International Journal of Environment, Agriculture and Biotechnology
Vol-10, Issue-4; Jul-Aug, 2025
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Isolation and Molecular Characterization of Biosurfactant Producing Bacteria

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Received: 08 Jun 2025; Received in revised form: 03 Jul 2025; Accepted: 10 Jul 2025; Available online: 17 Jul 2025

***Abstract*— *Biosurfactants are surface-active compounds produced by microorganisms with broad environmental and industrial significance. Due to their amphiphilic structure, biodegradability, and production from renewable sources, biosurfactants are emerging as sustainable alternatives to synthetic surfactants. This review provides a comprehensive overview of the recent advancements in the isolation of biosurfactant-producing bacteria, screening techniques, molecular characterization, and diverse applications in agriculture, bioremediation, and healthcare. Special attention is given to microbial strains isolated from oil-contaminated environments, their plant growth-promoting (PGP) attributes, metal tolerance, and antimicrobial potential. The review also outlines screening protocols and biochemical techniques to identify potent strains, and discusses their role in sustainable agriculture and ecological restoration.*

Keywords*— *Biosurfactants, Bioremediation, Agriculture, Antimicrobial, Sustainability.

I. INTRODUCTION

The increasing demand for eco-friendly and sustainable solutions in agriculture, industry, and environmental management has intensified interest in biosurfactants—surface-active compounds synthesized by microorganisms. Unlike synthetic surfactants, which are often derived from petroleum and associated with environmental toxicity and poor biodegradability, biosurfactants are biodegradable, less toxic, and can be produced from renewable substrates. Biosurfactants are naturally derived surfactants synthesized by bacteria, fungi, and yeasts. Compared to synthetic surfactants, they exhibit superior ecological compatibility, lower toxicity, and effectiveness at extreme pH, temperature, and salinity conditions. Their relevance in soil bioremediation, microbial enhanced oil recovery (MEOR), drug delivery, and as plant growth promoters has stimulated a surge in microbial exploration and characterization.

II. CLASSIFICATION AND PRODUCERS

Microbial biosurfactants are chemically diverse, categorized mainly into glycolipids, lipopeptides, phospholipids, fatty acids, polymeric surfactants, and particulate biosurfactants. Notable producers include *Pseudomonas aeruginosa* (rhamnolipids), *Bacillus subtilis* (surfactin, iturin), and *Candida* spp. (sophorolipids) (Ali *et al.*, 2024; Madaki *et al.,* 2025). These microorganisms are often isolated from hydrocarbon-rich or stressed environments such as oil fields or saline ecosystems.

III. ISOLATION FROM OIL-CONTAMINATED SOILS

Oil-contaminated soils offer a rich microbial diversity adapted to hydrocarbon degradation and biosurfactant production. Studies have reported successful isolation of *Bacillus*, *Pseudomonas*, *Acinetobacter*, and *Marinobacter* spp. from petroleum-contaminated environments (Sharma *et al.,* 2022; Al-Marri *et al.,* 2023). Minimal salt media supplemented with hydrocarbons are commonly used for enrichment and isolation.

IV. SCREENING TECHNIQUES

Biosurfactant-producing bacteria are screened using:

- **Drop collapse and oil displacement tests**: Qualitative assays indicating surface activity.
- **CTAB-methylene blue agar test**: Detects anionic biosurfactants via dark blue halo.
- **Emulsification index (E24%)**: Measures stability of oil-water emulsions.
- **Hemolytic activity**: Indicates surfactant-induced RBC lysis.
- **Surface tension measurement**: Quantifies the surfactant activity using tensiometers (Uyar and Sağlam, 2021; Kadiri *et al.,* 2024).

V. BIOCHEMICAL AND MOLECULAR CHARACTERIZATION

Biochemical profiling helps categorize microbial isolates based on metabolic traits such as catalase, oxidase, urease, citrate utilization, and sugar fermentation abilities (Mian *et al.,* 2021; Verma *et al.,* 2022). Molecular identification via 16S rRNA gene sequencing provides taxonomic resolution and phylogenetic affiliation (Wright *et al.,* 2012; Ijaz *et al.,* 2021). Techniques like ARDRA and rep-PCR further support genetic diversity studies (Achouak *et al.,* 2000).

VI. PLANT GROWTH-PROMOTING ATTRIBUTES

Biosurfactant producers often exhibit PGP traits including:

- **Indole-3-acetic acid (IAA)**: Enhances root elongation and development (Karthika *et al.,* 2020; Pantoja-Guerra *et al.,* 2023).
- **Siderophores**: Chelate iron and promote plant health (Nithyapriya *et al.,* 2021).
- **Hydrogen cyanide (HCN)** and **ammonia**: Inhibit pathogens and improve nitrogen availability (Ambust *et al.,* 2021; Xu *et al.,* 2020).
- **Phosphate, zinc, potassium, and silica solubilization**: Mobilize essential nutrients to enhance plant uptake (Ali *et al.,* 2023; Babu *et al.,* 2024).

VII. EXTRACTION AND STRUCTURAL CHARACTERIZATION

Biosurfactants are extracted using:

- **Acid precipitation** and **solvent extraction** (e.g., methanol, chloroform).
- **Liquid-liquid extraction (LLE)** for higher yield (Vecino et al., 2015). Characterization tools include FTIR, TLC, NMR, and GC–MS to identify rhamnolipids, lipopeptides, and glycolipids (Dalhat *et al.,* 2024; Sridharan *et al.,* 2025).

VIII. ANTIMICROBIAL ACTIVITY

Biosurfactants show strong inhibitory effects against Gram-positive and Gram-negative pathogens. Their mode of action includes membrane destabilization and cytoplasmic leakage (Giri *et al.,* 2023; Haque *et al.,* 2020). Activity against *S. aureus*, *E. coli*, and *S. pneumoniae* has been reported in several studies.

IX. HEAVY METAL TOLERANCE AND BIOREMEDIATION

Some biosurfactant-producing strains also show resistance to toxic metals like Pb, Zn, Cd, and Cr, indicating dual potential for biosurfactant production and heavy metal bioremediation (Govarthanan *et al.,* 2017; Al-Sajad *et al.,* 2024). Mechanisms include metal chelation, efflux, and biosorption.

X. FUTURE PERSPECTIVES

The multifunctionality of biosurfactant-producing bacteria makes them promising candidates for green technologies in agriculture, medicine, and environmental cleanup. However, optimization of production and large-scale industrial deployment require further research.

XI. CONCLUSION

Biosurfactant-producing bacteria represent a valuable resource for sustainable development. Their isolation from extreme environments, broad-spectrum activity, and compatibility with eco-friendly applications render them ideal for integrated environmental and agricultural management.

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