa, M., and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriology* 23, 130–135. doi: 10.1111/j.1365-2672.1960.tb00188.x
- Desai, J. D., and Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. Rev.* 61, 47–64. doi: 10.1128/mmb.61.1.47-64.1997
- Faccioli, Y. E., d., S., de Oliveira, K. W., Campos-Guerra, J. M., Converti, A., Soares da Silva, R., et al. (2024). Biosurfactants: chemical properties, ecofriendly environmental applications, and uses in the industrial energy sector. *Energies* 17, 5042. doi: 10.3390/en17205042
- Gudiña, E. J., Teixeira, J. A., and Rodrigues, L. R. (2011). Biosurfactant-producing lactobacilli: screening, production profiles, and effect of medium composition. *Appl. Environ. Soil Sci.* 2011, 1–9. doi: 10.1155/2011/201254
- Joshi, S., Bharucha, C., and Desai, A. J. (2008). Production of biosurfactant and antifungal compound by fermented food isolate *Bacillus subtilis* 20B. *Bioresource Technol.* 99, 4603–4608. doi: 10.1016/j.biortech.2007.07.030
- Koim-Puchowska, B., Kłosowski, G., Drózd-Afelt, J. M., Mikulski, D., and Zielinska, A. (2021). Influence of the medium composition and the culture conditions on surfactin biosynthesis by a native *Bacillus subtilis* natto BS19 strain. *Molecules* 26, 2985. doi: 10.3390/molecules26102985
- Li, H., Fang, C., Liu, X., Bao, K., Li, Y., and Bao, M. (2023). Quantitative analysis of biosurfactants in water samples by a modified oil spreading technique. *RSC Adv.* 13, 9933–9944. doi: 10.1039/D3RA00102D
- Madhu, A. N., and Prapulla, S. G. (2014). Evaluation and functional characterization of a biosurfactant produced by *Lactobacillus plantarum* CFR 2194. *Appl. Biochem. Biotechnol.* 172, 1777–1789. doi: 10.1007/s12010-013-0649-5
- Makkar, R. S., and Cameotra, S. S. (1999). Structural characterization of a biosurfactant produced by *Bacillus subtilis* at 45°C. *J. Surfact Detergents* 2, 367–372. doi: 10.1007/s11743-999-0091-6
- Makkar, R. S., and Cameotra, S. S. (2002). Effects of various nutritional supplements on biosurfactant production by a strain of *Bacillus subtilis* at 45°C. *J. Surfact Detergents* 5, 11–17. doi: 10.1007/s11743-002-0199-8
- Medhi, M. K., Ambust, S., Kumar, R., and Das, A. J. (2023). "Characterization and Purification of Biosurfactants," in *Advancements in Biosurfactants Research*. Eds. R. Aslam, M. Mobin, J. Aslam and S. Zehra (Springer International Publishing, Cham), 79–93. doi: 10.1007/978-3-031-21682-4_4
- Moraís, I. M. C., Cordeiro, A. L., Teixeira, G. S., Domingues, V. S., Nardi, R. M. D., Monteiro, A. S., et al. (2017). Biological and physicochemical properties of biosurfactants produced by *Lactobacillus jensenii* P6A and *Lactobacillus gasserii* P65. *Microb. Cell Fact* 16, 155. doi: 10.1186/s12934-017-0769-7
- Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y., and Imanaka, T. (2000). A study on the structure–function relationship of lipopeptide biosurfactants. *Biochim. Biophys. Acta (BBA) – Gen. Subj.* 1488, 211–218.
- Mouafo, H. T., Mbawala, A., and Ndjouenkeu, R. (2022). Biosurfactants from lactic acid bacteria: A critical review on production, extraction, structural characterization and food application. *Biotechnol. Rep.* 33, e00763. doi: 10.1016/j.fbio.2022.101598
- Mouafo, H. T., Mbawala, A., Somashekar, D., Tchougang, H. M., Harohally, N. V., and Ndjouenkeu, R. (2021). Biological properties and structural characterization of a novel rhamnolipid like-biosurfactants produced by *Lactobacillus casei* subsp. *casei* TM1B. *Biotech. App Biochem.* 68, 585–596. doi: 10.1002/bab.1966
- Nataraj, B. H., Ramesh, C., and Mallappa, R. H. (2021). Characterization of biosurfactants derived from probiotic lactic acid bacteria against methicillin-resistant and sensitive *Staphylococcus aureus* isolates. *LWT* 151, 112195. doi: 10.1016/j.lwt.2021.112195
- Okorie, I. K. M., and Ogunjobi, A. A. (2024). Characterisation of a unique manganese-containing biosurfactant produced from *Pseudomonas aeruginosa* strain S16, isolated from soil found in a mixed farm, located in Ibadan, Oyo State, Nigeria. *Preprint*. doi: 10.21203/rs.3.rs-4161420/v1
- Prajapati, J. B., Khedkar, C. D., Chitra, J., Suja, S., Mishra, V., Sreeja, V., et al. (2011). Whole-Genome Shotgun Sequencing of an Indian-Origin *Lactobacillus helveticus* Strain, MTCC 5463, with Probiotic Potential. *J. Bacteriol.* 4282–4283. doi: 10.1128/jb.05449-11
- Presti, I., D'Orazio, G., Labra, M., La Ferla, B., Mezzasalma, V., Bizzaro, G., et al. (2015). Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their *in vitro* effect. *Appl. Microbiol. Biotechnol.* 99, 5613–5626. doi: 10.1007/s00253-015-6482-8
- Rodrigues, L., Banat, I. M., Teixeira, J., and Oliveira, R. (2006). Biosurfactants: potential applications in medicine. *J. Antimicrobial Chemotherapy* 57, 609–618. doi: 10.1093/jac/dkl024
- Rogosa, M., Mitchell, J. A., and Wiseman, R. F. (1951). A selective medium for the isolation and enumeration of oral lactobacilli. *J. Dental Res.* 30, 682–689. doi: 10.1177/00220345510300051201
- Sakr, A. E., and Massoud, M. I. (2021). Impact of prebiotic potential of stevia sweeteners-sugar used as synbiotic preparation on antimicrobial, antibiofilm, and antioxidant activities. *LWT* 144, 111260. doi: 10.1016/j.lwt.2021.111260
- Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., and Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Crit. Rev. Biotechnol.* 30, 127–144. doi: 10.3109/07388550903427280
- Satpute, S. K., Kulkarni, G. R., Banpurkar, A. G., Banat, I. M., Mone, N. S., Patil, R. H., et al. (2016). Biosurfactant/s from Lactobacilli species: Properties, challenges and potential biomedical applications. *J. Basic Microbiol.* 56, 1140–1158. doi: 10.1002/jobm.201600143
- Shah, N., Nikam, R., Gaikwad, S., Sapre, V., and Kaur, J. (2016). Biosurfactant: types, detection methods, importance and applications. *Ind. Jour. Microb. Res.* 3, 5. doi: 10.5958/2394-5478.2016.00002.9
- Sharma, J., Kapley, A., Sundar, D., and Srivastava, P. (2022). Characterization of a potent biosurfactant produced from *Franconibacter* sp. IITDAS19 and its application in enhanced oil recovery. *Colloids Surfaces B: Biointerfaces* 214, 112453. doi: 10.1016/j.colsurfb.2022.112453
- Sharma, D., Saharan, B. S., Chauhan, N., Bansal, A., and Procha, S. (2014). Production and structural characterization of *Lactobacillus helveticus* derived biosurfactant. *Sci. World J.* 2014, 1–9. doi: 10.1155/2014/493548
- Sharma, R., Singh, J., and Verma, N. (2018). Optimization of rhamnolipid production from *Pseudomonas aeruginosa* PBS towards application for microbial enhanced oil recovery. *3 Biotech.* 8, 20. doi: 10.1007/s13205-017-1022-0
- Siddique, I. M. (2024). Exploring functional groups and molecular structures: A comprehensive analysis using FTIR spectroscopy. *Chem. Res. J.* 9, 70–76. doi: 10.5281/ZENODO.11281698
- Sim, L., Ward, O. P., and Li, Z.-Y. (1997). Production and characterisation of a biosurfactant isolated from *Pseudomonas aeruginosa* UW-1. *J. Ind. Microbiol. Biotechnol.* 19, 232–238. doi: 10.1038/sj.jim.2900450
- Singh, N., Hu, X.-H., Kumar, V., Solanki, M. K., Kaushik, A., Singh, V. K., et al. (2024). Microbially derived surfactants: an ecofriendly, innovative, and effective approach for managing environmental contaminants. *Front. Bioengineering Biotechnol.* 12. doi: 10.3389/fbioe.2024.1398210
- Singh, P., and Tiwary, B. N. (2016). Isolation and characterization of glycolipid biosurfactant produced by a *Pseudomonas otitidis* strain isolated from Chirimiri coal mines, India. *Bioresour. Bioprocess.* 3, 42. doi: 10.1186/s40643-016-0119-3

- Smyth, T. J. P., Perfumo, A., McClean, S., Marchant, R., and Banat, I. M. (2010). "Isolation and Analysis of Lipopeptides and High Molecular Weight Biosurfactants," in *Handbook of Hydrocarbon and Lipid Microbiology*. Ed. K. N. Timmis (Springer Berlin Heidelberg, Berlin, Heidelberg), 3687–3704. doi: 10.1007/978-3-540-77587-4_290
- Sonoki, T., Kajita, S., Uesugi, M., Katayama, Y., and Iimura, Y. (2011). Effective removal of bisphenol a from contaminated areas by recombinant plant producing lignin peroxidase. *JPEB* 105, 2. doi: 10.4172/2157-7463.1000105
- Tavares, J., Alves, L., Silva, T. P., and Paixão, S. M. (2021). Design and validation of an expeditious analytical method to quantify the emulsifying activity during biosurfactants/bioemulsifiers production. *Colloids Surfaces B: Biointerfaces* 208, 112111. doi: 10.1016/j.colsurfb.2021.112111
- Thavasi, R., Sharma, S., and Jayalakshmi, S. (2013). Evaluation of screening methods for the isolation of biosurfactant producing marine bacteria. *J. Pet Environ. Biotechnol.* 2, 1–7. doi: 10.4172/2157-7463.S1-001
- Tiwary, M., and Dubey, A. K. (2018). Characterization of biosurfactant produced by a novel strain of *pseudomonas aeruginosa*, isolate ADMT1. *J. Surfact Detergents* 21, 113–125. doi: 10.1002/jsde.12021
- Tuleva, B., Christova, N., Cohen, R., Stoev, G., and Antonova, D. (2002). Rhamnolipid biosurfactant production by *Pseudomonas fluorescens* strain 11 isolated from industrial wastewater. *World J. Microbiol. Biotechnol.* 18, 317–321.
- Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., and McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. *J. Microbiological Methods* 56, 339–347. doi: 10.1016/j.mimet.2003.11.001