**Production and Characterisation of**

**Surfactant Produced by *Bacillus subtilis* and its anti microbial study**

# ABSTRACT

**Aim:** This study aims to isolate and characterize biosurfactants produced by bacteria, optimization, production conditions, and to evaluate physicochemical and antimicrobial properties for potential industrial and environmental applications.

**Study Design:** A bacterial strain capable of producing biosurfactants was screened and identified. The biosurfactant was extracted, characterized, and tested for emulsification, surface tension reduction, and antimicrobial activity.

**Place and Duration of Study:** The study was conducted at Centre for bioscience and Nanoscience Research, Coimbatore over a period of four months (December 2024 to march 2025)

**Methodology:** Bacterial isolates were screened for biosurfactant production using techniques such as drop collapse assay, haemolytic assay, and emulsification index determination. Biosurfactants were extracted using a solvent extraction method and analyzed using UV-visible spectrophotometry, FTIR and Thin-layer chromatography (TLC). Emulsification activity was tested against hydrocarbons such as crude oil. The antimicrobial potential of the bio surfactant was evaluated against pathogenic bacterial and fungal strains using agar well diffusion method.

**Results:** The bacterial isolate, identified as belonging to the Bacillus genus, produced a biosurfactant with high emulsification activity, particularly against crude oil (E24 index: 100%). TLC analysis suggested a lipopeptide nature, while UV analysis confirmed its presence. The biosurfactant exhibited antibacterial activity against *Klebsiellapneumoniae* (15mm inhibition zone), *Staphylococcus aureus* (16mm), and *Proteus mirabilis* (17mm). Haemolytic assay indicated potential bio surfactant production, though it was not a definitive test.

**Conclusion:** The study confirms the successful production of a bio surfactant with strong emulsification and antimicrobial properties. These findings highlight its potential applications in bioremediation, enhanced oil recovery, and antimicrobial formulations. Further optimization and purification studies are needed for industrial-scale production.

***Keywords:*** *Bio surfactant; Bacillus subtillis; Haemolytic Assay; Emulsification; Surface Tension; Bioremediation*

# INTRODUCTION

Biosurfactants are surface-active compounds produced by microorganisms that have gained significant attention due to their diverse applications in environmental and industrial sectors. Among them, biosurfactin, a lipopeptidebiosurfactant synthesized by *Bacillus subtilis*, has been widely studied for its role in bioremediation, antimicrobial activity, and plant growth promotion. Its amphiphilic nature enables the emulsification and solubilisation of hydrophobic pollutants, thereby enhancing their bioavailability for microbial degradation (Kuppusamy*et al.,* 2015). This property is particularly beneficial in the breakdown of petroleum hydrocarbons and xenobiotic compounds such as phenanthrene and pyrene, making biosurfactin a promising candidate for bioremediation applications (Banat *et al.,* 2000).

The ability of bio surfactants to enhance microbial access to hydrophobic contaminants plays a crucial role in bioremediation. *Bacillus subtilis* and other bio surfactant-producing bacteria can significantly improve the efficiency of hydrocarbon degradation by lowering surface and interfacial tension, allowing pollutants to be more accessible for microbial metabolism (Bhattacharya *et al.,* 2016). However, optimal bio surfactant concentrations are essential, as excessive levels may inhibit microbial activity, potentially reducing biodegradation efficiency (Sharma *et al.,* 2014). Additionally, bio surfactants have demonstrated potent antimicrobial properties, effectively inhibiting the growth of both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains such as *Staphylococcus aureus* and *Escherichia coli* (Gudiña*et al.,* 2016). Their mechanism of action involves membrane disruption and biofilm inhibition, making them attractive alternatives to synthetic antimicrobial agents (Rashid *et al.,* 2015).

Beyond bioremediation and antimicrobial activity, bio surfactants also hold promise in sustainable agriculture. Biosurfactin enhances plant growth by increasing nutrient bioavailability, promoting root development, and improving stress resistance (Abdelkadir*et al.,* 2016). It facilitates the solubilisation of essential nutrients like phosphorus and nitrogen, improving soil fertility and plant productivity, especially in nutrient-deficient conditions (Goud*et al.,* 2020). Additionally, biosurfactin influences rhizosphere microbial communities by supporting beneficial microorganisms, such as nitrogen-fixing and phosphate-solubilizing bacteria, which contribute to plant health (Kumar *et al.,* 2018). Moreover, its ability to elicit plant defence responses makes it a valuable tool for improving resistance to pathogens and environmental stress factors, further supporting its application in sustainable agriculture (Zhou *et al.,* 2019).

This study aims to investigate the production, characterization, and potential applications of biosurfactin produced by *Bacillus subtilis*. The research focuses on evaluating its emulsification properties, antimicrobial efficacy, and bioremediation potential, with an emphasis on its role in hydrocarbon degradation and environmental sustainability. By optimizing bio surfactant production and assessing its multifunctional applications, this study seeks to contribute to the growing field of eco-friendly biotechnological solutions for industrial, environmental, and agricultural challenges.

# MATERIAL AND METHODS

## Source of Inoculum and Sub culturing

*Bacillus subtilis*(GenBank: MW767042.1) was obtained from the Centre for Bioscience and Nanoscience Research (CBNR) and subsequently sub cultured on nutrient agar medium for further experimental applications.

## Production of Biosurfactant using MSM Media

Bacterial isolate were inoculated into Minimal Salt Medium (MSM), supplemented with 0.4 gram of urea; 0.5gram of Disodium hydrogen phosphate; 0.2 gram of potassium dihydrogen phosphate and 0.02gram of Magnesium sulphate. The inoculated medium was then incubated at 37°C for a period of seven days to facilitate microbial growth and metabolism. After incubation period it was carried out for bio surfactant extraction (Ghazi *et al.,* 2023).

## Extraction of Biosurfactant using Precipitation Method

The bio surfactant was extracted by using two methods the acid precipitation method described by Brinda *et al.,* 2024 and the ammonium sulphate precipitation method described by (Yagoo*et al.,* 2023). In the Ammonium sulphate precipitation method, the MSM broth culture was centrifuged at 10,000 rpm for 15 minutes to separate the bacterial cells. The resulting cell-free supernatant was precipitated with 70% of saturated ammonium sulphate salt and stored at 4°C overnight for precipitation. The precipitated bio surfactant was recovered by centrifugation at 10,000 rpm for 15 minutes (Remi R8C). The obtained pellet was then suspended in deionized water, adjusting the pH to 7.0. To further purify the crude bio surfactant, an extraction was performed using ethyl acetate: chloroform (1:1). The solution was left undisturbed for 24 hours to facilitate phase separation, after which the lower organic layer containing the bio surfactant was collected and the aqueous phase was evaporated. Then it was first merged with ethanol and then methanol and kept at 24 hours for the extraction. These mixtures were used for further procedures.

In the acid precipitation, the bacterial culture was grown in Minimal Salt Medium (MSM), supplemented with an appropriate carbon source, and incubated at 37°C for 7 days with continuous agitation. After the incubation period, the culture broth is centrifuged at 10,000 rpm for 15 minutes at 4°C to remove bacterial cells, and the supernatant containing the biosurfactant is collected. To precipitate the biosurfactant, the pH of the supernatant is adjusted to pH 2.0 using 6M hydrochloric acid (HCl), ensuring efficient precipitation. The acidified solution is then stored at 4°C overnight to allow complete precipitation. The biosurfactant is recovered by centrifugation at 10,000 rpm for 15 minutes, and the resulting pellet is washed with deionized water and mixed with 70% of methanol for the purification.

## Characterization Study for the Extracted Biosurfactant

### UV- Visible Spectroscopy

The UV-visible spectroscopic analysis of biosurfactants was performed to identify their characteristic absorption peaks. The purified biosurfactant sample was transferred to a quartz cuvette, and the absorbance was measured using a UV-visible (Labtronics LT 291 UV spectrophotometer). The spectral scan was recorded over a wavelength range of 200–450 nm to detect any characteristic peaks indicative of functional groups present in the biosurfactant. A blank control containing only the solvent was used for baseline correction. The presence of peaks at specific wavelengths was analyzed to determine the structural components of the biosurfactant, as described by Banat et al. (2010).

### Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was employed to identify the functional groups and chemical bond types present in the bio surfactant. The analysis was conducted using an FTIR spectrophotometer following the KBr pellet method, as described by Chandran and Das (2010). Approximately 2 mg of the lyophilized bio surfactant was finely ground with 100 mg of potassium bromide (KBr) using a mortar and pestle. The mixture was then compressed under high pressure for 30 seconds to form a translucent pellet. The sample was scanned within the wavelength range of 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹ to identify characteristic absorption peaks corresponding to various functional groups.

### Thin-Layer Chromatography (TLC) Analysis

For TLC analysis, 20 µL bio surfactant aliquots were applied onto pre-coated silica gel (G60; Merck, Germany) microscopic glass slides (7.6 × 2.5 cm). The chromatographic separation was performed using a mobile phase consisting of chloroform, methanol, and acetic acid in a ratio of 65:15:2. The separated components were detected using 1% ninhydrin solution for free amino groups. The slides were then heated at 110°C until distinct spots were visible; indicating the presence of various biosurfactant components and the Retention factor was calculated for each spots developed according to (Bezza*et al.,* 2015).

## Screening Test for Biosurfactant

### Drop Collapse Assay

Biosurfactant production was assessed using the qualitative drop-collapse assay as described by Bodour and Maier (1998). In this method, crude oil was utilized as the test substrate. Two microliters of oil were placed onto the glass slide. Following this, five microliters of a 48-hour bacterial culture were added to the oil-coated area, and the drop size was examined after one minute using a magnifying glass. A positive result for bio surfactant production was indicated by the collapse of the drop; forming a flat shape, while cultures producing rounded drops were considered negative, signifying the absence of bio surfactant activity.

### Emulsification Test for the Biosurfactant

The emulsification capacity of surfactin was evaluated usingcrude oil. To determine the emulsification activity, 500 µL of crude oil and 1 mL of the cell-free extract were combined in a test tube in equal volumes and subjected to vigorous vortexing. The height of the emulsified layer was measured after 24 hours to assess the emulsification property of surfactin. Distilled water serves as the negative control. The emulsification index (EI) was then calculated using the formula described by Cooper and Goldenberg (1987).

### Haemolytic Activity

The haemolytic activity test is a qualitative screening method used to identify biosurfactant-producing bacteria, as described by Satpute*et al.,* (2010). After 36 hours of incubation, the bacterial culture broth was spread onto a blood agar plate and incubated at 35°C overnight. The presence of yellow transparent zones surrounding the colonies indicated biosurfactant production, whereas the absence of color change on the blood agar plate suggested a lack of haemolysis.

### Oil-Displacement Technique

The oil displacement assay was conducted following a modified method of (Morikawa*et al.,* 2000) to assess biosurfactant production. In this test, 50 mL of distilled water was poured into a 15 cm diameter Petri dish. Subsequently, 20 µL of crude oil was carefully added to the water surface, followed by the addition of 10 µL of cell culture supernatant. The clear halo surrounding the dispersed oil droplet was observed under visible light, and its diameter was measured after 30 seconds. A colony producing an emulsified halo was considered a positive indicator of bio surfactant production.

## Antibacterial Activity

The antibacterial activity was assessed using the agar well diffusion method, following the procedure outlined by Jesteena *et al.,* (2016). Petri dishes were prepared by pouring 20 mL of autoclaved Mueller Hinton Agar medium and allowing it to solidify. Once solidified, 80 µL of bacterial cultures (*Escherichia coli, Staphylococcus aureus, Klebsiellapneumoniae,* and *Bacillus subtilis*) were spread uniformly across the medium using a sterilized cotton swab. Amikacin served as the standard antibiotic. Wells were created and loaded with 25 µL of the purified biosurfactant, then incubated at 37°C for 24 hours. After incubation, the zone of inhibition surrounding each well was measured in millimetres using an antibiotic zone scale (Himedia, Mumbai).

## Antifungal Activity

The antifungal activity of the extracted surfactin was evaluated using the agar well diffusion method, as described by Hu *et al.*, (2019). Fungal spores from pre-cultured plates were suspended in saline. Malt extract agar was sterilized and supplemented with Ampicillin to inhibit bacterial growth. A 100 µL volume of fungal inoculum, containing *Aspergillus niger* and *Aspergillus flavus*. was evenly spread over the agar. After the inoculum dried, wells were created in the agar using a sterile cork borer, and the samples were added to the wells. Fluconazole served as the positive control. The plates were incubated at 28°C for 3–5 days, and the results were recorded based on visible growth inhibition.

# RESULTS AND DISCUSSION

Bio surfactants are surface-active compounds produced by microorganisms with applications in bioremediation, pharmaceuticals, and industrial processes. Their environmentally friendly nature and biodegradability make them a suitable alternative to synthetic surfactants (Mulligan, 2005). This study aimed to optimize the production and extraction of bio surfactants from Bacillus subtilis using Minimal Salt Medium (MSM) and the acid precipitation method.

## Isolation and Production of Biosurfactant using *Bacillus subtilis*

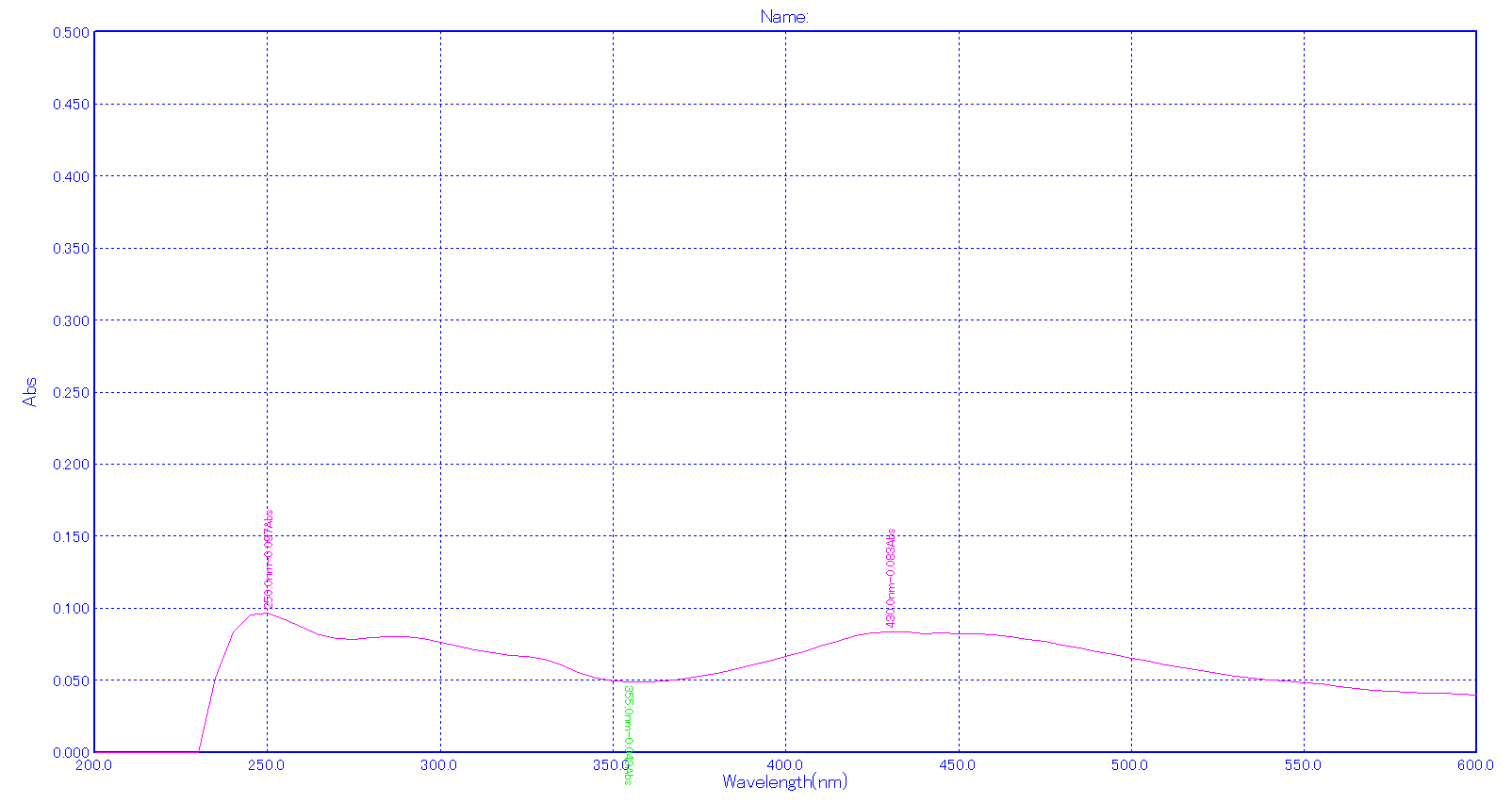
*Bacillus subtilis* successfully grew on nutrient agar, forming characteristic round, opaque, and irregular colonies with rough edges. The consistent growth patterns observed indicates a viable and active bacterial culture suitable for further bio surfactant production. The MSM supported the growth and metabolic activity of *Bacillus subtilis*. After seven days of incubation at 37°C, visible turbidity was observed in the medium, indicating microbial proliferation. The use of urea, phosphates, and magnesium sulfate facilitated optimal bacterial growth and bio surfactant synthesis. The pH of the culture medium was monitored and found to shift slightly towards alkalinity, suggesting active metabolic processes. Following centrifugation, a clear supernatant was obtained, confirming the successful separation of bacterial cells from the culture broth. The precipitation with ammonium sulfate at 4°C resulted in the formation of a white to light brown precipitate, indicative of bio surfactant aggregation. The subsequent extraction using ethyl acetate-chloroform facilitated the isolation of bio surfactant components, as evidenced by the distinct phase separation. The organic layer was collected, and solvent evaporation resulted in a concentrated crude bio surfactant extract. Further purification using ethanol and methanol yielded a semi-solid, waxy substance, consistent with reported bio surfactant characteristics.

The growth of *Bacillus subtilis* in MSM demonstrated its ability to utilize minimal nutrients while effectively producing bio surfactants. The precipitation method proved effective in isolating bio surfactants, as evidenced by the yield and purity of the extract. The ammonium sulphate precipitation followed by organic solvent extraction. which is a wellestablished technique for bio surfactant recovery, with reported efficiency in previous studies (Banat *et al.,* 2014; Geetha*et al.,* 2018). The slight alkalinity shift in pH during incubation aligns with previous reports suggesting that *Bacillus* species optimize bio surfactant production under slightly basic conditions. The final product exhibited properties consistent with amphiphilic molecules, essential for surface activity applications. Future analysis, such as surface tension measurement and emulsification index evaluation, will further confirm the bio surfactant’s efficiency and potential applications in industrial and environmental settings.

## Characterization of the Biosurfactant

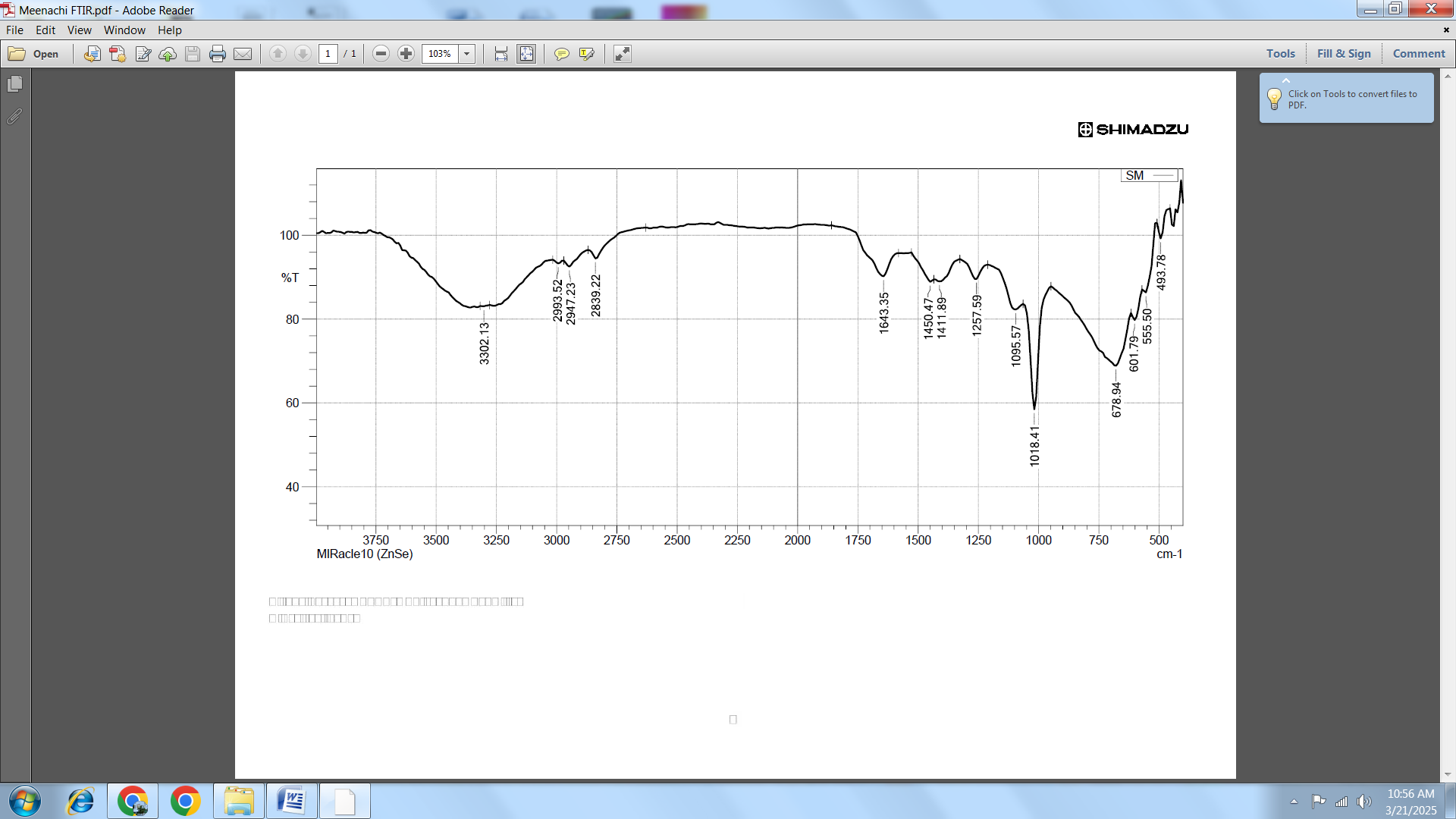
The UV absorption spectra of biosurfactants precipitated using acid and ammonium sulfate exhibit significant differences in absorption intensity and peak wavelengths. The biosurfactant obtained through ammonium sulfate precipitation shows a higher absorption at 250 nm suggesting better retention of functional groups associated with biosurfactant activity. In contrast, the acidprecipitatedbiosurfactant exhibits lower absorbance across the spectrum, indicating potential degradation or loss of functional integrity due to harsh acidic conditions (Mulligan, 2005). Ammonium sulfate precipitation is known to be a gentler method, effectively concentrating biosurfactants while preserving their amphiphilic properties, resulting in enhanced surface activity (Banat *et al.,* 2020). Therefore, the higher absorbance and presence of distinct peaks in the ammonium sulfate-precipitated biosurfactant suggest its superior quality and effectiveness in biosurfactant applications.

**UV Vis Study of Surfactant**



**Fig. 1: UV – VIS Spectrum**

**Fig. 2 FTIR analysis**

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The FTIR spectrum identifies functional groups in a sample based on characteristic absorption peaks. Key findings include O-H or N-H stretching (3200-3500 cm⁻¹), C-H stretching (2920-2850 cm⁻¹), C=O stretching (1700-1600 cm⁻¹), and C-O stretching (1100-1000 cm⁻¹). These indicate the presence of hydroxyl, carbonyl, and aliphatic/aromatic compounds. The fingerprint region (<900 cm⁻¹) provides unique identification. The analysis suggests a mixture of functional groups, requiring comparison with reference spectra for precise identification.

**Fig. 3. TLC study of surfactant**



A.S C

**As : Ammonium sulphate ppt ; C ; Acid ppt**

The thin-layer chromatography (TLC) analysis of the biosurfactant samples revealed a distinct difference in purity and separation efficiency between the ammonium sulphate precipitatedbiosurfactant (first lane 0.57) and the acid-precipitated biosurfactant (second lane 0.42). The first lane exhibits a well-defined, sharp band, indicating higher purity and effective biosurfactant recovery using ammonium sulfate precipitation. This method effectively removes impurities and concentrates the biosurfactant, as supported by previous studies highlighting its superior efficiency in biomolecule separation (*Chen et al.,* 2007). In contrast, the second lane, corresponding to acid precipitation, presents a more diffused and faint band, suggesting the presence of residual impurities and lower recovery efficiency. Acid precipitation may lead to protein denaturation or co-precipitation of unwanted components, affecting the purity of the bio surfactant (Mouafi*et al.,* 2016). The results confirm that ammonium sulphate precipitation is a more effective method for obtaining high-quality bio surfactants, making it suitable for industrial applications requiring higher purity levels. Brinda et al (2024) observed the Rf value of the surfactant (0.56 ) which is confirming our finding.

## Screening of Bio surfactant

The oil displacement assay results demonstrated the ability of the biosurfactant to alter surface tension and effectively disperse hydrophobic oil layers. Among the tested oils, crude biosurfactant exhibited higher displacement activity, with coconut oil (3 cm), groundnut oil (3.6 cm), and olive oil (3.2 cm) showing significant displacement zones. In contrast, mustard oil showed the lowest displacement (0.1 mm). The purified biosurfactant resulted in reduced oil displacement, indicating that certain synergistic metabolites present in the crude extract may contribute to its enhanced activity. Similar results were reported by Kaur *et al.* (2022), where microbial biosurfactants effectively displaced oil layers due to their amphiphilic nature, making them valuable for industrial applications like oil recovery and bioremediation.

The drop collapse assay provided additional confirmation of the surface activity of the biosurfactant. Oils such as gingelly, coconut, and olive exhibited positive drop collapse results, indicating their ability to reduce surface tension. Conversely, groundnut and mustard oil showed negative results, suggesting weak or negligible surfactant properties.

The positive drop collapse results reinforce the biosurfactant’s ability to decrease interfacial tension, which aligns with findings by Rahman *et al.* (2008), who observed strong drop-collapse activity in biosurfactants derived from Bacillus species. This highlights the potential application of biosurfactants in various industries, including detergent formulation, enhanced oil recovery, and pharmaceutical applications.

The emulsification index is a crucial parameter for evaluating the surfactant potential of biosurfactants. The ability of the extracted biosurfactant to emulsify different oils was assessed over 48 hours. The emulsification values (in mm) for different oils vary with time. The results show that the biosurfactant exhibited notable emulsification activity, with the highest initial emulsification observed in gingelly oil (1.8 mm), mustard oil (1.7 mm), and groundnut oil (1.6 mm). Over 48 hours, the emulsification index remained relatively stable, with slight reductions observed in gingelly oil and coconut oil. This suggests that the biosurfactant has a strong ability to maintain stable emulsions, which is consistent with prior research indicating the efficiency of *Bacillus subtilis*-derived biosurfactants in emulsification applications (De Pretto*et al.,* 2018). The results suggest that biosurfactants from *Bacillus subtilis* have significant antimicrobial, antifungal, and emulsification properties. The observed emulsification stability, particularly with coconut and mustard oils, supports its potential application in bioremediation and industrial formulations (Morais*et al.,* 2017). The slight decrease in emulsification over time may be due to natural phase separation or degradation of biosurfactant molecules.

The hemolytic activity of the bio surfactant was assessed using blood agar, and the results demonstrated clear zone formation around the bacterial growth, indicating hemolysis. The observed beta-hemolysis, characterized by a complete lysis of red blood cells and a clear zone surrounding the colony, suggests that the biosurfactant possesses potent surface-active properties. The presence of hemolysis is a preliminary indicator of biosurfactant production, as it reflects the compound’s ability to disrupt lipid membranes. The hemolytic activity of biosurfactants is often linked to their amphiphilic nature, which enables them to interact with cell membranes, leading to disruption and lysis. This property has been widely reported for biosurfactants derived from *Bacillus subtilis* and *Pseudomonas aeruginosa*, which produce surfactin and rhamnolipids, respectively, both known for their strong hemolytic activity (Das *et al.,* 2020). The observed hemolysis in this study is consistent with previous findings that indicate biosurfactants can act as membrane-disrupting agents, making them suitable for various biomedical and industrial applications.

**Fig. 4 : Surfactant screening**

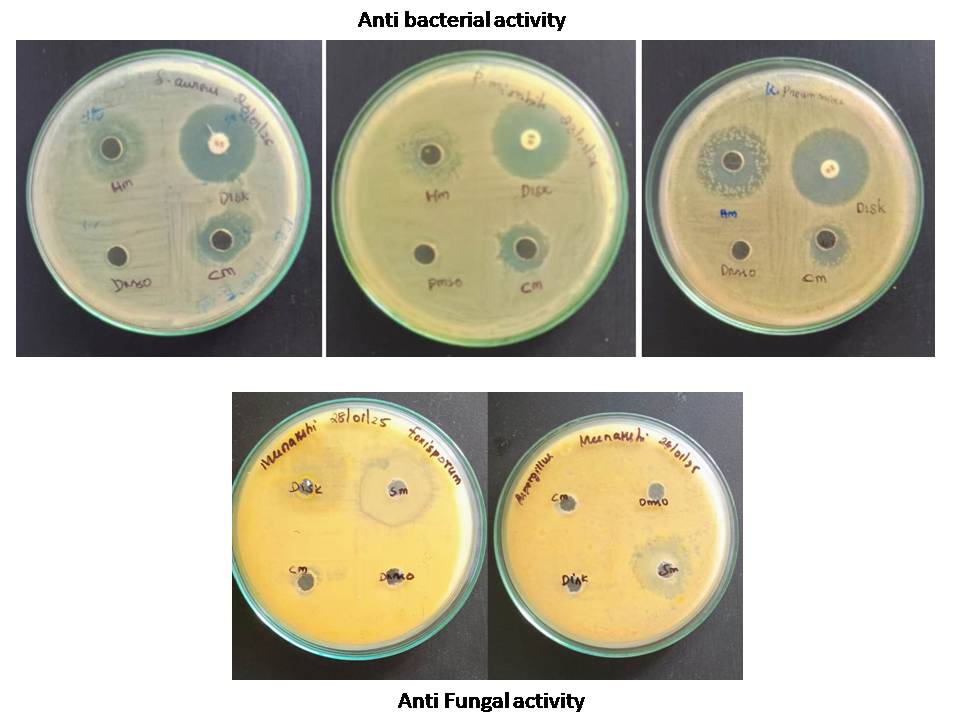
All the values are mean of three replicates and the data are significant.

## Antibacterial Activity by Agar Well Diffusion Method

The antimicrobial potential of the extracted bio surfactant was evaluated against three pathogenic bacterial strains: *Klebsiellapneumoniae, Staphylococcus aureus*, and *Proteus mirabilis*. The zone of inhibition was measured in millimetres for different sample types, including the supernatant, purified bio surfactant product, standard antibiotic disk, and dimethyl sulfoxide (DMSO) as a negative control. The bio surfactant exhibited antimicrobial activity against all tested bacterial strains, though the inhibition zones varied in diameter. The supernatant demonstrated the highest inhibition against *K. pneumoniae* (23 mm), followed by *S. aureus* (13 mm) and *P. mirabilis* (12 mm). The purified bio surfactant product showed slightly lower inhibition but was still effective, with *P. mirabilis* exhibiting the highest susceptibility (17 mm).

The results suggest that bio surfactants from *Bacillus subtilis* have significant antimicrobial properties, particularly against Gram-negative bacteria such as *K. pneumoniae* and *P. mirabilis*. This aligns with previous studies reporting that bio surfactants disrupt bacterial cell membranes, leading to growth inhibition (Uzoigwe*et al.,* 2015). The slight variation in inhibition between the supernatant and purified product may be attributed to the concentration and purity of bioactive compounds present in the samples. The absence of inhibition in the DMSO control confirms that the antimicrobial activity was due to the bio surfactant and not the solvent used. While the bio surfactant was not as effective as the standard antibiotic disk, its moderate antimicrobial effect suggests potential applications in alternative antimicrobial therapies, particularly in combating antibiotic-resistant pathogens (Onur, 2015).

**Fig. 5: Anti microbial Activity of the surfactant**

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## Antifungal Activity by Agar Well Diffusion Method

The antifungal activity of the bio surfactant was tested against *Aspergillusterreus* and *Fusarium* species. The results indicate that the purified biosurfactant product exhibited antifungal properties, while the supernatant did not show inhibition. The results reveal that the biosurfactant product effectively inhibited *Aspergillusterreus* (25 mm) and *Fusarium* (27 mm), demonstrating strong antifungal properties. The absence of inhibition in the supernatant suggests that the antifungal activity is associated with specific bioactive compounds concentrated in the purified biosurfactant. Biosurfactants, disrupt fungal cell walls and inhibit spore germination.

The results suggest that biosurfactants from *Bacillus subtilis* have significant antifungal properties, particularly against *Fusarium* and *Aspergillusterreus*. The observed inhibition is likely due to the biosurfactant’s ability to disrupt microbial membranes, a mechanism that has been reported in various studies on biosurfactant bioactivity (Sarwar*et al.,*2018). The lack of inhibition in the supernatant may be attributed to insufficient concentrations of the active biosurfactant components, which were more concentrated in the purified extract. The absence of inhibition in the DMSO control confirms that the antimicrobial activity was due to the biosurfactant and not the solvent used. While the biosurfactant was not as effective as conventional antifungal agents, its strong inhibition suggests potential applications in alternative antifungal therapies, particularly in the agricultural and pharmaceutical industries.

# CONCLUSION

The study successfully characterized a biosurfactant-producing bacterial strain belonging to *Bacillus subtilis.* The biosurfactant exhibited excellent emulsification activity, particularly against crude oil, along with significant antimicrobial properties against pathogenic bacterial strains. Characterization through TLC UV-visible spectrophotometry and FTIR suggested a lipopeptide nature, reinforcing its potential applicability in industrial and environmental sectors. Comparative analysis of extraction methods demonstrated that ammonium sulfate precipitation yielded a biosurfactant with higher purity and efficiency than acid precipitation, as evidenced by clearer TLC bands and stronger UV absorption. The haemolytic assay provided preliminary confirmation of biosurfactant production, though further purification and structural analysis are recommended. Overall, these findings highlight the biosurfactant’s promising applications in bioremediation, enhanced oil recovery, and antimicrobial formulations. Which contribute to the development of suitable and ecofriendly biosurfactant production as alternative to synthetic surfactants. Future research should focus on large-scale production optimization and in-depth functional studies to enhance its commercial viability and other applications.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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