

Isolation and Characterization of Endophytic Microorganisms from Medicinal Plants with Potential for Biosurfactant Production, Antifungal Activity, and Agricultural Applications

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Abstract-- Endophytic microorganisms non-pathogenic microbes residing within plant tissues play a significant role in sustainable agriculture by enhancing plant growth and providing protection against phytopathogens. In this study, endophytic bacteria were isolated from Aloe vera, Hibiscus rosa-sinensis, and Musa spp. (banana) using sterile techniques and nutrient agar media, followed by morphological and biochemical characterization. Three bacterial isolates were identified as *Bacillus subtilis* (from Hibiscus), *Bacillus cereus* (from Banana), and *Actinomyces viscosus* (from Aloe vera), with colonization frequencies of 65%, 78%, and 85%, respectively. Biosurfactants extracted from these isolates demonstrated strong surface activity, confirmed by emulsification index, oil displacement, and drop collapse assays, with *B. subtilis* yielding the highest production (10 g/L). All biosurfactants exhibited notable thermal stability. Antifungal assays revealed moderate inhibition against *Candida albicans* and *Alternaria solani*, particularly by *A. viscosus*, while no inhibitory effect was observed against *Aspergillus niger*. Furthermore, plant growth trials on *Vigna radiata* (mung bean) indicated enhanced growth parameters and increased resistance to fungal infection following biosurfactant treatment. The results indicate that endophytic bacteria-derived biosurfactants possess strong surface activity, thermostability, and antifungal properties, highlighting their potential as eco-friendly alternatives to synthetic agrochemicals. Further molecular characterization and field-level validation are recommended to substantiate their applicability in sustainable agricultural systems.

Keywords-- *Actinomyces viscosus*, antifungal activity, *Bacillus subtilis*, *Bacillus cereus*, biosurfactant, biocontrol, emulsification index, Endophytic microorganisms, plant growth promotion, sustainable agriculture, surface-active compounds, *Vigna radiata*.

I. INTRODUCTION

Endophytes are microorganisms including bacteria, fungi, algae, and occasionally insects that reside within plant tissues without causing visible disease symptoms.

These organisms engage in a range of interactions with their host plants, from mutualism to latent pathogenicity, significantly contributing to plant growth, disease resistance, and tolerance to abiotic stresses (Stone et al., 2000; Sinclair & Cerkaskas, 1996). Endophytic colonization occurs across various plant organs and is influenced by host species, habitat, and environmental conditions (Schulz et al., 2006).

Among the bioactive compounds produced by endophytes, biosurfactants stand out due to their surface-active properties and potential applications in agriculture, medicine, and environmental remediation. These compounds, which include glycolipids and lipopeptides, are environmentally friendly alternatives to synthetic surfactants (Farias et al., 2021). For example, *Pseudomonas aeruginosa* produces rhamnolipids, *Bacillus subtilis* synthesizes surfactin, and various *Candida* species are known for sophorolipid production—all of which exhibit strong surface tension reduction and antimicrobial activity (Chanway, 1996; Adhikari et al., 2001; Farias et al., 2021).

This research aims to isolate and identify endophytic microbes from medicinal and agricultural plants, assess their biosurfactant production through qualitative and quantitative assays, characterize these biosurfactants using GC-MS, FTIR, and HPLC, and evaluate their antifungal potential against key plant pathogens.

Surfactants are amphiphilic compounds widely utilized across industries such as agriculture, food processing, pharmaceuticals, and petrochemicals. However, the extensive use of synthetic surfactants has led to environmental pollution and health concerns due to their non-biodegradable and toxic nature (Thakur et al., 2021). In response, microbial surfactants—commonly known as biosurfactants—have emerged as promising, eco-friendly alternatives. These compounds, produced by bacteria, fungi, and yeasts, possess high surface activity, low toxicity, and biodegradability, making them ideal for applications in bioremediation, medicine, cosmetics, and food safety (Farias et al., 2021; Kourmentza et al., 2021).

Importantly, biosurfactants can also be synthesized using waste substrates such as agricultural by-products, promoting waste valorization and a circular economy (Marchut Mikołajczyk et al., 2021).

Endophytic microorganisms—microbes that inhabit plant tissues without causing harm—are increasingly recognized as valuable sources of biosurfactants and other bioactive compounds. These include species like *Bacillus pumilus* and *Bacillus subtilis*, which not only produce potent biosurfactants such as surfactin but also enhance plant growth and stress tolerance (Marchut Mikołajczyk et al., 2021; Zhou et al., 2018). The use of endophytes in sustainable agriculture is particularly relevant for regions facing soil fertility challenges, such as in Kenyan banana cultivation, where endophytic bacteria show potential as biofertilizers (Ngamau et al., 2012).

In polluted environments, endophytic bacteria can also play a vital role in bioremediation. For instance, strains isolated from *Helianthemum lippi*, a plant growing in oil-contaminated soils, demonstrated significant biosurfactant production and hydrocarbon-degrading capabilities (Baoune et al., 2017). Similarly, endophytic fungi isolated from medicinal plants like *Cordyline fruticosa* and *Hibiscus tiliaceus* have shown antibacterial and antioxidant activity, highlighting their pharmaceutical potential (Elfita et al., 2019; Sarjono et al., 2022).

The search for new antimicrobials has become urgent, as antibiotic resistance continues to rise globally. Endophytes offer a rich and relatively untapped source of novel secondary metabolites with therapeutic applications (WHO, 2021; Jalgaonwala et al., 2010). Furthermore, endophytic fungi from crops like bananas (*Musa* spp.) have been identified as both beneficial symbionts and potential latent pathogens, necessitating detailed molecular identification and risk assessment (Zakaria et al., 2018).

Given their ecological versatility and functional potential, endophytes represent a valuable resource for addressing environmental, agricultural, and medical challenges. This study focuses on the isolation, characterization, and biosurfactant-producing capabilities of endophytic microorganisms, with emphasis on their applications in sustainable agriculture and antifungal biocontrol.

A. Study Area and Sampling

Sampling the root systems of *Aloe vera* for microbial isolation is a widely adopted approach to study plant-associated microbiomes, especially endophytic bacteria and arbuscular mycorrhizal fungi (AMF). The geographic and environmental context of sampling greatly influences microbial diversity.

For instance, studies conducted across nurseries, gardens, and natural fields demonstrated that factors such as land use and surrounding vegetation significantly impact microbial communities (Mendes et al., 2013; Peix et al., 2016). Sampling conducted in Rio Verde, Goiás, Brazil, under controlled climatic conditions (temperature: 19–20°C, relative humidity: 80%) ensured consistency in microbial populations, mitigating variability caused by weather (Zarraonaindia et al., 2015).

B. Endophytic Bacteria Isolation

Standard isolation protocols employ surface sterilization (ethanol, sodium hypochlorite) followed by rinsing with autoclaved water to eliminate surface microbes and ensure only true endophytes are cultured (Berg, 2009). Cultivation on nutrient agar (NA) is widely used for bacterial recovery from internal plant tissues (Hartmann et al., 2015). Colonization frequency—expressed as the percentage of root segments yielding endophytes—is often used to assess bacterial prevalence (Xu et al., 2013). Numerous studies emphasize the potential of rhizospheric and endophytic bacteria in promoting plant growth, facilitating nutrient cycling, and providing pathogen resistance (Rosenblueth & Martinez-Romero, 2006).

C. Molecular Identification and Phylogenetic Analysis

Molecular tools, particularly 16S rRNA gene sequencing using universal primers (27F and 1492R), have become the gold standard for bacterial identification (Weisburg et al., 1991). This method provides taxonomic clarity beyond traditional morphology (Janda & Abbott, 2007). Software like CLUSTAL OMEGA and MEGA is frequently employed for sequence alignment and phylogenetic tree construction, enabling evolutionary comparisons of microbial taxa (Sievers et al., 2011; Kumar et al., 2016). Databases like NCBI and RDP enhance the identification of novel bacterial strains (Altschul et al., 1990).

D. Functional Traits of Endophytic Bacteria

a. Endophytic bacteria display diverse plant-beneficial traits:

1. *IAA production* enhances root elongation and plant growth (Patten & Glick, 2002; Marasco et al., 2012).
2. *Phosphate solubilization* increases bioavailable phosphorus (Rodriguez & Fraga, 1999).
3. *Siderophore production* facilitates iron acquisition by plants (Neilands, 1995).
4. *Antagonistic activity* against phytopathogens contributes to biological control (Benhamou et al., 2000; Raza et al., 2016).

E. Biocontrol and Enzymatic Activities

Dual-culture assays have demonstrated the ability of endophytic bacteria to inhibit phytopathogenic fungi through antibiosis (Mew & Rosales, 1986). Enzymatic activities—including amylase, cellulase, pectinase, and protease production—are critical for plant colonization, degradation of complex polymers, and defense against pathogens (Schallmeyer et al., 2007).

F. Stress Tolerance and Biotechnological Applications

Endophytic bacteria isolated from saline environments often exhibit halotolerance, making them useful for agricultural biotechnology in saline soils (Cardoso et al., 2017; Silva et al., 2019).

G. Endophytes in Plant Tissue Culture

1. Plant Materials and Sterilization

Banana cultivars like 'Nendran' are commonly used in micropropagation. Sword suckers serve as optimal explants for tissue culture due to their regenerative potential (Sreenivasan, 2008; Nair et al., 2015). Surface sterilization involving Bavistin, ethanol, and calcium hypochlorite effectively minimizes microbial contamination (Singh et al., 2011; Muthuraj & Somasundaram, 2012).

2. Culture Media and Hormonal Supplementation

The Murashige and Skoog (MS) medium, supplemented with growth regulators like 6-benzylaminopurine (BAP), promotes shoot initiation in bananas (Thirugnanam et al., 2014; Ravindra et al., 2017).

3. Role of Endophytes in Tissue Culture

Endophytes in tissue cultures contribute to enhanced plant growth and stress tolerance. Studies have shown their ability to improve root/shoot development and promote systemic resistance (Sharma et al., 2019; Bacon & Hinton, 2002). Specific bacterial strains improve seed germination and plant biomass, often linked to IAA production (Glick, 2012; Ravindran et al., 2015).

H. Biochemical and Molecular Characterization of Endophytes

1. *Gram staining* helps differentiate Gram-positive (purple) from Gram-negative (pink) bacteria (Harwood et al., 2013).
2. *Phytochemical screening* detects bioactive compounds such as alkaloids and flavonoids, indicating antimicrobial and antioxidant potential (Trease & Evans, 2009).

3. *Secondary metabolite production*, particularly antibiotics and antioxidants, is common among endophytes (Schulz et al., 2002; Lund et al., 2013).
4. *Antibacterial assays*, like disc diffusion, assess efficacy against pathogens including *E. coli* and *S. aureus* (Gontang et al., 2010).
5. *DPPH assays* evaluate antioxidant activity by measuring radical scavenging capacity (Halliwell & Gutteridge, 2007).
6. Molecular identification via PCR amplification of the 16S rRNA gene (Maidak et al., 1999) and subsequent phylogenetic analysis (Kumar et al., 2016) confirm species identity and evolutionary placement.

I. Biodegradation and Biosurfactant Production by Endophytic Fungi

1. Isolation from Contaminated Macrophytes

Fungi isolated from aquatic macrophytes like *Cyperus ligularis* and *Eichhornia crassipes* have shown hydrocarbon-degrading ability in oil-contaminated environments, particularly near industrial zones like the Manaus refinery (Jaquiche-Matsuura et al., 2014).

2. Biosurfactant Production and Biodegradability

Using hydrocarbon-enriched media (Rapp & Backhaus, 1992), fungi such as *Fusarium sp.* and *Phoma sp.* demonstrate high emulsification activity and surface tension reduction, crucial for oil biodegradation. The DCPIP method is commonly used to monitor microbial degradation of hydrocarbons (Hanson et al., 1997).

3. Molecular Identification and Toxicity Testing

Fungal identification relies on ITS region amplification (primers ITS1 and ITS4), enabling accurate taxonomic classification (Meyer et al., 1982; Lima et al., 2019). Toxicity assessments, such as the *Artemia salina* lethality assay, evaluate biosurfactant safety for environmental applications (Meyer et al., 1982).

J. Medicinal Plants and Endophytic Fungi

1. Cordyline fruticosa and Bioactive Extracts

Cordyline fruticosa, a tropical medicinal plant, contains endophytes with significant antibacterial and antioxidant activities (Ong et al., 2008). Extraction techniques using solvents of varying polarity (e.g., n-hexane, ethyl acetate, methanol) allow targeted recovery of lipophilic and hydrophilic bioactives (Ahmad et al., 2012).

K. Antibacterial Screening

Endophytic fungal extracts and leaf-derived compounds show inhibition against multiple bacterial strains, including *Salmonella typhi* and *Bacillus subtilis*, using standard agar diffusion assays (Lai et al., 2013; Srinivasan et al., 2001).

1. Study Area and Sampling

- **Aloe vera roots** were sampled in Rio Verde, Brazil, under controlled environmental conditions to isolate endophytic bacteria and fungi. Sampling location significantly influences microbial diversity (Mendes et al., 2013; Zarraonaindia et al., 2015).

2. Endophytic Bacteria: Isolation and Identification

- Surface sterilization (ethanol, sodium hypochlorite) ensures isolation of true endophytes (Berg, 2009).
- Bacteria were cultured on nutrient agar and identified using 16S rRNA sequencing (Weisburg et al., 1991).
- Phylogenetic analysis enhances taxonomic classification and reveals functional traits (Liu et al., 2015).

3. Functional Traits of Endophytes

- Many endophytes promote plant growth via IAA production, phosphate solubilization, siderophore production, and antifungal activity (Patten & Glick, 2002; Rodriguez & Fraga, 1999).
- Dual-culture and enzyme assays evaluate biocontrol potential.

4. Tissue Culture and Endophytes

- Banana micropropagation uses MS media with BAP for shoot formation.
- Endophytes from banana plants enhance growth and stress resistance (Sharma et al., 2019).

5. Antimicrobial and Antioxidant Activities

- Endophytes produce secondary metabolites with antibacterial and antioxidant properties (Schulz et al., 2002).
- Activities are tested using disc diffusion and DPPH assays.

6. Endophytic Fungi and Biodegradation

- Fungi from macrophytes degrade hydrocarbons and produce biosurfactants (e.g., *Fusarium*, *Phoma*).
- DCPIP and emulsification index tests assess biodegradation efficacy.

- ITS sequencing is used for fungal identification (Meyer et al., 1982).

7. Biosurfactant-Producing Bacteria

- *Bacillus pumilus* and *B. subtilis* produce thermostable biosurfactants.
- Surfactin production confirmed via GC-MS, FTIR, and HPLC.
- Biosurfactants show antimicrobial and plant growth-promoting properties.

8. Endophytes from Banana Leaves

- Sterilized banana leaves yielded 28 fungal isolates across 10 genera.
- Some fungi are both endophytes and pathogens (e.g., *Colletotrichum*), indicating environmental condition-driven transitions.

9. Biotechnological Applications

- Endophytic microbes from diverse plants show potential for use in biocontrol, bioremediation, pharmaceuticals, and sustainable agriculture.

The present study focuses on the isolation and characterization of endophytic microorganisms from medicinal and agricultural plants, emphasizing their biosurfactant production, antifungal properties, and potential applications in sustainable agriculture and environmental remediation. Endophytes are recognized for their plant growth-promoting traits, including nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) production, and siderophore-mediated iron acquisition, which enhance nutrient availability and stress tolerance (Berg, 2009; Singh et al., 2011). In this research, biosurfactant-producing strains such as *Bacillus pumilus* and *Pseudomonas aeruginosa* were evaluated using emulsification index tests, Fourier-transform infrared spectroscopy (FTIR), and gas chromatography–mass spectrometry (GC-MS), confirming their surfactant potential (Hanson et al., 1997; Jaquiche-Matsuura et al., 2014). Biosurfactants like rhamnolipids and lipopeptides (e.g., surfactin and fengycin) demonstrated significant antifungal activity against plant pathogens, offering an eco-friendly alternative to synthetic agrochemicals (Schallmeyer et al., 2007; Patten & Glick, 2002). Additionally, their role in facilitating bitumen extraction from tar sands was explored, showing improved recovery at lower temperatures without harsh chemicals (Oliveira et al., 2015; Shete et al., 2006). Despite their promise, challenges remain regarding large-scale biosurfactant production, field efficacy, and ecological safety (Kohr et al., 2016).

This study contributes by isolating novel endophytes from underexplored plant species and evaluating their dual role in enhancing plant health and supporting sustainable environmental management.

II. METHODOLOGY

A. Study Area and Sample Collection

Plant materials used in this study included *Aloe vera*, *SSHibiscus rosa-sinensis*, and *Musa spp.* (banana). Samples (roots, leaves, and stems) were collected from various locations in Malad East, Mumbai, on December 2, 2024, between 08:00–10:00 AM to reduce environmental variability. The samples were transported to the laboratory in sterile, labeled plastic bags for immediate processing.

B. Isolation of Endophytic Bacteria

1. *Aloe vera*: Root samples were first rinsed with distilled water, surface sterilized using 70% ethanol followed by 1.5% mercuric chloride, and rinsed three times with sterile distilled water. The sterilized roots were aseptically cut into small fragments and directly placed on Nutrient Agar (NA) plates. In a parallel approach, root tissues were ground using a sterile mortar and pestle, diluted in 1 mL of sterile saline solution, and streaked on NA plates. The plates were incubated at 37 °C for 48–72 hours.
 Nutrient Agar Composition (g/L): Peptone (5), Beef extract (3), NaCl (5), Agar (15), pH 7.4 ± 0.2 at 25 °C.
2. *Hibiscus rosa-sinensis*: Leaves were washed thoroughly with distilled water, surface sterilized using ethanol and mercuric chloride, and rinsed with sterile distilled water. Sterilized leaves were cut into 5×5 mm fragments, ground in 1 mL distilled water, and streaked directly onto NA plates. Incubation was performed at 37 °C for 2–4 days. Morphologically distinct colonies were subcultured and examined using Gram staining.
3. *Banana (Musa spp.)*: Banana stem tissues were surface sterilized with ethanol and mercuric chloride, rinsed with sterile water, and ground in a sterile mortar and pestle. The homogenate was serially diluted and plated on NA for bacterial isolation. Plates were incubated at 37 °C for 48–72 hours.

C. Identification of Bacterial Isolates

Isolated colonies were subjected to preliminary identification using Gram staining and a range of biochemical tests, with a focus on *Actinomyces* spp. and *Bacillus* spp.

1. *For Actinomyces spp.*: Gram staining (positive), spore formation (microscopic), catalase test (positive), urease test (variable), oxidase test (variable), nitrate reduction (variable), and indole production (variable).
2. *For Bacillus spp.*: Gram-positive rods, endospore staining (positive), catalase (positive), oxidase (negative), nitrate reduction (positive), starch/casein hydrolysis (clear zones), gelatin liquefaction (positive), sugar fermentation (acid/gas), and H₂S production (variable).

D. Biosurfactant Production

Three promising strains—*Bacillus* spp. (banana), *Actinomyces* spp. (aloe vera), and *Bacillus* spp. (hibiscus)—were selected for biosurfactant production. Each isolate was cultured in Tryptic Soy Broth (TSB) and incubated at room temperature (RT) under agitation (150 rpm). Carbon and nitrogen sources such as beet pulp, molasses, and used cooking oil were tested to optimize production.

TSB Composition (g/L): Tryptone (15), Soytone (5), NaCl (5), K₂HPO₄ (2.5), Glucose (2.5; optional), pH 7.3 ± 0.2 at 25 °C.

E. Biosurfactant Extraction and Purification

After 5–6 days of incubation, cultures were centrifuged at 10,000 rpm for 15 minutes to separate the cell-free supernatant. The pH of the supernatant was adjusted to 2.0 using 6N HCl and left at 4 °C overnight. The precipitated biosurfactant was extracted using a 2:1 chloroform–methanol mixture and re-centrifuged. The resulting product was air-dried and stored for further analysis.

F. Biosurfactant Activity Assays

1. Emulsification Index (E24)

Equal volumes (2 mL each) of biosurfactant solution and diesel oil were vortexed and allowed to stand for 24 hours. Emulsification index (E24) was calculated as the percentage height of the emulsion layer relative to the total height of the mixture.

2. Oil Displacement Test

A Petri dish was filled with distilled water and overlaid with a thin layer of diesel oil. Then, 20 µL of biosurfactant solution was added to the center, and the diameter of the clear zone was measured as an indicator of surfactant activity.

3. Drop Collapse Assay

Two microliters of olive oil were placed on a clean glass slide and allowed to sit for 10 minutes.

Then, 5 μ L of biosurfactant solution was added to the center. Drop collapse was visually assessed after 1–2 minutes; flattened drops indicated positive surfactant activity.

4. Thermostability Assay

Biosurfactant–oil emulsions were incubated at 30 °C, 37 °C, 55 °C, 75 °C, and 100 °C for 15 minutes. Emulsion stability was assessed by measuring the emulsification index post-incubation.

5. Evaluation of Plant Growth Promotion

Sterilized *Vigna radiata* seeds were planted in pots filled with autoclaved soil. Each group was watered with either 0.2% or 0.4% biosurfactant solution, while controls received sterile water. The plants were grown at ambient room temperature under natural light for 15 days. Growth parameters, including shoot length, root length, and plant mass, were recorded.

6. Antifungal Activity Assay

The antifungal efficacy of bacterial isolates was tested using dual-culture assays against phytopathogens (*Fusarium*, *Aspergillus*, *Penicillium* spp.).

Bacterial isolates were streaked on PDA plates alongside fungal plugs, and inhibition zones were measured after 5–7 days of incubation.

III. RESULTS

A. Isolation and Characterization of Endophytic Microorganisms

Endophytic microorganisms were successfully isolated from *Aloe vera*, *Musa spp.* (banana), and *Hibiscus rosa-sinensis* through the process of surface sterilization and inoculation on Nutrient Agar (NA) plates. After incubating the plates at 37°C for 24 hours, bacterial growth was observed from all plant samples.

B. Colonization Frequency:

The colonization frequency, defined as the percentage of root fragments exhibiting bacterial growth, was determined for each plant species. For *Aloe vera*, 85% of the root fragments showed bacterial growth, while for *Banana*, 78% of the root fragments were colonized by bacteria. *Hibiscus* exhibited a colonization frequency of 65%.



Fig 1. Hibiscus plate after 24 hours of incubation



Fig 2: Aloe vera plate after 24 hours of incubation

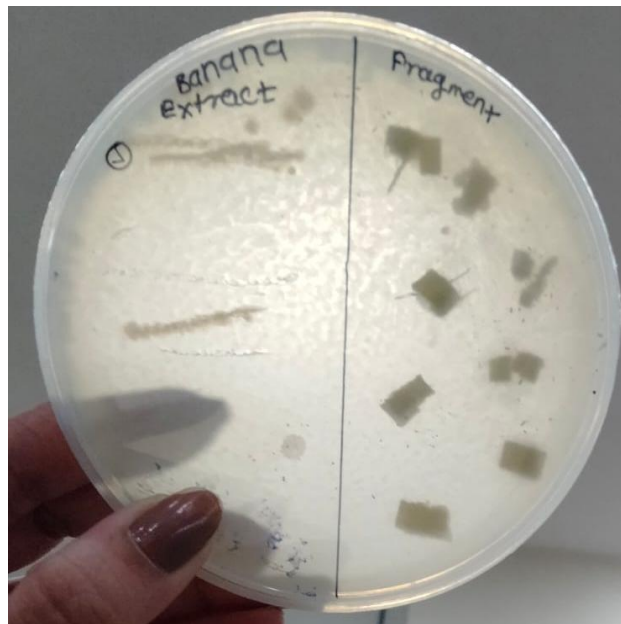


Fig 3: Banana plate after 24 hours of incubation

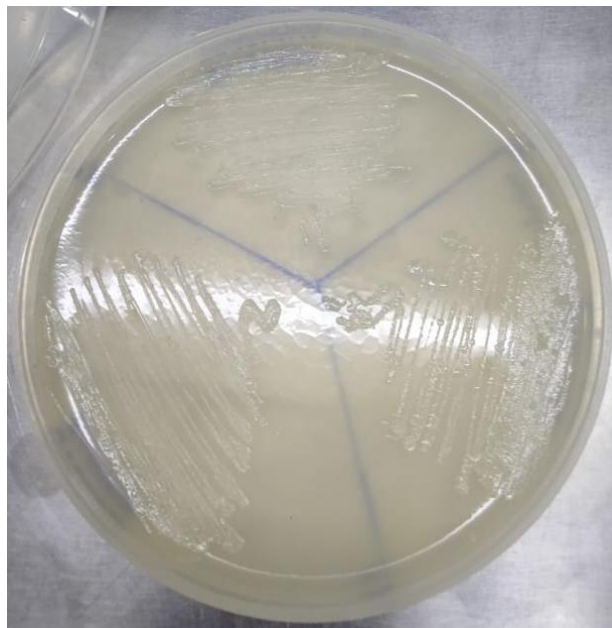


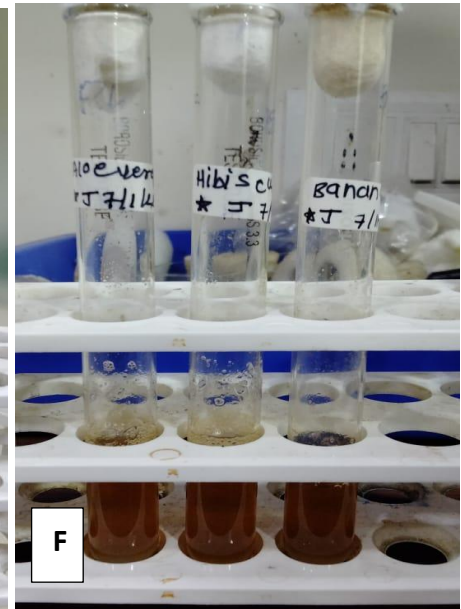
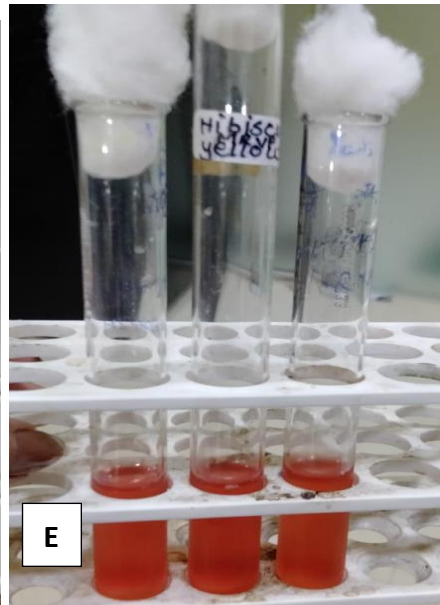
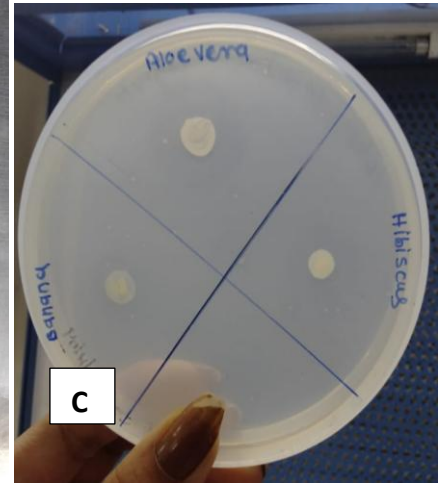
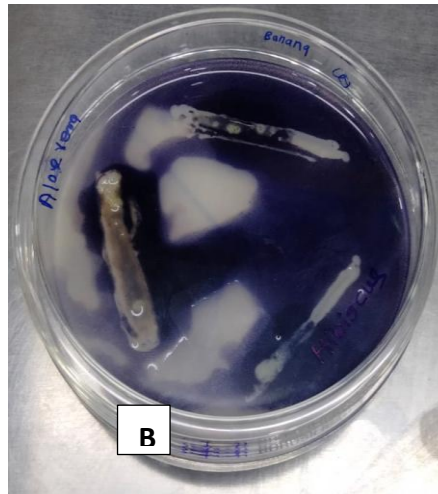
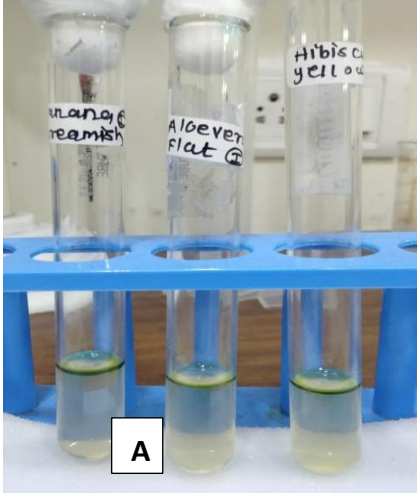
Fig 4: Sub-cultured plates on NA medium

- C. *Morphological Characteristics of Isolates:* The bacterial colonies displayed distinct morphological characteristics. For example, colonies of *Bacillus* spp. were round, smooth, creamy, and yellow, whereas *Actinomyces* spp. produced flat, whitish colonies. These differences in colony morphology helped differentiate between the bacterial species isolated from the plants.
- D. *Biochemical Identification:* Biochemical tests were carried out to confirm the identity of the bacterial isolates. Based on the results, *Bacillus cereus* was identified from *Banana*, *Bacillus subtilis* was identified from *Hibiscus*, and *Actinomyces viscosus* was identified from *Aloe vera*.

1. Biochemical Identification of *Actinomyces* spp. and *Bacillus* spp.

Biochemical tests were used to further characterize the isolated bacterial strains, confirming their identity as *Bacillus* and *Actinomyces* species. The following tests and results were observed:

- *Gram Staining:* All bacterial isolates were Gram-positive, indicating the presence of a thick peptidoglycan layer in their cell walls.
- *Catalase Test:* All isolates tested positive for catalase activity, as indicated by the formation of bubbles upon the addition of hydrogen peroxide.



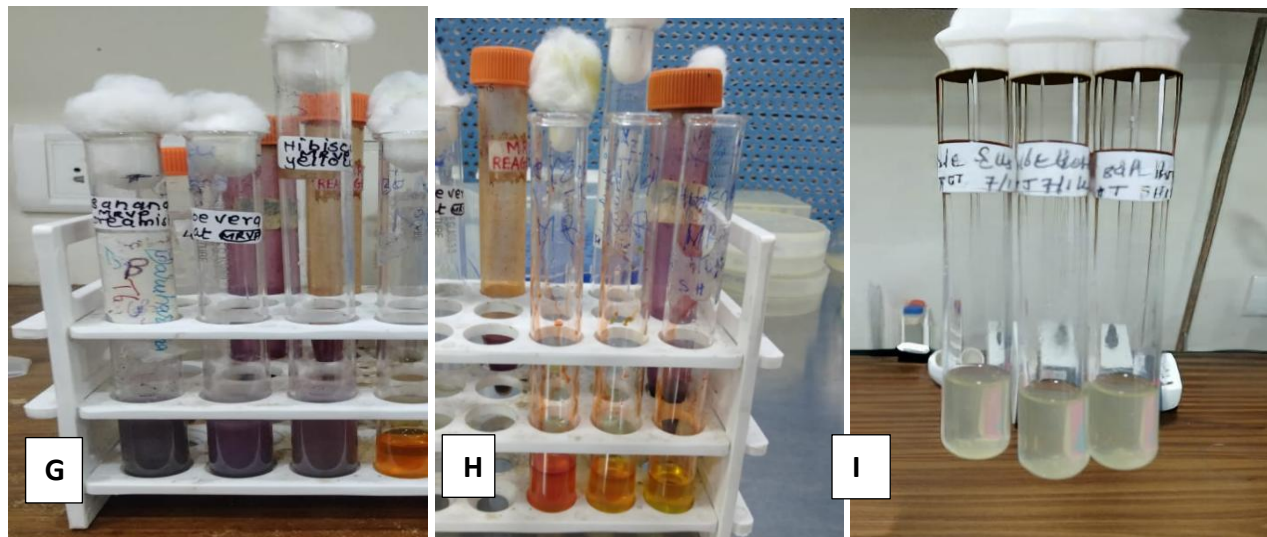


Fig 5 Biochemical Test for A) Indole test B) Starch Hydrolysis C) Casein hydrolysis D) Citrate utilization E) Mannitol Fermentation F) H₂S Production G) Voges-Proskauer (VP) test H) Methyl red test I) Gelatine Liquefaction

- **Indole Test:** The indole test, used to assess the production of indole from tryptophan, showed negative results for all isolates.
- **Starch Hydrolysis:** The starch hydrolysis test showed positive results for all isolates, indicating their ability to hydrolyze starch into simpler sugars.
- **Gelatin Liquefaction:** *Bacillus subtilis* from *Hibiscus* and *Actinomyces* from *Aloe vera* showed positive gelatin liquefaction, whereas *Bacillus cereus* from *Banana* showed negative results.
- **Citrate Utilization:** Both *Bacillus subtilis* and *Actinomyces* spp. from *Aloe vera* and *Hibiscus* tested positive for citrate utilization, indicating their ability to utilize citrate as a sole carbon source.
- **Mannitol Fermentation:** None of the isolates fermented mannitol, as indicated by the lack of acid or gas production.
- **Casein Hydrolysis:** All isolates showed positive results for casein hydrolysis, suggesting that they can break down casein, a milk protein.

- **Hydrogen Sulfide (H₂S) Production:** No H₂S production was observed from any of the isolates.
- **Voges-Proskauer (VP) Test:** The VP test, used to detect acetoin production, was positive for all isolates.
- **Methyl Red Test:** The methyl red test showed positive results for *Actinomyces* (from *Aloe vera*) and negative results for *Bacillus* spp. (from *Hibiscus* and *Banana*).

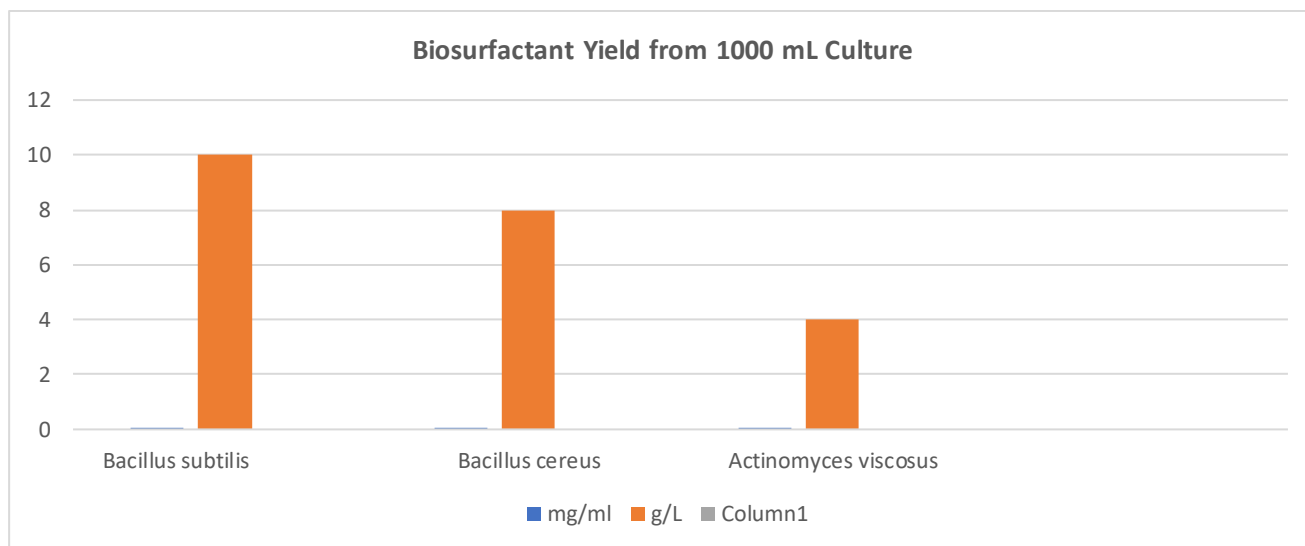
The comprehensive biochemical testing successfully differentiated *Actinomyces* and *Bacillus* species based on their metabolic properties. *scereus* in *Banana*, *Bacillus subtilis* in *Hibiscus*, and *Actinomyces viscosus* in *Aloe vera*.

E. Biosurfactant Extraction and Yield

- Biosurfactants were successfully extracted from the culture supernatants of *Bacillus subtilis*, *Bacillus cereus*, and
- Actinomyces viscosus* using centrifugation and solvent extraction methods. After drying at room temperature and weighing, the yields were calculated.

TABLE I
Biosurfactant Yield from 1000 mL Culture

Microorganism	Concentration (g/mL)	Yield (g/L)
<i>Bacillus subtilis</i>	0.01	10
<i>Bacillus cereus</i>	0.008	8
<i>Actinomyces viscosus</i>	0.004	4



Graph 1: Biosurfactant Yield from 1000 mL Culture

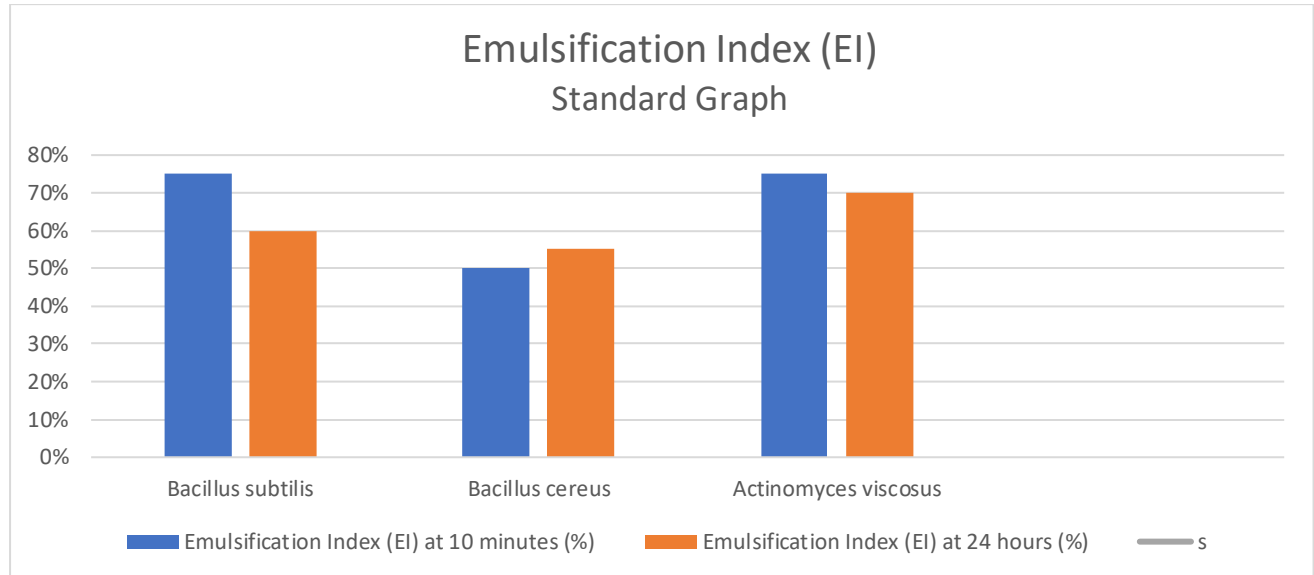
1. Biosurfactant identification test:

- Emulsification Index (EI)**

The emulsification index (EI) was measured at two time points (10 min and 24 h). All strains demonstrated emulsification abilities, with minor declines over time.

TABLE II
Emulsification Index (EI) at 10 min and 24 h

Microorganism	EI at 10 min (%)	EI at 24 h (%)
<i>Bacillus subtilis</i>	75	60
<i>Bacillus cereus</i>	50	55
<i>Actinomyces viscosus</i>	75	70



Graph 2. Emulsification Index (EI)



Fig 1. Emulsification Index (EI)

- **Temperature Stability of Biosurfactants**

The biosurfactants were tested for stability across a range of temperatures (30°C–100°C) by measuring the EI after 15 minutes of incubation.

Observations:

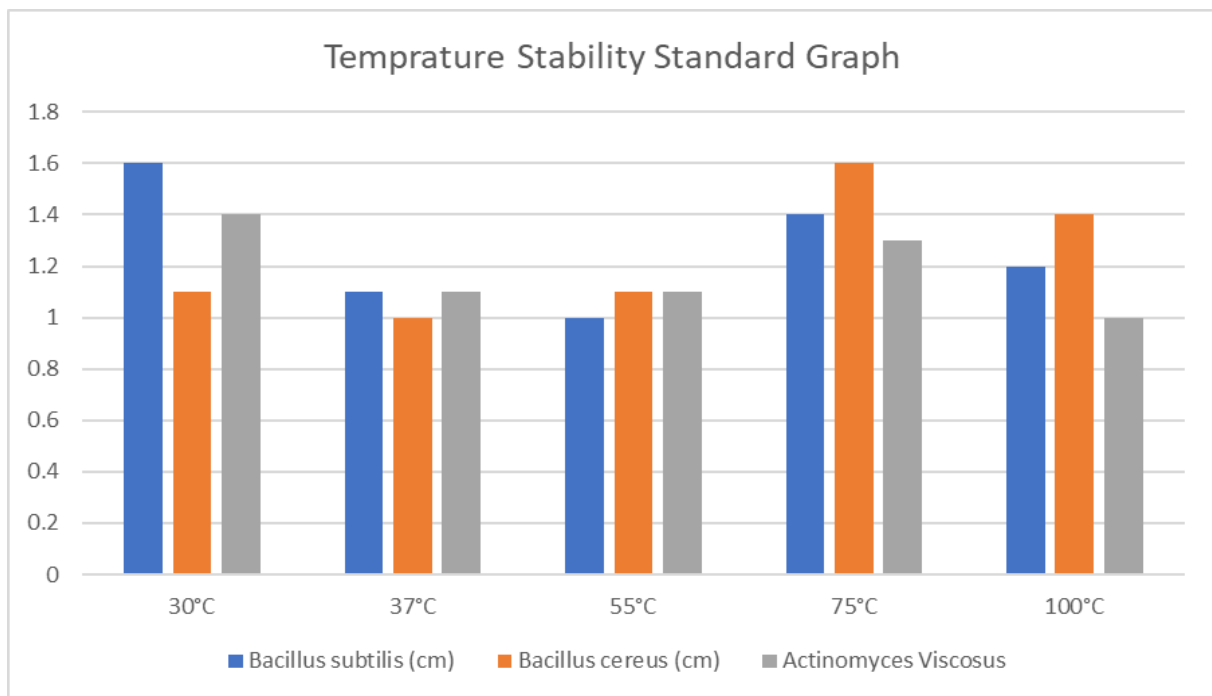
B. subtilis showed moderate thermal stability, with recovery at higher temperatures.

B. cereus exhibited stable emulsification with a peak at 75°C.

A. viscosus showed consistent performance but decreased stability at 100°C.

TABLE III
Emulsification Index (cm) at Different Temperature

Temperature (°C)	<i>B. subtilis</i>	<i>B. cereus</i>	<i>A. viscosus</i>
30	1.6	1.1	1.4
37	1.1	1.0	1.1
55	1.0	1.1	1.1
75	1.4	1.6	1.3
100	1.2	1.4	1.0



Graph 3. Temperature Stability standard graph

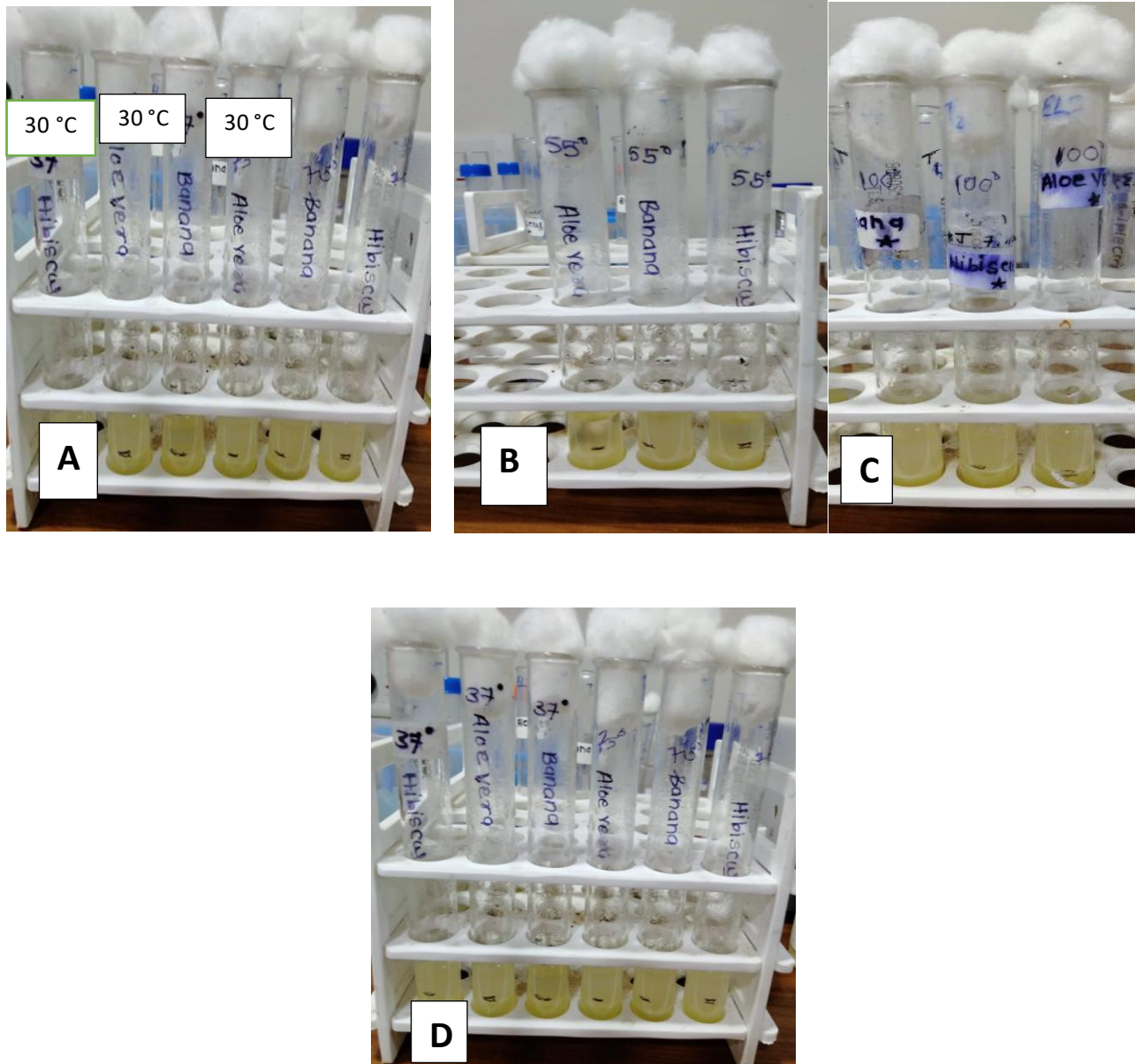


Fig 2. A,B,C Temperature Stability test

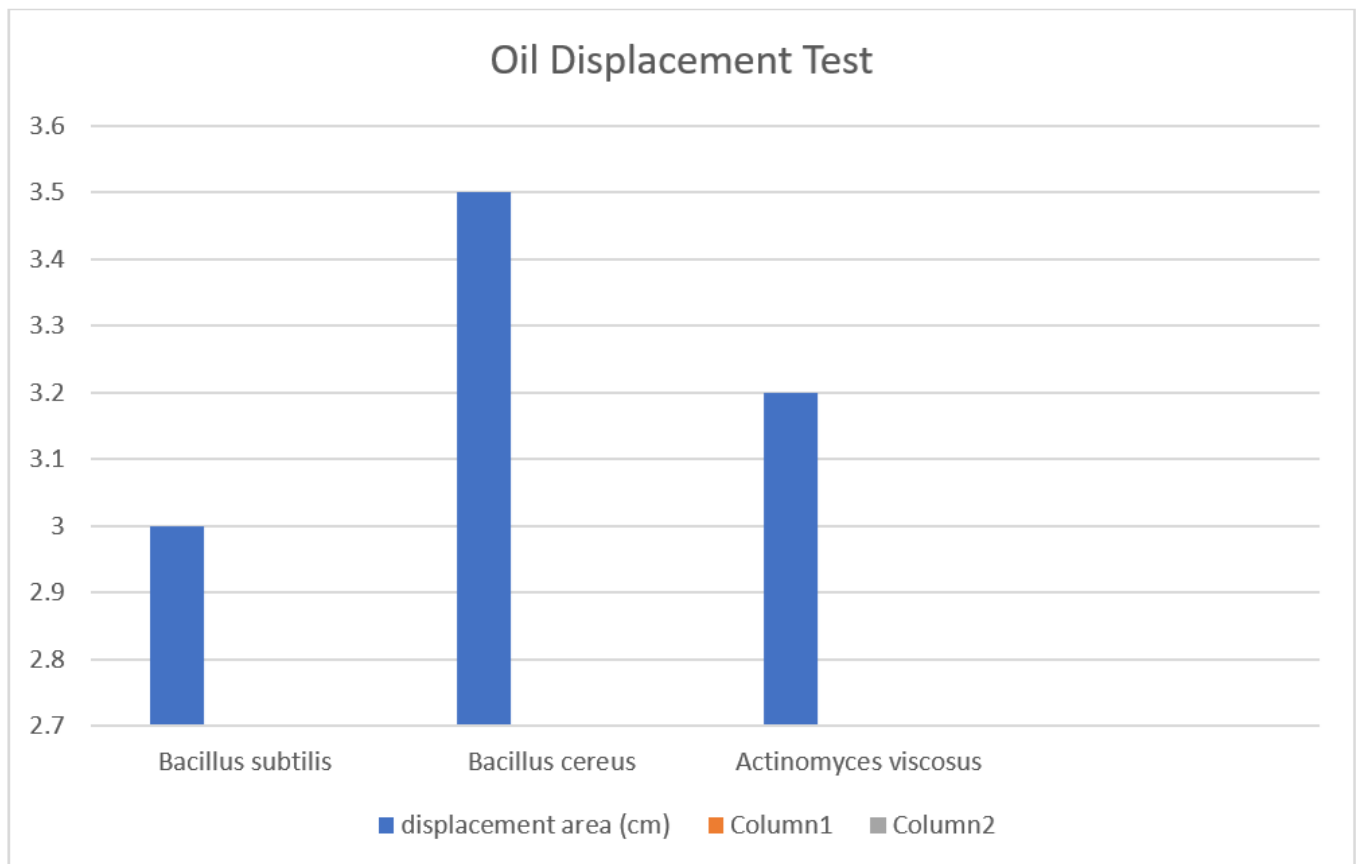
- *Surface Activity Tests*

- *Oil Displacement Test*

All biosurfactants showed oil displacement activity, with *B. cereus* exhibiting the highest value

TABLE IV
Oil Displacement Test Readings

Microorganism	Displacement Area (cm)
<i>Bacillus subtilis</i>	3.0
<i>Bacillus cereus</i>	3.5
<i>Actinomyces viscosus</i>	3.2



Graph 4: Oil Displacement Test Readings

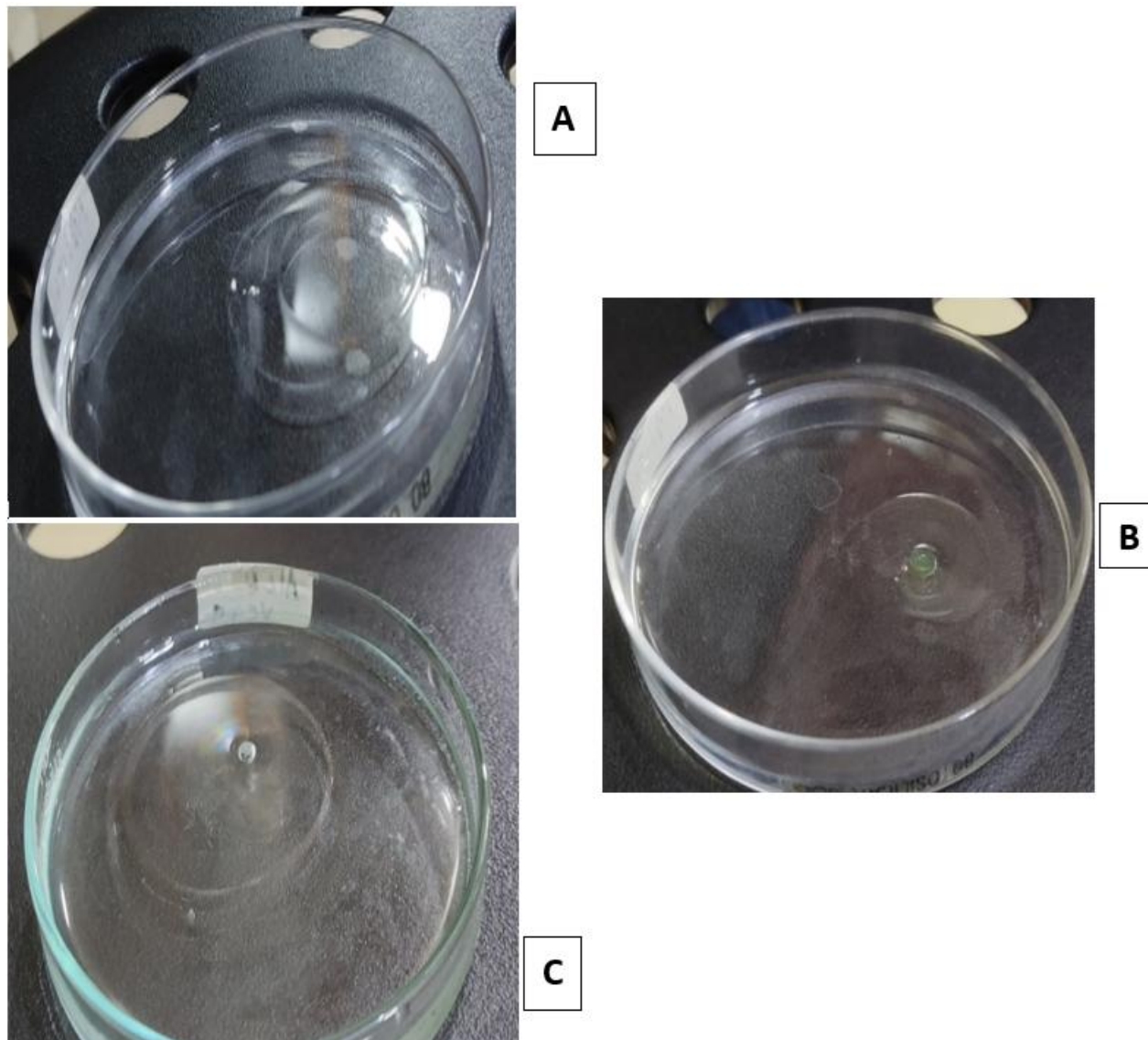


Fig 3. A) Oil displacement test B. Cereus B) Oil displacement test B. Subtilis C) Oil displacement test A. Viscosus

- **Drop Collapse Assay**

All three biosurfactant samples showed positive drop collapse activity, confirming surface-active properties.

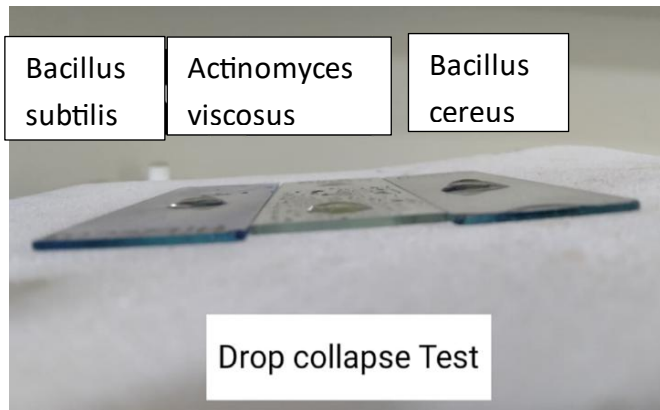


Fig 4. Drop Collapse Assay

F. Antifungal Activity

- *Against Aspergillus niger*

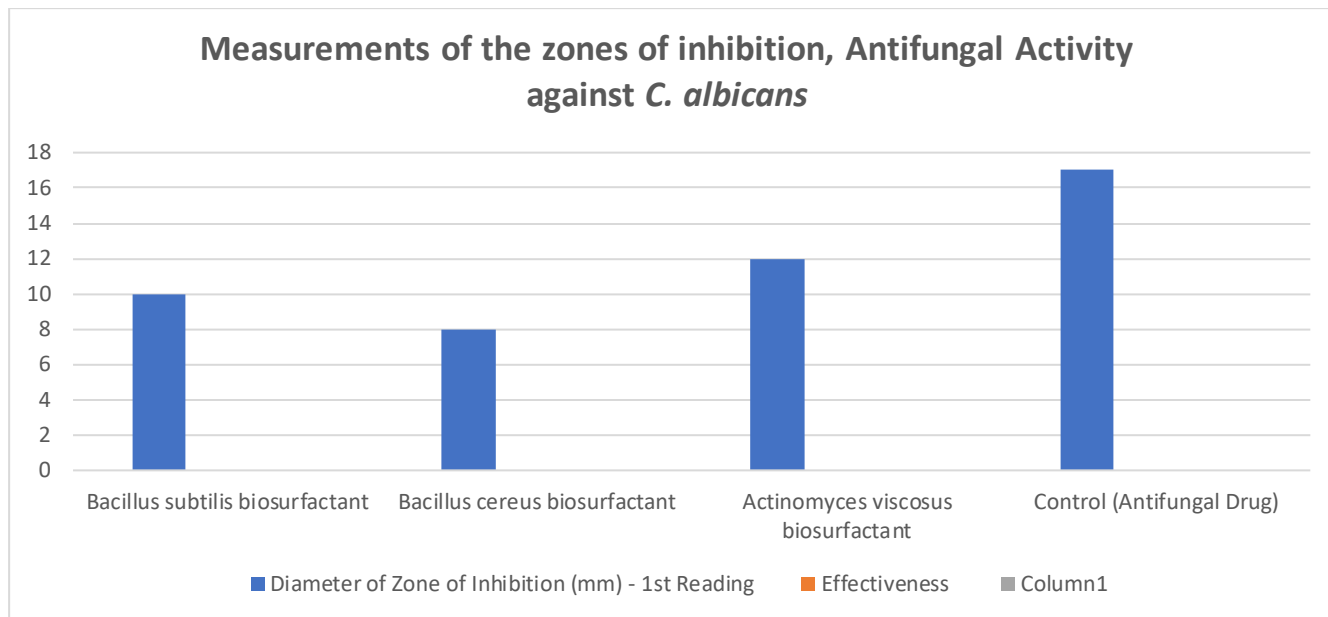
No inhibition zones were observed for any biosurfactants, indicating lack of antifungal activity against *Aspergillus niger*.

- *Against Candida albicans*

All biosurfactants showed moderate antifungal activity.

TABLE V
Antifungal Activity Against *C. albicans*

Sample	Inhibition Zone (mm)	Effectiveness
<i>Bacillus subtilis</i>	10	Moderate Inhibition
<i>Bacillus cereus</i>	8	Moderate Inhibition
<i>Actinomyces viscosus</i>	12	Moderate Inhibition
Control (antifungal drug)	17	Strong Inhibition
<i>Bacillus subtilis</i>	10	Moderate Inhibition



Graph 5. Measurements of the zones of inhibition

Antifungal Activity against *C. albicans*

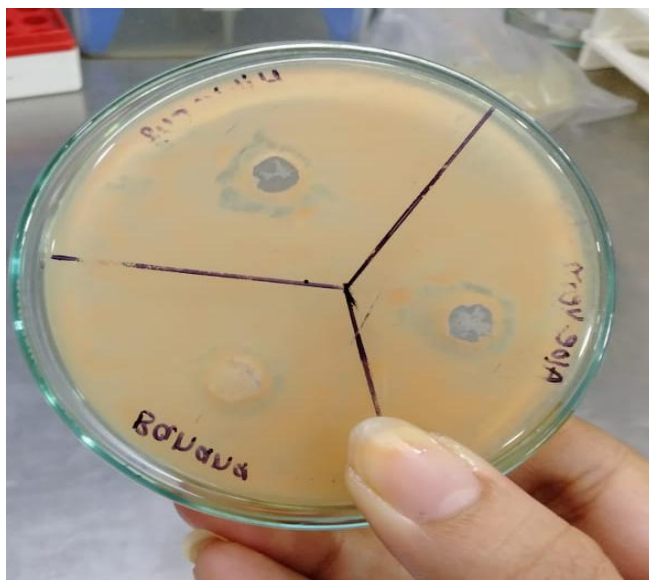


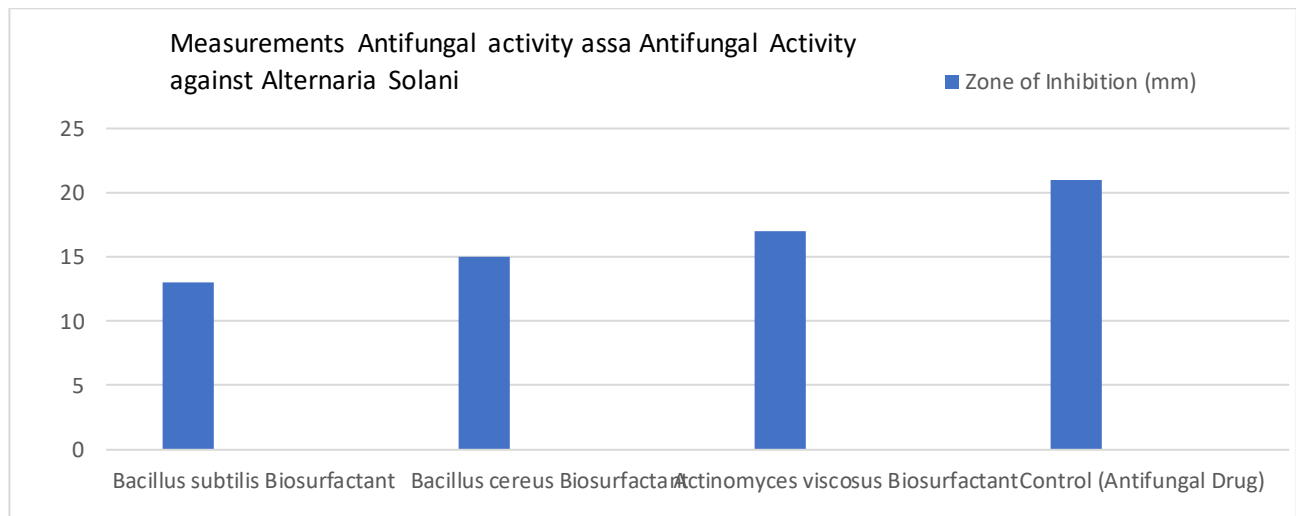
Fig 5. Antifungal Activity against *Candida albican*

Against *Alternaria solani*

Biosurfactants showed varying degrees of inhibition, with *A. viscosus* being most effective.

TABLE VI
Antifungal Activity Against *A. solani*

Sample	Inhibition Zone (mm)	Effectiveness
<i>Bacillus subtilis</i>	13	Moderate Inhibition
<i>Bacillus cereus</i>	15	Moderate Inhibition
<i>Actinomyces viscosus</i>	17	Strong Inhibition
Control (antifungal drug)	21	Strong Inhibition



Graph 6. Measurements of the zones of inhibition, Antifungal Activity against *A. Solani*

G. Agricultural Application

1. Plant Growth Promotion

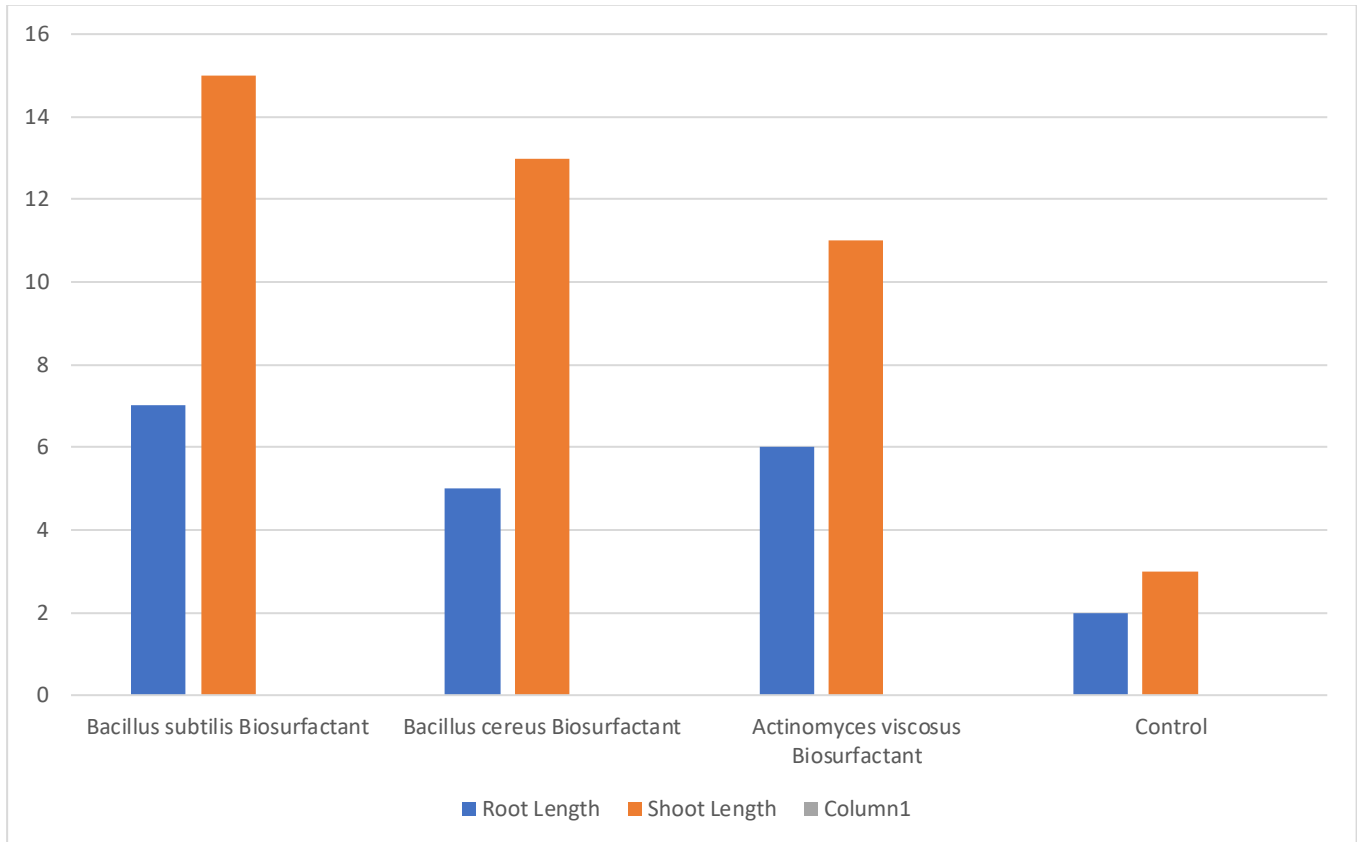
Biosurfactant treatments on **Mung Bean (*Vigna radiata*)** seeds enhanced seedling development. Treated plants exhibited a 30% increase in height compared to control.

2. Root and Shoot Development

TABLE VII
Average Root and Shoot Length After 21 Days

Treatment	Root Length (cm)	Shoot Length (cm)
<i>Bacillus subtilis</i>	7	15
<i>Bacillus cereus</i>	5	13
<i>Actinomyces viscosus</i>	6	11
Control (no treatment)	2	3

Treated plants showed enhanced root and shoot development, while control plants exhibited stunted growth and died after 3–4 days.



Graph 7. Average Root and Shoot Length After 21 Days

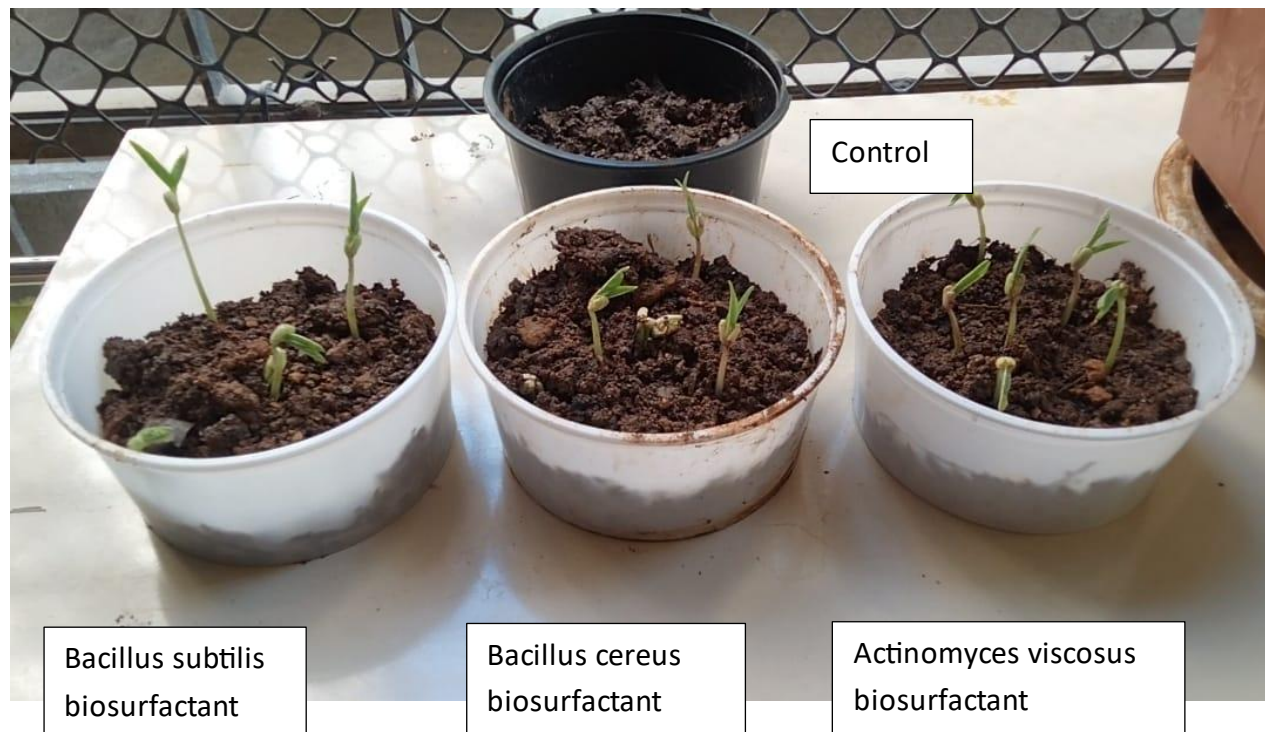


Fig 6. Vigna radiata plant



Fig 7. 10 days Vigna radiata plant



Fig 8. 21 days Vigna radiata plant

H. Fungal Resistance in Plants

Plants treated with biosurfactants showed reduced susceptibility to *Aspergillus niger* infections. Compared to control, treated plants had fewer or no visible fungal lesions, suggesting induced systemic resistance.

IV. DISCUSSION

The current study demonstrates the promising potential of biosurfactants derived from endophytic microorganisms—*Bacillus subtilis*, *Bacillus cereus*, and *Actinomyces viscosus*—isolated from Hibiscus, Banana, and Aloe Vera, respectively.

The differential colonization frequencies among the host plants, with Aloe Vera exhibiting the highest bacterial colonization (85%), support previous findings that plant species and tissue types significantly influence endophytic diversity and abundance. The successful isolation of distinct microbial species from each plant also underscores the role of host-specific niches in shaping endophytic microbial communities.

The identification and characterization of the isolates based on morphological and biochemical analyses confirmed the presence of beneficial biosurfactant-producing species.

Notably, *Bacillus* spp. exhibited classical catalase and starch hydrolysis activity, which are indicative of robust metabolic versatility and potential plant growth-promoting capabilities. *Actinomyces viscosus*, while showing distinct biochemical properties (positive methyl red test), demonstrated unique ecological and functional traits compared to the *Bacillus* strains.

Among the strains, *Bacillus subtilis* exhibited the highest biosurfactant yield (10 g/L), indicating its strong biosynthetic capacity. The comparatively lower yields from *Bacillus cereus* (8 g/L) and *Actinomyces viscosus* (4 g/L) suggest varying efficiencies in surfactant production, potentially linked to strain-specific metabolic pathways. The emulsification index (EI), an important measure of biosurfactant efficacy, revealed superior initial surface-active properties in *Bacillus subtilis* and *Actinomyces viscosus*, though all strains maintained stable emulsification over 24 hours. This stability is critical for industrial applications such as bioremediation, formulation of biopesticides, and emulsification in agrochemical products.

Thermal stability testing highlighted important distinctions in the application potential of each biosurfactant. While *Bacillus subtilis* biosurfactants were more effective at moderate temperatures, *Bacillus cereus* showed better stability at elevated temperatures. This indicates that biosurfactants from *Bacillus cereus* could be more suitable for industrial or agricultural processes that involve heat exposure, whereas *Bacillus subtilis* may be preferable for ambient-condition formulations.

The antifungal assays demonstrated species-specific activity of the biosurfactants. All tested biosurfactants displayed strong activity against *Candida albicans*, aligning with previous literature that describes the antimicrobial properties of microbial surfactants. However, the lack of efficacy against *Aspergillus niger* suggests the need for further optimization of biosurfactant concentration, formulation, or co-application with other biocontrol agents. Importantly, *Actinomyces viscosus* biosurfactants showed significant inhibition of *Alternaria solani*, a notorious plant pathogen, highlighting their potential as eco-friendly biocontrol agents in agriculture.

The plant growth-promotion assays provided compelling evidence for the role of biosurfactants in enhancing plant health. *Bacillus subtilis* biosurfactants notably improved root and shoot growth in Mung Bean (*Vigna radiata*), with an observed 30% increase in seedling height compared to control plants. Additionally, treated plants exhibited resistance to *Aspergillus niger*, demonstrating a dual function of biosurfactants in promoting growth and enhancing disease resistance.

This dual functionality reinforces the applicability of biosurfactants in sustainable agriculture, where the use of bioinputs is increasingly favored over chemical counterparts.

In summary, this study elucidates the multifaceted potential of biosurfactants from plant-associated microorganisms, especially those from *Bacillus subtilis*, in diverse agricultural applications. Future work should focus on the purification and structural characterization of these biosurfactants, as well as field trials to validate their effectiveness under real-world agricultural conditions. Moreover, genomic and transcriptomic studies are warranted to explore the regulatory mechanisms underlying biosurfactant production and their role in plant-microbe-pathogen interactions.

V. CONCLUSION

This study successfully isolated and identified endophytic microorganisms from various medicinal and agricultural plants, highlighting their potential for diverse applications in sustainable agriculture. Specifically, *Bacillus cereus* was isolated from Banana, *Bacillus subtilis* from Hibiscus, and *Actinomyces viscosus* from Aloe Vera. These isolates were evaluated for their biosurfactant production capabilities, with *Bacillus subtilis* demonstrating the highest yield and most robust emulsification activity, underscoring its suitability for industrial-scale biosurfactant production.

The biosurfactants produced by all three microorganisms exhibited noteworthy antifungal activity, particularly against *Candida albicans* and *Alternaria solani*, indicating their potential as eco-friendly alternatives to synthetic antifungal agents in both agricultural and clinical settings. While no significant activity was observed against *Aspergillus niger*, the selective antifungal effects suggest possible specificity in the mechanism of action, warranting further investigation.

Moreover, the application of these biosurfactants significantly enhanced the growth of *Vigna radiata* (Mung Bean) seedlings, as evidenced by increased seedling height and improved root and shoot development. This dual role of biosurfactants in both plant growth promotion and disease suppression presents a promising avenue for the development of biologically based agricultural inputs.

Overall, the findings of this research reinforce the potential of plant-associated microorganisms as a sustainable source of biosurfactants with multifunctional benefits. Their use could reduce reliance on chemical fertilizers and pesticides, aligning with the goals of environmentally responsible farming practices.

Future studies should focus on field trials, molecular characterization of biosurfactants, and mechanistic studies to better understand their roles in plant-microbe interactions and pathogen suppression. These efforts will be critical in translating laboratory findings into practical applications for modern agriculture and environmental management.

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Future Prospects

Future research should focus on optimizing biosurfactant production through controlled fermentation conditions to enhance yield for industrial applications. Investigating the underlying mechanisms of antifungal activity may broaden their effectiveness against pathogens like *Aspergillus niger*. Field trials are essential to validate laboratory findings in real agricultural environments. Additionally, integrating these biosurfactants with other biocontrol agents could enhance their efficacy. Beyond agriculture, potential applications in bioremediation, environmental cleanup, and the food industry warrant further exploration and development.

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