International Journal of Agricultural Technology 2025 Vol. 21(4):1313-1328
DOI: https://doi.org/10.63369/ijat.2025.21.4.1313-1328
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

# Biosurfactant from *Streptomyces zaomyceticus* HUS20 isolated from the Indian Himalayan Region (IHR) and its antibacterial activity

**Kishorekumar, A.[1], Radhakrishnan, M.[1*], Manigundan, K.[2], Ranjani, S.[1], Thenmozhli, G. S.[1], Gopikrishnan, V.[1], Anita, P.[3] and Soytong, K[4].**

[1]Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai – 600 119. Tamil Nadu, India; [2]Dong-A University, Pusan, South Korea; [3]Department of Biotechnology, Graphic Era Deemed to be University, Dehradun, India; [4]Research Institute of Modern Organic Agriculture, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Kishorekumar, A., Radhakrishnan, M., Manigundan, K., Ranjani, S., Thenmozhli, G. S., Gopikrishnan, V., Anita, P. and Soytong, K. (2025). Biosurfactant from *Streptomyces zaomyceticus* HUS20 isolated from the Indian Himalayan Region (IHR) and its antibacterial activity. International Journal of Agricultural Technology 21(4):1313-1328.

**Abstract** Actinobacteria, which are known to synthesize bioactive molecules with significant industrial and medicinal applications. In order to assess the possibility for producing biosurfactant (BS), this study identified culturable actinobacteria from the high-altitude soils of Drama Valley, Dugtu Village in the Indian Himalayan Region (IHR). Actinobacteria were isolated from pre-treated soil samples using nutrient agar, starch casein nitrate agar, and actinomycetes isolation agar, which were enhanced with 1% crude oil. Using the haemolytic assay, oil displacement test, emulsification activity, drop collapse assay, and penetration assay, morphologically different colonies were proved for the production of BS. In this study, a promising biosurfactant-producing strain, strain HUS20 showed considerable emulsification index (65%) and a 30 mm zone in the oil displacement test. Using 16S rRNA sequencing, the strain HUS20 was shown to be 100% identical to *Streptomyces zaomyceticus* (GenBank accession: OQ996835.1). in HR LC MS analysis, the crude biosurfactant extract showed the presence of more than 164 metabolites. Additionally, the crude biosurfactant demonstrated strong antibacterial action against foodborne pathogens, with *Bacillus cereus* showing the greatest suppression (24 mm zone at 100 mg/ml). The potential of actinobacteria from harsh Himalayan habitats as a useful resource for the synthesis of biosurfactants is highlighted by this work. *Streptomyces zaomyceticus* HUS20 is found to be promising strain for isolation of potential biosurfactant molecule.

**Keywords:** Actinobacteria, Biosurfactant (BS), HR-LC-MS, Indian Himalayan Region (IHR), *Streptomyces zaomyceticus*

[*]**Corresponding Author**: Radhakrishnan, M.; **Email**: mrkactinos@gmail.com

## Introduction

Actinobacteria, a group of Gram-positive bacteria, is known for their diverse array of metabolic products, including antibiotics, enzymes, pigments and biosurfactants, etc. The advancement of biotechnology is significantly dependent on these microorganisms, particularly in the drugs and other high value products like biosurfactants, pigments, enzymes, etc., (Kumari and Menghani, 2021). Biosurfactants have distinguished themselves due to their biodegradability, environmental friendliness, and potential to serve as alternatives to synthetic surfactants across various sectors. The antimicrobial action, oil recovery, and bioremediation represent only a fraction of the diverse applications for these amphiphilic compounds (Sharma *et al*., 2022). Identifying potential actinobacteria capable of producing biosurfactant presents an ongoing challenge, particularly in extreme and unexplored environments.

The Indian Himalayan Region (IHR) represents a significant biodiversity hotspot, distinguished by its extensive altitudinal variation, climatic extremes, and a wide array of ecosystems. The IHR extends over 2,500 kilometers and encompasses ten states of India, showcasing a distinctive collection of flora and fauna. The extreme environmental conditions of the region—characterized by low temperatures, elevated UV radiation, and fluctuating nutrient availability—present challenges that have influenced the evolution of highly specialized and diverse microbial communities (Sharma *et al*., 2021). These microorganisms are essential for ecosystem functioning and present exciting possibilities for biotechnological applications. The microbial diversity in the IHR includes bacteria including actinobacteria, fungi and archaea, all of which play a crucial role in nutrient cycling, soil fertility, and interactions between plants and microbes. Among these, psychrophilic and psychrotolerant bacteria have been thoroughly examined for their paucity to endure and flourish in low-temperature environments (Kumari and Menghani, 2021). Actinobacteria represent an essential group that makes up a substantial portion of the microbial population within the IHR. Actinobacteria from the IHR are recognized for their capacity to generate a diverse range of bioactive secondary metabolites, such as antibiotics and biosurfactants. Members of this phylum have been pinpointed as a valuable source of numerous high value product (Mishra *et al*., 2022). Investigations have documented the identification of various novel actinobacterial taxa from this area, highlighting its significance as an unexplored source of biotechnologically important microorganisms.

Biosurfactants produced by actinobacteria have become essential biomolecules because of their functions in lowering surface and interfacial tension, facilitating emulsification, and exhibiting antimicrobial properties. The

significance of these properties is especially pronounced when tackling environmental issues like oil spills and contamination (Kaur and Vyas, 2023). Moreover, biosurfactants have shown promise in pharmaceutical applications, such as inhibiting biofilm formation and serving as drug delivery agents. Discovering actinobacteria that produce biosurfactants in distinctive ecosystems like the IHR may offer promising opportunities for both industrial and environmental uses.

The objective of this investigation was to isolate culturable actinobacteria from the Indian Himalayan Region (IHR) and assess their potential for biosurfactant production using a range of qualitative and quantitative assays. A methodical approach was utilized, starting with the gathering of soil samples from a secluded Himalayan site, and subsequently leading to the isolation and characterization of actinobacterial strains. Various assays were employed to screen for biosurfactant activity, such as hemolytic activity, oil displacement, emulsification, drop collapse, and penetration assays. The investigation further included the molecular characterization of the most promising strain to determine its phylogenetic identity and to explore its biosynthetic potential via metabolite profiling.

## Materials and methods

### *Description of the strain*

*Streptomyces zaomyceticus* HUS20 was isolated from the Drama Valley, Dugtu Village in the Indian Himalayan Region using Starch Casein Nitrate (SCN) agar. Then preserved in 20% glycerol stock and ISP2 agar slants at 4°C for future use.

### *Screening of strain HUS20 biosurfactant (BS) activity*

The *Streptomyces zaomyceticus* HUS20 were evaluated for biosurfactant production using a series of standard assays, as detailed below:

#### Hemolytic assay

A preliminary screening method utilizing the hemolytic assay was employed to identify biosurfactant production. Actinobacterial spores from HUS20 was inoculated to freshly prepared blood agar plates (composition per 100 ml: malt extract, 1.0 g; yeast extract, 0.4 g; NaCl, 1.0 g; and 5 ml sheep blood) and incubated at 28°C for a duration of 72–96 hours. Subsequently, the plates were examined for clear zones surrounding the colonies, which would

suggest hemolysis and the potential production of biosurfactants (Ravindran *et al*., 2020).

**Oil displacement test**

The oil displacement method assessed the ability of biosurfactants to reduce surface tension. Strain HUS20 were grown in 25 ml ISP2 broth for 48–76 hours at 150 rpm. A petri dish (15 cm in diameter) was filled with 20 ml of distilled water, and 2 ml of crude engine oil was spread evenly on the water's surface. Subsequently, 10 µl of cell-free supernatant was added to the center of the oil layer. The formation of a transparent halo or oil displacement zone indicated biosurfactant activity. The size of the halo was measured as an indirect estimate of the activity (Rodrigues *et al*., 2021).

**Emulsification activity**

The emulsification activity was evaluated by combining 2 ml of cell-free supernatant of HUS20 with an equal volume of kerosene in a 1:1 ratio. The solution underwent vigorous vortexing for a duration of 2 minutes and was subsequently permitted to settle for a period of 24 hours. The height of the emulsified layer was recorded, and the emulsification index (E24) was determined using the formula: Emulsification activity (%) = [height of the emulsified layer / total height of the liquid] X100 Distilled water served as a negative control, and all experiments were performed in triplicates to guarantee precision (Sharma *et al*., 2022).

**Drop collapse assay**

The drop collapse assay was conducted to evaluate the production of biosurfactants by HUS20. A 96-well microtiter plate was utilized for the assay, with 2 µl of crude oil added to each well to establish a thin oil layer. The plate was equilibrated for one hour at room temperature. Subsequently, 5 µl of cell-free supernatant HUS20 was meticulously pipetted onto the oil surface. After a duration of 1 minute, the configuration of the drop was meticulously examined. A flat or collapsed drop signified the presence of biosurfactant, whereas a stable, rounded drop indicated its absence. Distilled water served as the negative control, for biosurfactant activity (Jadhav and Baghela, 2021).

**Penetration assay (PA)**

The penetration assay was performed to evaluate the biosurfactant production by strain HUS20. This method was followed by a slight modification (Kumar *et al.,* 2021) thin oil layer was prepared by mixing oil (e.g., vegetable or crude oil) with silica gel to create a hydrophobic paste. This mixture was added

to the wells of a 96-well microtiter plate, forming a stable oil layer on the surface. Cell-free supernatants of the strain HUS20 were obtained by growing the isolates in ISP2 broth until the late log phase, followed by centrifugation at 10,000 rpm for 10 minutes. Ten microliters of the cell-free supernatant were then carefully added to the center of each well containing the oil layer. The plate was observed for any changes in the oil layer. The disruption or spreading of the aqueous supernatant into the oil phase was considered a positive result, indicating the presence of biosurfactant activity. Wells with sterile distilled water instead of supernatant were used as negative controls. This method provided a qualitative assessment of biosurfactant production and was effectively utilized as a rapid screening technique (Kumar *et al*., 2021). Figure 1 represents different BS screening.

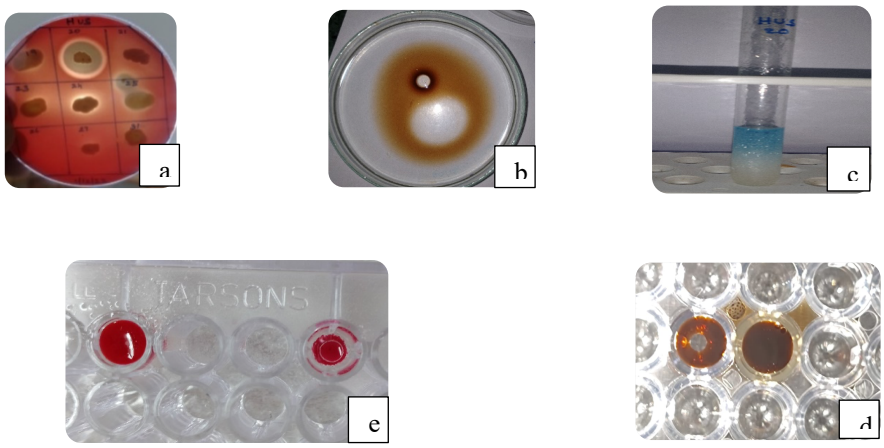


**Figure 1.** Hemolysis in blood agar (a), oil displacement (b), emulsification of the hydrocarbon(c), drop collapse (d) and penetration assay (e)

### *Characterization and identification of actinobacterial strain HUS 20*

The actinobacterial strain HUS20, recognized for its biosurfactant activity through various assays, was subjected to comprehensive taxonomic analysis and investigations into biosurfactant production. The microscopic, cultural, and physiological characteristics of the strain were examined in accordance with the methodologies established by Kumar *et al*. (2013) and Shirling and Gottlieb (1966). The process of molecular characterization included sequencing of the 16S rRNA gene. Genomic DNA extraction was carried out utilizing the GenElute™ Genomic DNA Kit, followed by PCR amplification conducted with an Eppendorf Master Cycler Gradient. The primers employed were 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG

ACT T-3′), in accordance with the methodology outlined by Lane (1991). The 50 µl reaction mixture included 2 µl genomic DNA (100 ng), 5 µl 10× buffer, 4 µl BSA (1 mg/ml), 1 µl dNTP mixture (2.5 mM), 1 µl of each primer (10 µM), and 3.5 U Taq DNA polymerase. The thermal cycling conditions comprised an initial denaturation at 94°C for 6 minutes, followed by 35 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 minutes, with a final extension at 72°C for 8 minutes. The purified PCR product underwent sequencing via the Sanger method at Eurofins Genomics, Bangalore. Sequence editing and contig assembly were performed utilizing DNA Baser software (version 3), and sequences were analyzed against those in the GenBank database through BLAST. The 16S rRNA gene sequence was aligned with sequences retrieved from GenBank using CLC Sequence Viewer 6.0, and a phylogenetic tree was constructed employing the neighbor-joining algorithm in MEGA software (version 7.0) following the methodology of (Saitou and Nei, 1987). Bootstrap analysis involving 1000 replications was utilized to ascertain confidence values for the tree branches (Felsenstein, 1985). The partial 16S rRNA nucleotide sequence of strain HUS20 has been deposited in the GenBank database.

### *Extraction and characterization of BS*

A fresh inoculum of strain HUS20 was prepared by culturing in 50 ml of ISP2 broth. Following a 48-hour incubation, the inoculum was transferred into 500 ml of ISP2 broth in a 2000 ml conical flask and incubated on a rotary shaker at 28°C and 150 rpm for 7 days. Following incubation, the culture underwent centrifugation at 8000 × g for 15 minutes to yield a cell-free supernatant. The extraction of the biosurfactant from the supernatant was performed utilizing a liquid-liquid extraction method, employing an equal volume of methanol over a period of 24 hours. The organic phase that contained the biosurfactant was concentrated with a rotary evaporator (Eppendorf), and the resulting crude biosurfactant was kept in an airtight container under refrigeration (Kumar *et al*., 2015).

### *Metabolite profiling of crude biosurfactant*

The metabolite profile of the crude extract derived from strain HUS20 was examined through high-resolution liquid chromatography-mass spectrometry (HR-LC-MS) at the SAIF, IIT Bombay, India. The analysis utilized a Thermo LTQ Orbitrap XL mass spectrometer in conjunction with an Agilent 1100 LC system, which included a solvent reservoir, in-line degasser, binary pump, and a chilled autosampler. Methanol (MeOH) served as the solvent for sample infusion

into the electrospray ionization (ESI) source to obtain spectra, adhering to protocols akin to those outlined by Tanaka *et al*. (2010).

The separation utilized a 5 µm Kinetex C18 column (50 mm × 2.1 mm), held at a temperature of 25°C. A gradient elution system utilizing water and methanol, each with 0.1% formic acid, was employed at a flow rate of 200 µL/min. The gradient program commenced with an initial phase of 10% methanol for 3 minutes, followed by a ramping phase increasing from 10% to 90% methanol over a duration of 30 minutes, and concluded with a final phase at 90% methanol for 3 minutes. The mass spectrometer functioned in positive ion mode, utilizing a spray voltage of 5 kV, a capillary temperature of 230°C, a sheath gas (N2) flow rate of 12 units, and an auxiliary gas flow rate of 5 units, aligning with the conditions documented by Zhang *et al*. (2011). MS/MS spectra were acquired in a data-dependent acquisition mode. Selected ions underwent collision-induced dissociation (CID) with an isolation width of 2.0, normalized collision energy of 35, activation Q of 0.250, and an activation time of 30 ms, following established methodologies (Smith *et al*., 2005).

### *Testing of biosurfactant producing HUS20 for antibacterial activity*

The evaluation of the antibacterial activity of the extracted biosurfactant was conducted against specific foodborne pathogens, such as Staphylococcus aureus, Bacillus cereus, Aeromonas caviae, and Aeromonas salmonicidae, employing the well diffusion assay method. This approach, commonly employed for evaluating antimicrobial efficacy, was performed on Muller-Hinton agar (MHA) plates, prepared in accordance with established protocols. Wells measuring 7 mm in diameter were created using a sterile cork borer, guaranteeing consistency among all test wells for precise zone measurements (Balouiri *et al*., 2016). Each well received 100 µL of the biosurfactant dissolved in 10% dimethyl sulfoxide (DMSO), a widely used solvent for hydrophobic compounds, recognized for its capacity to preserve compound stability and bioactivity (Kumar *et al*., 2021). The plates were subsequently incubated at 37°C for a duration of 24 hours to facilitate the diffusion of the biosurfactant into the agar and its interaction with the bacterial cells. After incubation, the zones of inhibition were measured in millimeters, offering a quantitative assessment of the antibacterial efficacy of the biosurfactant (Choi *et al*., 2019).

## Results

### *Description of the strain*

HUS20 demonstrated distinctive morphological features and was preserved on ISP2 agar slants at 4°C for further study. Its colony characteristics, including pigmentation, texture, and growth pattern were consistent with actinobacterial profiles and indicated its potential as a promising biosurfactant producer. These preliminary traits guided its selection for comprehensive biochemical and molecular characterization.

### *Screening of actinobacteria for BS production*

Identifying potential biosurfactant-producing microorganisms necessitates a thorough screening strategy. This study revealed that strain HUS20 exhibited notable potential for biosurfactant production.

#### Hemolytic assay

Strain HUS20 exhibited hemolytic activity upon being streaked onto blood agar plates. Following incubation at 28°C for a duration of 72–96 hours, distinct clear zones of hemolysis were noted surrounding the colonies, signifying the synthesis of biosurfactants that can lyse red blood cells. The observation of unique hemolytic zones provided additional confirmation of HUS20's capability as a biosurfactant producer.

#### Oil displacement test

The oil displacement assay revealed that the culture supernatant of HUS20 demonstrated notable biosurfactant activity. The addition of 10 µL of the cell-free supernatant to the oil layer resulted in the formation of a transparent halo, accompanied by a displacement zone that measured approximately 30 mm in diameter. This result demonstrated the ability of the biosurfactant generated by strain HUS20 to reduce surface tension, aligning with observations from comparable research that employed this approach for both qualitative.

#### Emulsification activity

The emulsification activity assay underscored the effectiveness of the biosurfactant produced through HUS20. The cell-free supernatant, when combined with kerosene in a 1:1 ratio, resulted in a stable emulsion following vigorous vortexing. The emulsification index (E24) was determined to be around 65%, reflecting a significant degree of biosurfactant activity. The degree of emulsification is observed noteworthy, indicating robust hydrophobic and hydrophilic interactions facilitated by the biosurfactant molecules.

#### Drop collapse assay

The drop collapse assay revealed that the supernatant from strain HUS20 resulted in a total flattening of the drop within just 1 minute upon contact with

the oil layer in the microtiter plate wells. The swift decline observed serves as a favorable sign for biosurfactant generation, given that surface-active substances diminish the interfacial tension between water and oil phases. The lack of a consistent rounded drop in the control wells underscored the assay's specificity.

**Penetration assay (PA)**

The penetration assay demonstrated that the cell-free supernatant of HUS20 was capable of effectively disrupting the oil layer prepared with silica gel, spreading swiftly upon application. This activity validates the capacity of the biosurfactant to incorporate into and alter hydrophobic phases, showcasing its potential in applications such as oil recovery or environmental remediation. The qualitative assessment aligned with findings on biosurfactant efficacy that were evaluated using comparable methods. The results of the assays indicated that strain HUS20 demonstrated robust biosurfactant activity consistently across various tests. The hemolytic activity, extensive oil displacement zone, elevated emulsification index, drop collapse, and penetration capabilities underscored its promise as a significant microbial source of biosurfactants for both industrial and environmental uses.

***Identification of actinobacterial strain HUS20***

Strain HUS20 displayed both substrate and aerial mycelium upon microscopic examination, validating its distinctive growth pattern. The observation of robust mycelium, free from any indications of fragmentation, indicated that the strain upholds standard morphological characteristics pertinent to its classification. The results proved with investigation into *Streptomyces* species, which typically exhibited both aerial and substrate mycelia as integral components of their growth morphology. A detailed microscopic observation of strain HUS20, showcasing the clearly visible distinct mycelial arrangement is presented in Figure 2.

The additional molecular characterization via 16S rRNA gene amplification confirmed the morphological identification, demonstrating a complete identity with *Streptomyces zaomyceticus* (GenBank accession number OQ996835.1). The molecular match confirmed the classification of HUS20 within the *Streptomyces*, recognized for its varied metabolic capabilities, which is encompassed the production of important secondary metabolites like antibiotics and biosurfactants. The alignment of the 16S rRNA gene sequence is illustrated in Figure 3), showcasing a significant level of homology with the reference strain. The molecular evidence substantiates the hypothesis that HUS20 is part of the *Streptomyces* species, recognized for their intricate

secondary metabolite profiles, potentially encompassing biosurfactant production.

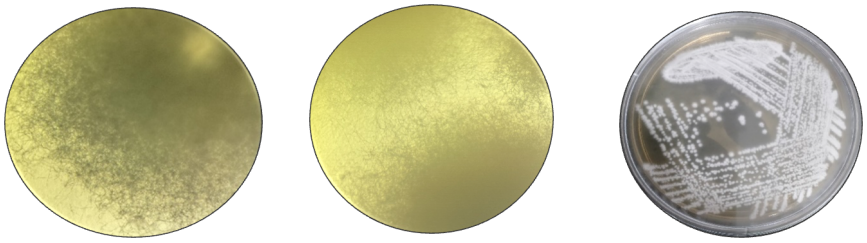

**Figure 2.** Aerial and substrate mycelium of the strain Hus20

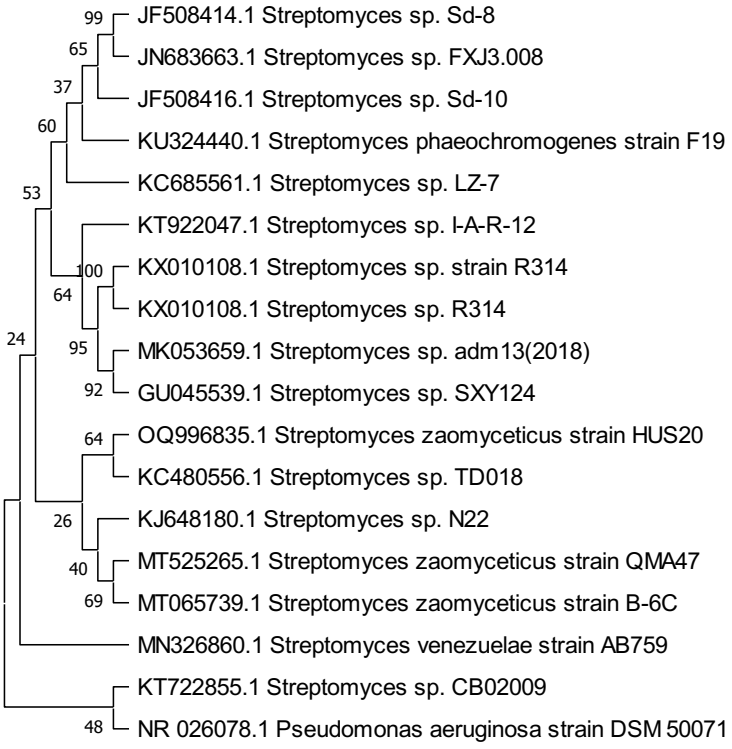


**Figure 3**. Phylogenetic analysis of the strain Hus20

***Testing of biosurfactant producing HUS20 for antibacterial activity***

The antimicrobial efficacy of strain HUS20 was evaluated against various foodborne pathogens, such as *Staphylococcus aureus*, *Aeromonas salmonicidae*, *Bacillus cereus*, and *Aeromonas caviae*. The results revealed that strain HUS20

exhibited significant antimicrobial activity at various concentrations, with the most substantial inhibition zone of 12 mm recorded for *Staphylococcus aureus* when the biosurfactant was applied at a concentration of 50 mg/ml. At a concentration of 100 mg/ml, the inhibition zone increased to 15 mm in *Aeromonas salmonicidae*. The *Bacillus cereus* pathogen demonstrated considerable inhibition at a concentration of 100 mg/ml, leading to a prominent zone of 24 mm, the largest recorded among all tested organisms all the results were mentioned in Table 1.

**Table 1.** Antibacterial activity of strain Hus20 using different pathogens

| Pathogen Name | Zone of Inhibition | | | | |
|---|---|---|---|---|---|
| | 10mg/ ml | 25mg/ ml | 50mg/ml | 100mg/ml | Solvent Control |
| ***S. aureus*** | - | 11 | 12 | - | - |
| ***A. salmonicidae*** | - | 12 | 13 | 15 | - |
| ***B. cereus*** | 15 | 17 | 18 | 24 | - |
| ***A. caviae*** | - | 12 | 11 | 12 | - |

### *Characterization of biosurfactant*

The crude biosurfactant extracted from *Streptomyces* sp. HUS20 displayed unique physical characteristics, manifesting as a brown, viscous, sticky, oily residue. The compound demonstrated solubility in various aqueous solutions as well as in a diverse array of organic solvents, such as chloroform, methanol, hexane, diethyl ether, and ethyl acetate. The extraction of crude biosurfactant using ethyl acetate yielded 0.0433 g per 100 ml of medium. The bioactive compounds found in the crude biosurfactant underwent further examination through High-Resolution Liquid Chromatography-Mass Spectrometry (HRLCMS). The chromatogram illustrated the diverse bioactive secondary metabolites found in the sample (Figure 4). Every peak in the chromatogram is shown indicative of a particular compound, with the area beneath the peak reflecting the amount of that compound present. The intensity of the peaks provided valuable information regarding the relative abundance of each metabolite, while the mass spectrometry data delivered comprehensive details about the molecular weight, chemical formula, and structural characteristics of these compounds, facilitating their identification and quantification. The HRLCMS analysis offers essential data for pinpointing the bioactive components in the crude extract, which may play a role in its antimicrobial properties, as noted in earlier studies. This information is contributed to a deeper

comprehension of the potential applications of the biosurfactant derived from *Streptomyces* sp. HUS20.

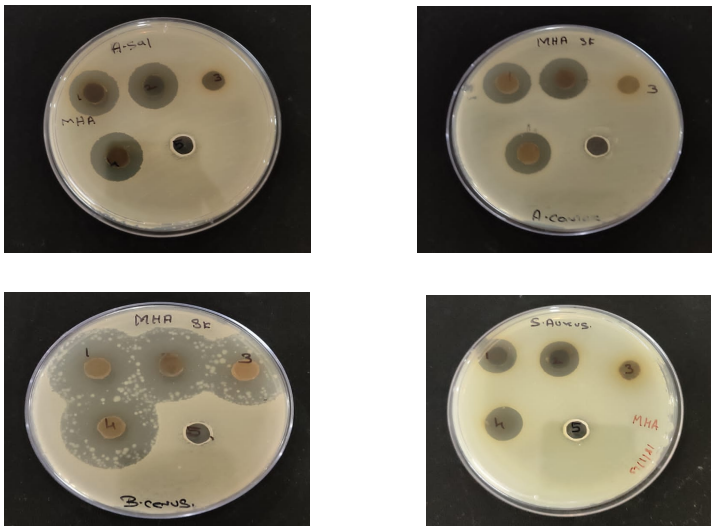


**Figure 4.** Antibacterial activity of Biosurfactant producing strain Hus20

***List of suspected compounds from potential Hus20 identified through HRLCMS/MS at NegativeESI and PossitiveESI Mode***

This paper is presented the findings from HRLCMS/MS analysis conducted in both Positive ESI and Negative ESI modes, revealing the identification of several molecules linked to biosurfactants (Figure 5). These encompassed substances that recognized for their surfactant and bioactive characteristics, including fatty acids, phenolic compounds, and amino acids.

***Fatty acids and derivatives***

Stearic acid (C18 H36 O2, 284.2722) found to be a common fatty acid, found in biosurfactants such as rhamnolipids and sophorolipids. Palmitoleic acid (C16 H30 O2, 254.2252) found to be a monounsaturated fatty acid with known antimicrobial properties, found to be proved in biosurfactants like glycolipids. Pentadecanoic acid (C15 H30 O2, 242.2252) found to be a straight-chain fatty acid associated with potential antimicrobial biosurfactant production. Dodecanedioic acid (C12 H22 O4, 230.1523) found to be a diacid that contributed to surfactant formation through esterification or other modifications.

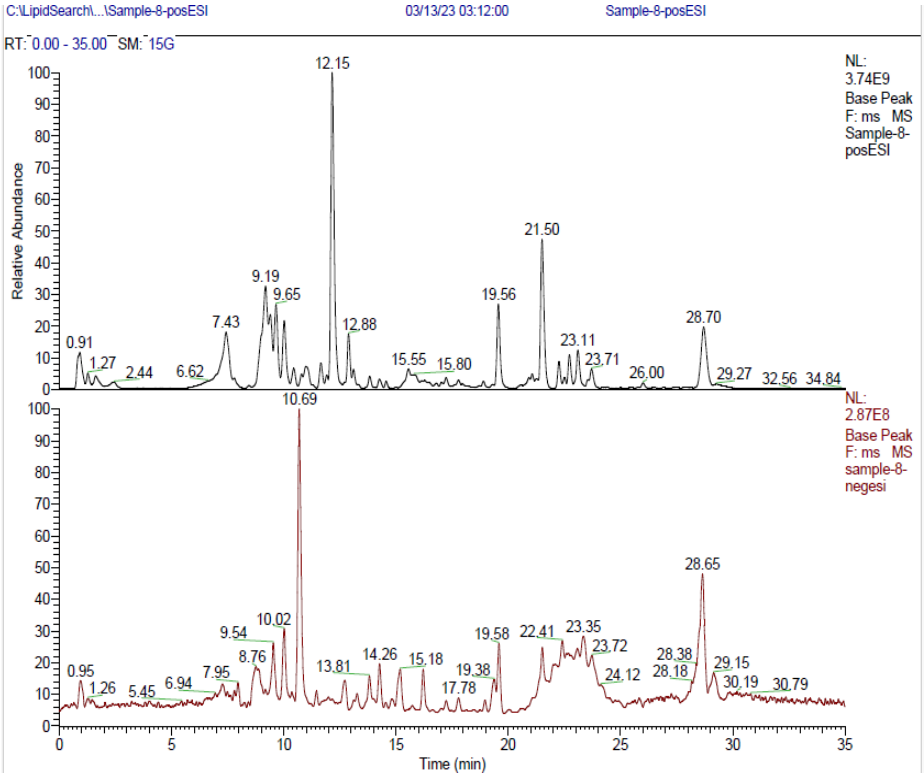


**Figure 5.** Bioactive secondary metabolites from the sample Hus20 by HRLCMS Chromatogram

### *Phenolic compounds*

Phenol (C6 H6 O, 94.04208) is exhibited biosurfactant like properties and antimicrobial activities, particularly in plant-derived surfactants. 4-Hydroxybenzoic acid (C7 H6 O3, 138.0321) found to be a phenolic compound that enhanced the antimicrobial activity of biosurfactants. Caffeic acid (C9 H8 O4, 180.0428) found to be a bioactive phenolic acid with antimicrobial properties, potentially enhancing the effectiveness of biosurfactants.

### *Amino acids and derivatives*

L-Tyrosine methyl ester (C10 H13 N O3, 195.09) found to be an amino acid derivative that can play a role in the synthesis of surfactants with bioactive properties. L-Tryptophan (C11 H12 N2 O2, 204.12) found to be a precursor for the production of bioactive molecules such as indole-based surfactants. DL-Tryptophan (C18 H22 N2 O3, 314.164) found to be an essential amino acid known for its role in microbial biosurfactant production, especially in the context of indole-based surfactants.

### *Miscellaneous compounds*

Adenine (C5 H5 N5, 135.0549) found to be a purine base that may contribute to the growth of surfactant-producing microorganisms. Uracil (C4 H4 N2 O2, 112.0276) found to be a nitrogenous base that influenced microbial activity related to surfactant production. These molecules reflected the diversity of compounds that contributed to biosurfactant properties, either by direct surfactant activity or by promoting the production of such compounds in microbial cultures.

## Discussion

The morphological and molecular identification confirmed strain HUS20 as a member of the *Streptomyces*, known for its prolific biosynthetic capacity. The consistent performance in biosurfactant screening assays including hemolytic activity, oil displacement, emulsification, drop collapse, and penetration tests points to its strong surface-active potential. These methods are widely recommended for preliminary biosurfactant detection (Satpute *et al*., 2008) and help validate the biosurfactant-producing ability of microbial isolates.

The antimicrobial screening results further highlight the utility of HUS20-derived biosurfactants, with inhibitory effects against multiple pathogens, especially *Bacillus cereus*. This confirms the presence of potent antimicrobial compounds in the crude extract, supported by HRLCMS/MS findings. Fatty acids, phenolics, and amino acid derivatives identified in the extract are well-documented contributors to surfactant activity and antimicrobial action (Kumar *et al.*, 2021; Singh *et al*., 2018). Together, these findings support the biotechnological potential of *Streptomyces zaomyceticus* HUS20 as a source of multifunctional biosurfactants. Its ability to reduce surface tension, form stable emulsions, inhibit bacterial growth, and produce chemically diverse bioactive compounds suggests applications across industrial, pharmaceutical, and environmental domains.

The current study is highlighted the potential of *Streptomyces zaomyceticus* strain HUS20, isolated from the high-altitude soils of Drama Valley, Dugtu Village in the Indian Himalayan Region, as a prolific biosurfactant producer with applications in industrial and environmental domains. Comprehensive screening through hemolytic activity, oil displacement, emulsification index, drop collapse, and penetration assays confirmed the robust biosurfactant production capabilities of strain HUS20. These assays collectively revealed the ability of the biosurfactant to reduce surface and interfacial tensions, emulsify hydrophobic substrates, and disrupt hydrophobic phases, emphasizing its efficiency in multiple applications. Morphological characterization of strain HUS20 demonstrated well-developed aerial and substrate mycelia typical of *Streptomyces* spp., corroborated by 16S rRNA gene sequencing, which identified

the strain as *Streptomyces zaomyceticus* with a high degree of genetic similarity. The extraction and characterization of the biosurfactant further confirmed its chemical stability and suitability for industrial applications.

Antibacterial assays demonstrated the biosurfactant's efficacy against key foodborne pathogens, including *Staphylococcus aureus*, *Bacillus cereus*, Aeromonas caviae, and Aeromonas salmonicidae. These findings suggest that the biosurfactant is possessed significant antimicrobial activity, making it a promising candidate for applications in food safety and pathogen control. Additionally, metabolite profiling of the crude biosurfactant via HR-LC-MS revealed a complex composition of surface-active compounds, consistent with known secondary metabolites produced by Streptomyces. This chemical complexity underpins its multifaceted applications in bioremediation, enhanced oil recovery, and other industrial sectors. In conclusion, *Streptomyces zaomyceticus* strain HUS20 is found to be a valuable microbial resource with significant potential in biosurfactant production, environmental remediation, and antibacterial applications. The results of this study pave the way for future exploration of this strain's biosynthetic pathways, optimization of biosurfactant production, and potential commercialization.

## Acknowledgements

We sincerely express our gratitude to the Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, for providing the necessary facilities and support to carry out this research. We extend our heartfelt thanks to the Department of Biotechnology, Graphic Era Deemed to be University, Dehradun, for their collaborative efforts and valuable contributions to this work.

## References

Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6:71-79.

Choi, S. H., Jang, Y. S., Jang, J. H., Bae, T. S., Lee, S. J. and Lee, M. H. (2019). Enhanced antibacterial activity of titanium by surface modification with polydopamine and silver for dental implant application. Journal of applied biomaterials & functional materials, 17:2280800019847067.

Felsenstein, J. (1985). Phylogenies and the comparative method. The American Naturalist, 125:1-15.

Jadhav, R. and Baghela, A. (2021). Yeast Research in India: A Perspective on Taxonomy and Applications. Progress in Mycology: An Indian Perspective, 591-626.

Kaur, P. and Vyas, M. (2023). Microbial surfactants: Environmental contamination management techniques for sustainable ecosystem. In Relationship between microbes and the environment for sustainable ecosystem services (pp.221-232). Elsevier.

Kaur, A., Kaur, M. and Vyas, P. (2023). Precise tuning of SiO2 thickness in SiO2@ Sr0. 4Ti0. 4Mg0. 2Fe2O4. 4 core–shell nanocomposites for augmenting photocatalytic and antibacterial activity. ACS Sustainable Chemistry & Engineering, 11:15577-15590.

Kumari, N. and Menghani, E. (2021). Evaluation of antibacterial activity and identification of bioactive metabolites by GCMS technique from Rhizospheric Actinomycetes. Indian Journal of Natural Products and Resources, 11:287-294.

Kumar, V., Muratani, M., Rayan, N. A., Kraus, P., Lufkin, T., Ng, H. H. and Prabhakar, S. (2013). Uniform, optimal signal processing of mapped deep-sequencing data. Nature biotechnology, 31:615-622.

Kumar, A., Singh, S. K., Kant, C., Verma, H., Kumar, D., Singh, P. P. and Kumar, M. (2021). Microbial biosurfactant: a new frontier for sustainable agriculture and pharmaceutical industries. Antioxidants, 10:1472.

Kumar, G., Kumar, R. and Sharma, A. (2015). Characterization of biosurfactants from indigenous soil bacteria recovered from oil contaminated sites. Journal of Environmental Biology, 36:1101.

Kumari, N. and Menghani, E. (2021). Evaluation of antibacterial activity and identification of bioactive metabolites by GCMS technique from Rhizospheric Actinomycetes. Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)], 11:287-294.

Mishra, A. K., Mohanty, R. B., Ghosh, R., Mishra, K., Shukla, U. K. and Kar, R. (2022). Modern pollen–vegetation relationships along an altitudinal transect in the Western Higher Himalaya, India: Palaeoclimatic and anthropogenic implications. The Holocene, 32:835-852.

Ravindran, A., Sajayan, A., Priyadharshini, G. B., Selvin, J. and Kiran, G. S. (2020). Revealing the efficacy of thermostable biosurfactant in heavy metal bioremediation and surface treatment in vegetables. Frontiers in microbiology, 11:222.

Rodrigues, A. I., Gudiña, E. J., Teixeira, J. A. and Rodrigues, L. R. (2021). Biosurfactants as biocontrol agents against mycotoxigenic fungi. Biosurfactants for a sustainable future: production and applications in the environment and biomedicine, 465-490.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4:406-425.

Satpute, S. K., Bhawsar, B. D., Dhakephalkar, P. K. and Chopade, B. A. (2008). Assessment of different screening methods for selecting biosurfactant producing marine bacteria. Indian Journal of Geo-Marine Sciences, 37:243-250.

Shirling, E. T. and Gottlieb, D. (1966). Methods for characterization of Streptomyces species. International Journal of Systematic and Evolutionary Microbiology, 16:313-340.

Sharma, V., Singh, D., Manzoor, M., Banpurkar, A. G., Satpute, S. K. and Sharma, D. (2022). Characterization and cytotoxicity assessment of biosurfactant derived from Lactobacillus pentosus NCIM 2912. Brazilian Journal of Microbiology, 53:327-340.

Sharma, S., Tiwari, P. and Pandey, L. (2021). Optimization of culture conditions for the production of biosurfactants. In Microbial Enhanced Oil Recovery: Principles and Potential. Singapore: Springer Singapore, pp.149-178.

Singh, R., Glick, B. R. and Rathore, D. (2018). Biosurfactants as a biological tool to increase micronutrient availability in soil: A review. Pedosphere, 28:170-189.

Smith, C. A., O'Maille, G., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R. and Siuzdak, G. (2005). METLIN: a metabolite mass spectral database. Therapeutic drug monitoring, 27:747-751.

Tanaka, H., Sugita-Konishi, Y., Takino, M., Tanaka, T., Toriba, A. and Hayakawa, K. (2010). A survey of the occurrence of Fusarium mycotoxins in biscuits in Japan by using LC/MS. Journal of Health Science, 56:188-194.

Zhang, G., Huang, G., Xiao, L. and Mitchell, A. E. (2011). Determination of advanced glycation end products by LC-MS/MS in raw and roasted almonds (*Prunus dulcis*). Journal of agricultural and food chemistry, 59:12037-12046.

(Received: 24 December 2024, Revised: 30 June 2025, Accepted: 3 July 2025)