ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 1, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ECO-FRIENDLY BIOSURFACTANT LIPOPEPTIDES AND THEIR CYTOTOXICITY EVALUATION FOR ENVIRONMENTAL AND PHARMACEUTICAL APPLICATIONS

RITU BALA[1], SOURBH SUREN GARG[2], ARUN KARNWAL[3], RATTAN DEEP SINGH[4]*

[1]Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India. [2]Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India. [3]Department of Microbiology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India. [4]Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India.

***Corresponding author: Rattan Deep Singh; Email: rattan.19383@lpu.co.in**

Received: 20 August 2025, Revised and Accepted: 25 November 2025

ABSTRACT

Objectives: This study compares biosurfactants produced by *Bacillus subtilis* microbial type culture collection 2423 (OBS), *Bacillus* spp. RZ2MS9 (O10), *Enterobacter* spp. TSSAS2-48 (O16), and standard surfactin based on their structural characteristic and cytotoxicity profile to evaluate their suitability for pharmaceuticals and environmental applications.

Methods: Structural components of the biosurfactants were analyzed using thin-layer chromatography (TLC) and Fourier-transform infrared spectroscopy (FTIR), enabling identification of functional groups such as amines, fatty acids, and ester linkages. Cytotoxicity was assessed using the MTT assay on L929 fibroblast cells to determine IC_{50} values for each biosurfactant and the surfactin standard.

Results: TLC and FTIR analyses revealed that the biosurfactants contained core structural units such as amines, fatty acids, and ester linkages, confirming their characteristic surface-active properties. The IC_{50} values obtained were 108.14±0.45 µg/mL (OBS), 160.70±0.21 µg/mL (O10), 214.61±0.54 µg/mL (OBS cream), 239.83±0.30 µg/mL (O10 cream), and 349.72±0.23 µg/mL (surfactin). Comparative analysis indicated that O10 demonstrated a more favorable cytotoxicity profile, suggesting higher biocompatibility.

Conclusion: *Bacillus* spp. RZ2MS9 (O10) demonstrates promising structural and biocompatibility attributes, supporting its potential for pharmaceutical and environmental applications. Further optimization and scale-up could enhance its commercial viability.

Keywords: Biosurfactant, Surfactin, Cytotoxicity, Antimicrobial activity, Pharmaceutical applications, Environmental sustainability.

© 2026 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i1.56586. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Biosurfactants are amphiphilic biological molecules containing both hydrophilic and hydrophobic components, which enables them to position themselves at oil-water, water-air, and solid-water interfaces. This structural arrangement allows them to lower interfacial tension and enhance the solubilization and dispersion of materials that are otherwise immiscible. The hydrophilic region typically contains charged groups, while the hydrophobic region is composed of fatty acids or hydrophobic peptides, together creating a balanced interaction with water [1]. Their amphipathic nature contributes to strong surface activity and a range of biological functions, making biosurfactants highly relevant in industrial biotechnology, environmental remediation, and drug delivery. Unlike many chemical surfactants that may cause skin irritation or environmental toxicity, biosurfactants are non-toxic and biodegradable.

Among biosurfactants, lipopeptides, especially surfactin, are widely studied due to their strong surface activity and multiple biological properties. Surfactin, primarily produced by *Bacillus* species, is a cyclic lipoheptapeptide (~1.36 kDa) consisting of a cyclic lactone ring linked to 3-hydroxy fatty acids. Variations in fatty acid chain length or amino acid composition led to structural diversity, which affects its physicochemical and biological properties. The presence of hydrophobic residues (2, 3, 4, 6, 7) and negatively charged residues (Glu^1 and Asp^5) supports its amphipathic character [2]. These structural features contribute to its strong surface activity, antimicrobial properties, and ability to interact with cell membranes, making surfactin a promising molecule for biomedical and cosmetic applications.

The skin, the largest organ of the human body, forms a primary protective barrier that maintains internal homeostasis. It prevents excessive water loss and shields against microbial and chemical insults. Interactions between epidermal cells and the skin microbiota support immune balance and host defense. However, long-term exposure to synthetic surfactants in personal-care products can cause irritation and disruption of the skin barrier. Because of this, biosurfactants are being explored as safer, skin-compatible alternatives and are now used across food, pharmaceutical, and environmental industries [3].

Despite their advantages, commercial use is still limited by high production costs. Research therefore focuses on developing cost-effective production methods using renewable or waste feedstocks such as canola oil, soybean oil, waste frying oil, wheat husk, rice husk, orange peel, and sugarcane bagasse. Using these substrates can make biosurfactant production both economical and eco-friendly.

Even with their promising properties, thorough cytotoxicity evaluation is essential, especially for biosurfactants intended for pharmaceutical or cosmetic use. Such assessments help determine their safety and cellular responses. Although early studies indicate that surfactin and related biosurfactants are biocompatible, further research is required to understand how different structures and concentrations influence biological effects [4]. This integrated approach highlights the potential

of lipopeptide biosurfactants as safe, effective, and environmentally sustainable agents for pharmaceutical and environmental applications.

METHODS

Biosurfactant extract production

Soil samples contaminated with hydrocarbons and heavy metals were used as potential sources for biosurfactant producing bacteria, since such environments often select strains with strong surface-active properties. The standard strain *Bacillus subtilis* microbial type culture collection (MTCC) 2423 was obtained from the MTCC (IMTECH MTCC) [5].

The strain was revived in nutrient broth containing beef extract 1 g, yeast extract 2 g, peptone 5 g, and sodium chloride 5 g, prepared as per the Hi Media protocol. The culture was incubated at 37°C in a rotary shaker at 190 rpm overnight. A fresh inoculum was then transferred into nutrient broth and later streaked onto nutrient agar slants, followed by incubation at 37°C for 24–48 h. The slants were subcultured every 2 weeks, and a single isolated colony was used to prepare fresh inoculum.

The bacterial culture was incubated at 30°C at 200 rpm overnight and then diluted to 10% volume. *B. subtilis* MTCC 2423 and the isolated strains were cultivated in minimal medium consisting of glucose 2.5 g, monosodium glutamate 1 g, yeast extract 0.3 g, magnesium sulfate heptahydrate 0.1 g, dipotassium phosphate and potassium chloride 0.05 g (Sigma Aldrich), adjusted to pH 7.0. The cultures were incubated at 30°C at 160 rpm for 48 h. After incubation, the broth was collected for biosurfactant extraction [6].

For purification, the culture supernatant was acidified to pH 2 with hydrochloric acid to allow biosurfactant precipitation. The precipitate was collected by centrifugation at 10,000 rpm for 20 min. A solvent mixture of chloroform: methanol (2:1, v/v) was used for extraction, and the organic layer was separated and evaporated using a rotary evaporator to obtain purified biosurfactant.

Surfactin (Sigma Aldrich) was used as the standard biosurfactant reference. Surfactin is a cyclic lipopeptide produced by *B. subtilis*, known for antibacterial, antiviral, and antitumor properties. It has the molecular formula $C_{53}H_{93}N_7O_{13}$, a molecular weight of 1036.34 g per mole, CAS number 24730-31-2 and PubChem Substance ID 24899562 (Fig. 1). The molecule contains a cyclic heptapeptide linked to a beta hydroxy fatty acid, forming a lactone ring which contributes to its amphiphilic nature and high surface activity. Surfactin is widely used in drug delivery, environmental applications and formulation of environmentally safe detergents [7]. As per Sigma Aldrich instructions, surfactin was stored at 2–8°C to maintain its structural stability and biological activity.

Characterization of biosurfactant with thin layer chromatography (TLC)

Crude biosurfactant extracts (OBS: *B. subtilis* MTCC 2423; O10: *Bacillus* spp. RZ2MS9; O16: *Enterobacter* spp. TSSAS2-48) and a standard surfactin sample were applied onto silica gel 60 F_{254} plates (Merck) (4 µL per spot; approximately 0.5–1.0 mg mL^{-1}). The plates were developed in a saturated chamber using chloroform: methanol:water (65:25:4, v/v/v) as the mobile phase (development distance 9–12 cm; chamber pre-saturation for 15 min).

After development, the plates were dried and visualized using iodine vapor and ninhydrin reagent (0.2% w/v in n-butanol: acetic acid: water, 12:3:1; heated at 110°C for 5 min). The retention factor (Rf) was calculated as distance travelled by the spot divided by the distance travelled by the solvent front and is reported to two decimal places. All samples were analyzed in triplicate, and values are presented as mean Rf ± standard deviation [8].

2.3 Chemical identification of biosurfactant with Fourier transform infrared spectroscopy (FTIR)

FTIR was employed to identify the chemical bonds and functional groups present in the biosurfactant samples. Spectra were recorded in the 400–4,000 cm^{-1} range using an FTIR-Nicolet iS50 instrument (Thermo Fisher Scientific) operating in attenuated total reflection mode. FTIR is widely recognized as a reliable and discriminative analytical technique capable of precisely detecting functional groups in complex natural biosurfactant mixtures [9]. The obtained infrared spectra enabled the identification of both hydrophilic moieties (amines, alcohols, sulfonic groups) and hydrophobic components (alkyl chains, unsaturated fatty acids). The presence of these structural units contributes to the surface-active behavior of the biosurfactants, which governs their emulsification, oil-solubilizing, and bioremediation properties. These physicochemical characteristics support the industrial applicability of biosurfactants in food processing, cosmetics, and environmental remediation [10].

Preparation of cream sample for *in vitro* cytotoxicity analysis

The optimized anti-aging cream formulation (2 g base) was prepared using excipients selected to ensure structural stability, emulsification efficiency, and skin compatibility. The composition is as follows: Stearic acid (17% w/w), which provides consistency and contributes to the cream's structural integrity [10]; cetearyl alcohol (10% w/w), incorporated to enhance texture and formulation stability; liquid paraffin (11% w/w), included to impart smoothness and form a protective layer on the skin; glycerin (8.5% w/w), serving as an effective humectant to prevent dryness; propylene glycol (41% w/w), functioning as a solvent, humectant, and co-emulsifier to support uniform dispersion of ingredients; methyl paraben (1.75% w/w), added as a preservative to inhibit microbial growth; and L-ascorbic

Fig. 1: Structure of surfactin from *Bacillus subtilis* ≥98.0% (high performance liquid chromatography), CAS no. 24730-31-2

acid (10% w/w), serving as the active anti-aging agent with established antioxidant and skin-brightening properties [11]. This combination ensures a stable and functional anti-aging formulation.

A pollution-free emulsification method was employed for cream preparation. The oil phase (stearic acid, liquid paraffin, propylene glycol) and the aqueous phase (glycerin and distilled water) were heated separately to 70°C. Once both phases reached the same temperature, the oil phase was slowly added to the aqueous phase under continuous stirring. The mixture was then homogenized to obtain a uniform emulsion. Upon cooling to 40°C, L-ascorbic acid was incorporated to preserve its stability and biological activity. The resulting formulation was smooth, homogeneous, and suitable for subsequent *in vitro* cytotoxicity evaluation.

Cytotoxicity assay

To evaluate the cell cytotoxicity, L929 cells (mouse fibroblast skin cell line) were seeded at 1×10^4 cells/well in 96-well plate and incubated at 37°C in 5.0% CO_2, for 24 h to allow attachment. The cream formulation was insoluble in dimethyl sulfoxide (DMSO) but dispersed well in water; therefore, an aqueous stock solution (10 mg/mL) was prepared by dispersing the cream in sterile distilled water, followed by vortexing and sonication for 10 min. The resulting solution was centrifuged, and the supernatants were collected and filtered through a 0.22 µm filter. The final aqueous samples were diluted in complete culture medium before treatment (no DMSO or organic solvent was present in the wells).

From this stock, final concentrations of 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL (cream equivalent per mL) were prepared and treated to each well and incubated [12]. After 24 h of treatment, 20 µL of MTT solution (5 mg/mL in PBS; final concentration 0.5 mg/mL per well) was added to each well and incubated for 4 h at 37°C. The media was carefully aspirated, and the resulting formazan crystals that are formed due to the metabolic reduction of MTT by active cells were dissolved in 100 µL of DMSO and they were incubated again at 37°C for 30 min to solubilize the formazan crystals [13]. The absorbance of each well was then determined by enzyme-linked immunosorbent assay plate reader at 540 nm. The IC_{50} values represent the concentration required to reduce cell viability by 50%.

Cell viability (%) = (Sample OD/control OD)×100.

Table 1: Retention factor (Rf) values of observed samples stained with ninhydrin and iodine

Sample	Rf (iodine)	Rf (ninhydrin)
OBS (*Bacillus subtilis* MTCC 2423)	0.70±0.01	0.63±0.01
O10 (*Bacillus* spp. RZ2MS9)	0.78±0.01	0.64±0.01
O16 (*Enterobacter* spp. TSSAS2-48)	0.56±0.02	0.52±0.01
Surfactin (standard)	0.60±0.01	0.60±0.01

MTCC: Microbial type culture collection

RESULTS AND DISCUSSION

TLC

All biosurfactant samples produced iodine-positive bands, indicating the presence of lipid-containing moieties, and ninhydrin-positive bands, confirming peptide or amine-containing components, consistent with the structural characteristics of lipopeptide biosurfactants. The Rf values obtained under the chloroform-methanol-water solvent system are presented in Table 1 and shown in Fig. 2a [14]. Under iodine visualization, OBS showed an Rf of 0.70±0.01, O10 showed 0.78±0.01, O16 showed 0.56±0.02, and the surfactin standard showed 0.60±0.01. Under ninhydrin staining, OBS showed 0.63±0.01, O10 showed 0.64±0.01, O16 showed 0.52±0.01, and the surfactin standard showed 0.60±0.01.

Iodine-based Rf values generally increase with increasing hydrophobicity, reflecting longer or more non-polar fatty acyl chains. O10 exhibited the highest iodine-Rf (0.78), indicating the greatest hydrophobic character among the tested biosurfactants. In contrast, O16 consistently showed the lowest Rf values (iodine: 0.56; ninhydrin: 0.52), suggesting a more polar structure, likely associated with either shorter fatty acid chains or a more hydrophilic head group (Fig. 2b).

Importantly, the comparable ninhydrin-Rf values of OBS (0.63) and O10 (0.64) indicate similar polarity within the peptide/amine regions, whereas the elevated iodine-Rf of O10 supports the presence of a more hydrophobic lipid component. Overall, the dual-staining TLC profiles confirm the presence of structurally distinct lipopeptide mixtures, with compositional variation attributable to differences in lipid chain length and hydrophilic group substitutions, consistent with earlier reports on biosurfactant heterogeneity [15].

FTIR analysis

B. subtilis MTCC 2423 (OBS)

The FTIR spectrum of the biosurfactant produced by *B. subtilis* MTCC 2423 (Fig. 3a) shows characteristic absorption bands typical of lipopeptide-type biosurfactants. The broad N–H stretching band at 3326.26 cm^{-1} corresponds to secondary amines, which are structural features of amino-lipids and lipopeptides. The C–H stretching band at 2980.82 cm^{-1} reflects the presence of alkyl chains that contribute to hydrophobic interactions and emulsification ability.

The band near 1643.71 cm^{-1}, attributed to C=C stretching, indicates unsaturated fatty acids, a common feature in many biosurfactant lipopeptides that enhances surface activity. The C–H bending at 1393.62 cm^{-1} further supports the presence of fatty acyl or alkyl groups associated with hydrophobicity [16]. The C–O stretching band at 1251.02 cm^{-1} corresponds to alkyl–aryl ether linkages, which are typically observed in glycolipids and lipopeptides and contribute to surface-tension reduction.

A C–N stretching band at 1012.10 cm^{-1} confirms amino-group-containing moieties that enhance hydrophilic interactions. The low-

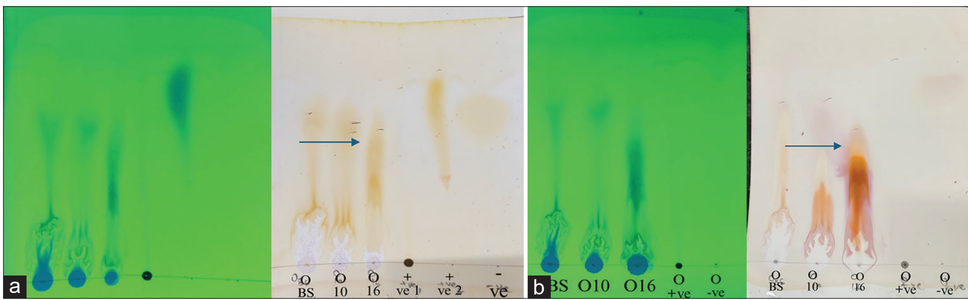

Fig. 2: (a) Thin layer chromatography (TLC) profile of biosurfactant extracts stained with iodine vapor. Lane 1=OBS (*Bacillus subtilis* microbial type culture collection [MTCC] 2423), Lane 2=O10 (*Bacillus* spp. RZ2MS9), Lane 3=O16 (*Enterobacter* spp. TSSAS2-48), Lane 4=positive, Lane 5=positive, Lane 6=negative. (b) Thin TLC of biosurfactant extract visualized with ninhydrin for peptide/amine detection. Lane 1=OBS (*Bacillus subtilis* MTCC 2423), Lane 2=O10 (*Bacillus* spp. RZ2MS9), Lane 3=O16 (*Enterobacter* spp. TSSAS2-48), Lane 4=control (positive), Lane 6=control (negative). Rf values (mean±standard deviation, n=3) are provided in Table 1

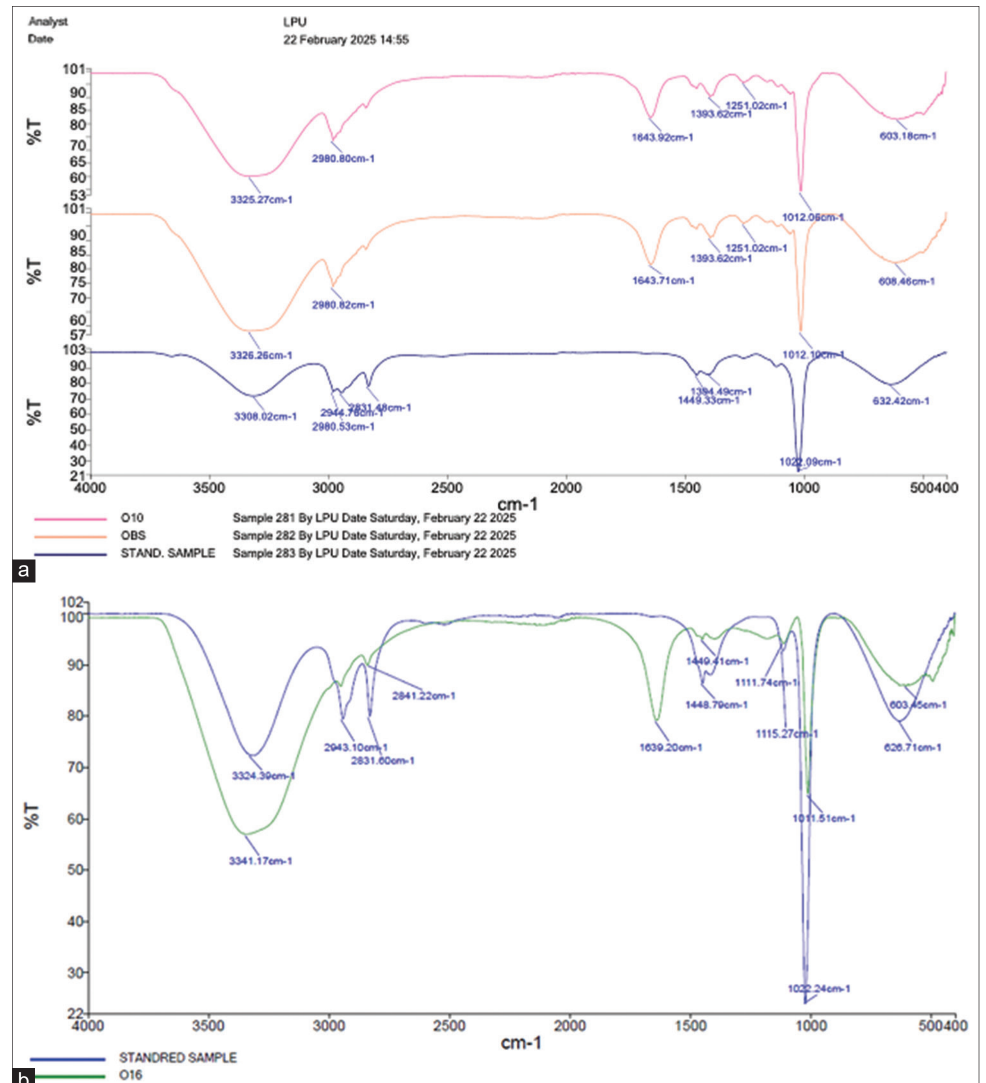

Fig. 3: (a) Fourier transform infrared spectroscopy (FTIR) spectra illustrating the functional group profiles of samples O10 (*Bacillus* spp. RZ2MS9) in pink, OBS (*Bacillus subtilis* microbial type culture collection 2423) in orange, and the standard surfactin sample in blue over the wavenumber range of 4,000–400 cm^{-1}. (b) FTIR spectra showing the functional group characteristics of O16 (*Enterobacter* spp. *TSSAS2-48*) in green and the standard surfactin sample in blue over the wavenumber range of 4,000–400 cm^{-1}

frequency peak at 608.46 cm^{-1} is commonly associated with aromatic ring deformations or other structural vibrational modes. Overall, the FTIR profile indicates that the biosurfactant produced by *B. subtilis* MTCC 2423 contains both hydrophilic and hydrophobic functional groups, supporting its application potential in emulsification, bioremediation, and other surface-active processes.

Frequency FTIR analysis for sample Bacillus spp. RZ2MS9 (O10)

The FTIR spectrum of the biosurfactant obtained from *Bacillus* spp. RZ2MS9 (O10) (Fig. 3a) shows characteristic absorption bands corresponding to functional groups commonly associated with lipopeptide and glycolipid biosurfactants [17]. The N–H stretching band at 3326.27 cm^{-1} indicates the presence of aliphatic amines, which are structural components of lipopeptides or amino-lipid molecules and contribute to hydrophilic interactions involved in emulsification. The C–H stretching band at 2980.80 cm^{-1} confirms the presence of long-chain alkyl groups that provide the hydrophobic domain essential for surface activity.

An absorption band at 1643.92 cm^{-1}, attributed to C=C stretching, suggests the presence of unsaturated fatty acids. The C–H bending at 1393.62 cm^{-1} further supports the existence of fatty-acyl chains characteristic of biosurfactant molecules. The band at 1251.02 cm^{-1} corresponds to ester functional groups, typically found in glycolipids and lipopeptides, where ester linkages contribute to reducing interfacial tension [18].

The C–O stretching band at 1012.06 cm^{-1} indicates phenolic or aromatic ether components, while the absorption near 608.18 cm^{-1} is consistent with aromatic hydrocarbon vibrations. These aromatic features suggest the presence of structurally stable biosurfactant constituents with enhanced emulsifying and solubilizing capabilities in complex environmental systems.

FTIR analysis for standard sample (surfactin)

The FTIR spectrum of the standard surfactin sample (Fig. 3b) displays characteristic absorption bands associated with lipopeptide biosurfactants [19]. The N–H stretching band at 3308.02 cm^{-1} indicates the presence of primary amines, which contribute to the hydrophilic domain of the molecule and play a central role in emulsification and surface interaction processes. The C–H stretching absorptions at 2980.53, 2944.76, and 2831.48 cm^{-1} reflect long-chain alkyl groups that represent the hydrophobic region responsible for interaction with nonpolar substrates and enhancement of emulsifying ability [20].

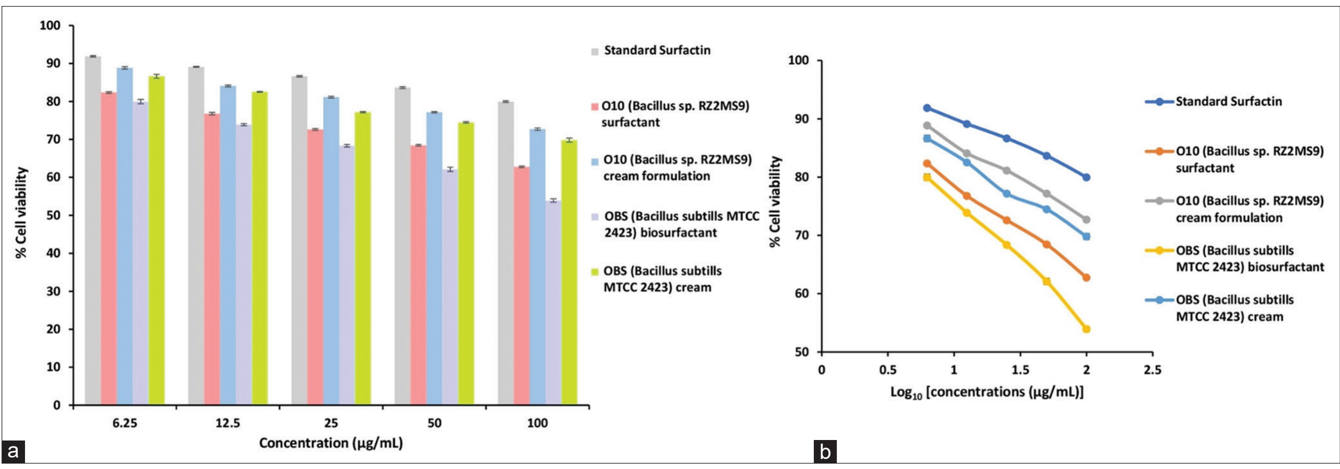

Fig. 4: Cytotoxicity assessment of biosurfactants and their cream formulations. (a) Percentage cell viability of L929 fibroblasts after 24-h exposure to standard surfactin, O10 biosurfactant, O10 cream, OBS biosurfactant, and OBS cream across concentrations of 6.25–100 μg/mL. (b) Log_{10} concentration–response curves derived from the MTT data, illustrating the cytotoxicity profile and concentration-dependent decline in cell viability for each tested sample

A distinct absorption band at 1022.09 cm^{-1} corresponds to S=O stretching, suggesting the presence of sulfoxide groups that are known to influence surface activity by lowering interfacial tension [18]. These functional groups collectively account for the strong emulsifying, bioremediation, and antimicrobial properties of surfactin, supporting its classification as an effective biosurfactant [21].

FTIR analysis for sample Enterobacter spp. TSSAS2-48 (O16)

As mentioned in Fig. 3b, the FTIR spectrum of *Enterobacter* spp. TSSAS2-48 (O16) reveals several functional domains associated with biosurfactant production [22]. The N–H stretching band at 3341.17 cm^{-1} confirms the presence of amine groups, whose hydrophilic nature contributes to emulsifying properties. The C–H stretching vibration at 2841.22 cm^{-1}, characteristic of alkyl chains, imparts hydrophobicity and enhances oil compatibility by reducing interfacial tension. The C=C stretching signal at 1639.20 cm^{-1} indicates unsaturated fatty acids, while the C–H bending signal at 1449.41 cm^{-1} further supports the presence of alkyl fragments, highlighting the structure–activity dualism underlying surfactant behavior [23].

Hydrophilic components are supported by the C–O stretching resonance at 1111.74 cm^{-1} and the C–N stretching band at 1011.51 cm^{-1}, which strongly suggest glycolipid or lipopeptide characteristics. Collectively, these findings support the potential of *Enterobacter* spp. TSSAS2-48 (O16) as a biosurfactant exhibiting essential emulsifying and surface-active properties for applications such as bioremediation, oil spill mitigation, and antimicrobial enhancement.

The comparative analysis of the key functional groups found in the biosurfactants (OBS, O10, O16) relative to standard surfactin further highlights the contributions of amines (N–H), alkyl chains (C–H), unsaturated fatty acids (C=C), ester linkages (C–O), and glycolipid-like structures (C–N). These insights provide a clearer understanding of the molecular features that govern the surface-active behavior of the biosurfactants and their potential applications in bioremediation and pharmaceuticals.

Cytotoxicity assessment of anti-aging cream formulations and derived biosurfactants

The cytotoxicity of standard surfactin, biosurfactants from *Bacillus* spp. RZ2MS9 (O10) and *B. subtilis* MTCC 2423 (OBS), and their corresponding cream formulations was evaluated in L929 fibroblast cells using the MTT assay [25]. All samples exhibited a concentration-dependent reduction in cell viability across the tested range compared to standard surfactin; however, they maintained more than 50% viability at all tested concentrations, as mentioned in Fig. 4a and b.

At the lowest concentration (6.25 μg/mL), cell viability ranged from 79.97±0.57% (OBS biosurfactant) to 91.87±0.17% (standard surfactin). With increasing concentrations, viability decreased progressively, reaching 53.91±0.45% (OBS biosurfactant), 62.75±0.21% (O10 biosurfactant), 69.81±0.54% (OBS cream), 72.70±0.30% (O10 cream), and 79.96±0.23% (standard surfactin) at 100 μg/mL. At all tested concentrations, the viability of cells treated with O10, OBS, and their cream formulations was significantly lower compared to the standard surfactin group [25].

A clear difference in IC_{50} values was observed among the samples. OBS biosurfactant showed the strongest cytotoxic effect with an IC_{50} of 108.14±0.45 μg/mL, followed by O10 biosurfactant (160.7±0.21 μg/mL) [26]. In contrast, cream formulations demonstrated significantly reduced cytotoxicity, with IC_{50} values of 214.61±0.54 μg/mL (OBS cream) and 239.83±0.3 μg/mL (O10 cream). Notably, standard surfactin exhibited the highest IC_{50} (349.72±0.23 μg/mL), indicating minimal cytotoxicity toward normal fibroblast cells.

The reduced cytotoxicity of the O10 cream formulation may be attributed to the partial encapsulation of biosurfactants within the cream matrix, which limits direct interaction with cell membranes. In contrast, crude biosurfactants, particularly OBS, may contain impurities or structurally diverse metabolites that increase cytotoxicity, whereas the purified nature of standard surfactin contributes to its superior safety. Previous studies have shown that some microbial biosurfactants, such as rhamnolipids and sophorolipids, maintain high cell viability (>90%), while synthetic surfactants like SDS exhibit significantly higher cytotoxicity [26]. These observations support the relative biocompatibility of the O10 cream formulation. Among the tested samples, OBS biosurfactant was the most cytotoxic, and standard surfactin was the least cytotoxic, followed by the O10 cream formulation.

CONCLUSION

This study investigated the biosurfactant producing ability of *B. subtilis* MTCC 2423 (OBS), *Bacillus* spp. RZ2MS9 (O10), *Enterobacter* spp. TSSAS2-48 (O16), and standard surfactin. Structural and functional characterization through TLC and FTIR confirmed the presence of amphiphilic molecules with essential functional groups such as amines, fatty acids, and ester linkages. All biosurfactants showed emulsification potential, reduction in surface tension, and antimicrobial activity. Purified samples retained their functional properties, indicating good stability and quality.

Cytotoxicity evaluation revealed that O10 from *Bacillus* spp. RZ2MS9 demonstrated the highest biocompatibility, making it suitable

for topical and pharmaceutical applications. Standard surfactin also showed excellent safety and serves as a strong reference for biosurfactant performance. The OBS biosurfactant exhibited moderate cytotoxicity, suggesting possible use in selected industrial applications. The O16 biosurfactant showed balanced activity and biocompatibility, indicating potential for environmental applications such as bioremediation.

Metabolite analysis suggested that O10 contained more aromatic phenolic compounds which may enhance stability and emulsifying ability, while O16 showed a higher proportion of fatty acids and hydrocarbons supporting its relevance in oil degradation and related environmental uses. In summary, the three tested strains produced biosurfactants with distinct structural features and functional capacities. O10 emerged as the most promising candidate due to its strong biocompatibility and functional profile, while standard surfactin confirmed its value as a safe and effective biosurfactant. OBS and O16 remain useful options depending on specific industrial or environmental requirements. Overall, the findings highlight the potential of these biosurfactants as safe, ecofriendly and effective alternatives to chemical surfactants with scope for further development and commercial application.

AUTHOR CONTRIBUTIONS

Ritu Bala contributed to the development of the methodology, experimental design, execution of laboratory experiments, data acquisition, data analysis, and preparation of the original manuscript draft. Rattan Deep Singh conceptualized the study, supervised all research activities, and provided critical review and editing of the manuscript. Sourbh Suren Garg contributed to the cytotoxicity evaluation, including performing the experimental procedures and assisting in the interpretation of the cytotoxicity data.

ACKNOWLEDGMENT

I am obliged to Lovely Professional University and CIF of LPU, Phagwara, to help me conduct my research.

STATEMENT OF COMPETING INTEREST

There is no conflict of interest between the authors regarding this paper.

FUNDING

The authors did not receive any specific grant or support from funding agencies. The authors are thankful to the Lovely Professional University for providing the required research facilities to carry out the research work.

REFERENCES

1. Phulpoto IA, Yu Z, Hu B, Wang Y, Ndayisenga F, Li J, *et al*. Production and characterization of surfactin-like biosurfactant produced by novel strain *Bacillus nealsonii* S2MT and it's potential for oil contaminated soil remediation. Microb Cell Fact. 2020 Jul 20;19(1):145. doi: 10.1186/s12934-020-01402-4, PMID 32690027
2. Haidar CN, Taddia A, Sortino M, López D, Malpiedi LP. Potential applications of biosurfactants produced by different *Pseudomonas syringae*: A comparative study on interfacial activity, antimicrobial efficacy, and cytotoxicity. Biocatal Agric Biotechnol. 2024 Jun 1;58:103166. doi: 10.1016/j.bcab.2024.103166
3. Abruzzo A, Parolin C, Corazza E, Giordani B, Di Cagno MP, Cerchiara T, *et al*. Influence of *Lactobacillus* biosurfactants on skin permeation of hydrocortisone. Pharmaceutics. 2021 May 31;13(6):820. doi: 10.3390/pharmaceutics13060820, PMID 34073138
4. Hoffmann M, Mück D, Grossmann L, Greiner L, Klausmann P, Henkel M, *et al*. Surfactin from *Bacillus subtilis* displays promising characteristics as O/W-emulsifier for food formulations. Colloids Surf B Biointerfaces. 2021 Jul 1;203:111749. doi: 10.1016/j.colsurfb.2021.111749, PMID 33862574
5. Çelik PA, Manga EB, Çabuk A, Banat IM. Biosurfactants' potential role in combating COVID-19 and similar future microbial threats. Appl Sci. 2020 Dec 31;11(1):334. doi: 10.3390/app11010334
6. Geissler M, Heravi KM, Henkel M, Hausmann R. Lipopeptide biosurfactants from *Bacillus* species. In: Biobased Surfactants. United States: AOCS Press; 2019 Jan 1. p. 205-40. doi: 10.1016/b978-0-12-812705-6.00006-x
7. Kwaśniewska D. Biosurfactants as determinants of the quality anti-aging products. Tenside Surfactants Deterg. 2024 May 27;61(3):259-64. doi: 10.1515/tsd-2023-2576
8. Ceresa C, Fracchia L, Williams M, Banat IM, Díaz De Rienzo MA. The effect of sophorolipids against microbial biofilms on medical-grade silicone. J Biotechnol. 2020 Feb 10;309:34-43. doi: 10.1016/j.jbiotec.2019.12.019, PMID 31887325
9. Rincón-Fontán M, Rodríguez-López L, Vecino X, Cruz JM, Moldes AB. Study of the synergic effect between mica and biosurfactant to stabilize Pickering emulsions containing vitamin E using a triangular design. J Colloid Interface Sci. 2019 Mar 1;537:34-42. doi: 10.1016/j.jcis.2018.10.106, PMID 30419372
10. Adu SA, Naughton PJ, Marchant R, Banat IM. Microbial biosurfactants in cosmetic and personal skincare pharmaceutical formulations. Pharmaceutics. 2020 Nov;12(11):1099. doi: 10.3390/pharmaceutics12111099, PMID 33207832
11. Pogorzelski S, Watrobska-Swietlikowska D, Sznitowska M. Surface tensometry studies on formulations of surfactants with preservatives as a tool for antimicrobial drug protection characterization. J Bio Phys Chem. 2012;3(4):324-33. doi: 10.4236/jbpc.2012.34040
12. Nasser M, Sharma M, Kaur G. Advances in the production of biosurfactants as green ingredients in home and personal care products. Front Chem. 2024 Mar 26;12:1382547. doi: 10.3389/fchem.2024.1382547, PMID 38595700
13. Lilge L, Ersig N, Hubel P, Aschern M, Pillai E, Klausmann P, *et al*. Surfactin shows relatively low antimicrobial activity against *Bacillus subtilis* and other bacterial model organisms in the absence of synergistic metabolites. Microorganisms. 2022 Apr 5;10(4):779. doi: 10.3390/microorganisms10040779, PMID 35456828
14. Brinda CM, Ragunathan RR, Johney J. Diversity and distribution of potential biosurfactant producing *Bacillus* sp MN 243657, GC-MS analysis and its antimicrobial study. Biosci Biotechnol Res Asia. 2023 Mar 1;20(1):271-91. doi: 10.13005/bbra/3088
15. Zargar AN, Mishra S, Kumar M, Srivastava P. Isolation and chemical characterization of the biosurfactant produced by *Gordonia* sp. IITR100. PLOS One. 2022 Apr 14;17(4):e0264202. doi: 10.1371/journal.pone.0264202, PMID 35421133
16. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev. 1997 Mar;61(1):47-64. doi: 10.1128/mmbr.61.1.47-64.1997, PMID 9106364
17. Sarethy IP, Bhatia N, Maheshwari N. Antibacterial activity of plant biosurfactant extract from *Sapindus mukorossi* and *in silico* evaluation of its bioactivity. Int J Pharm Pharm Sci. 2015;7(10):419-21.
18. Rodríguez-López L, López-Prieto A, Lopez-Álvarez M, Pérez-Davila S, Serra J, González P, *et al*. Characterization and cytotoxic effect of biosurfactants obtained from different sources. ACS Omega. 2020 Nov 24;5(48):31381-90. doi: 10.1021/acsomega.0c04933, PMID 33324849
19. Chang PH, Li SY, Juang TY, Liu YC. Mg-Fe layered double hydroxides enhance surfactin production in bacterial cells. Crystals. 2019 Jul 12;9(7):355. doi: 10.3390/cryst9070355
20. Dasankoppa FS, Sagare RD, Sureban MN, Sholapur HN, Ramesh AG, Murugesan V. Screening, design and characterization of algae-based topical dosage form as a remedy for digital ageing. Int J Appl Pharm. 2025;17(4):166-77. doi: 10.22159/ijap.2025v17i4.51784
21. Chitra B, Vijayakumar AB. Assessment on antimicrobial properties of surfactin from *Bacillus subtilis* on protoplasts and spheroplasts of pathogenic bacteria. Int J Curr Pharm Res. 2016;9(1):67-9. doi: 10.22159/ijcpr.2017v9i1.16612
22. Fa I, Mir JM. Surfactant material properties and coronavirus surface: A surface interaction for Covid-19 treatment and vaccination. Int J Chem Res. 2021 Oct 1;5:1-6.
23. Poomalai P, Krishnan J, Ravichandran A, Sureshkumar R. Biosurfactants: Sustainable alternative to synthetic surfactants and their applications. Int J Appl Pharm. 2024;16:34-43. doi: 10.22159/ijap.2024v16i2.50061
24. Zhu P, Zhang S, Kumar R, Zhang Z, Zhang Z, Wang Y, *et al*. Rhamnolipids from non-pathogenic *Acinetobacter calcoaceticus*: Bioreactor-scale production, characterization and wound healing

potency. N Biotechnol. 2022 Mar 25;67:23-31. doi: 10.1016/j.nbt.2021.12.001, PMID 34890838
25. Xu R, Ma L, Chen T, Wang J. Sophorolipid suppresses LPS-induced inflammation in RAW264.7 cells through the NF-κB signaling pathway. Molecules. 2022 Aug 8;27(15):5037. doi: 10.3390/molecules27155037, PMID 35956987
26. Rusanov AL, Luzgina NG, Lisitsa AV. Sodium dodecyl sulfate cytotoxicity towards HaCaT keratinocytes: Comparative analysis of methods for evaluation of cell viability. Bull Exp Biol Med. 2017 Jun;163(2):284-8. doi: 10.1007/s10517-017-3785-z, PMID 28726210