



## Plant Phytoremediation and Biosurfactant-Producing *Pseudomonas Sp. S3* Enhances Growth in Petroleum-Contaminated Soil

Dipali Girase and Mukesh Tiwari

Department of Microbiology

Dr. D. Y. Patil Arts Commerce and Science College, Akurdi, Pune-44

Corresponding Author – Dipali Girase

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### Abstract:

Petroleum hydrocarbon contamination severely affects soil fertility, microbial diversity, and crop productivity due to the toxic and phytotoxic effects of hydrocarbons. The present study evaluates the bioremediation and plant growth-promoting potential of a biosurfactant-producing *r* strain, *Pseudomonas sp. S3*, isolated from petroleum-contaminated soil. The strain exhibited strong biosurfactant activity, including a 42–43% emulsification index (E24), positive oil displacement and drop collapse tests, and significant surface tension reduction from 61.9 mN/m to 34.5 mN/m (Ambust, S., Das, A. J., & Kumar, R. (2021). Strain S3 demonstrated multiple plant growth-promoting (PGP) traits such as phosphate solubilization (94.46 µg/mL), siderophore production (64.41% SU), indole-3-acetic acid and (IAA) production (80.41 µg/mL). Pot experiments conducted in petroleum-contaminated soil (1%, 3%, and 5%) showed that treatments amended with S3 and crude biosurfactant significantly enhanced seed germination, root and shoot length, compared to untreated contaminated controls. Among all treatments, EX5 (1 kg soil + 1 L water + *Pseudomonas sp S3* + 300 mL crude biosurfactant) showed the highest improvement (10–15%) in plant growth parameters. The findings confirm that biosurfactant-producing plant growth-promoting (PGP) such as *Pseudomonas sp. S3* effectively reduce petroleum-induced phytotoxicity, enhance hydrocarbon bioavailability and degradation, and restore soil fertility, providing a sustainable strategy for remediation of petroleum-contaminated agricultural soils.

**Keywords - Biosurfactant; Petroleum hydrocarbon contamination; *Pseudomonas sp. S3*; Phytoremediation; Plant growth-promoting (PGP); Surface tension reduction; Soil restoration**

### Introduction:

Petroleum hydrocarbons are major environmental pollutants that adversely affect soil fertility, microbial diversity, and plant productivity. Hydrocarbon contamination reduces nutrient availability, creates anaerobic conditions, and inhibits plant physiological processes such as photosynthesis and root development.

Plant Growth Promoting biosurfactant producing; *Pseudomonas sp. S3* (PGP) enhances plant growth through mechanisms such as phosphate solubilization, siderophore production, and IAA synthesis. Biosurfactant-producing bacteria

improve hydrocarbon bioavailability by reducing surface and interfacial tension, thereby enhancing biodegradation. This study evaluates the biosurfactant-producing *Pseudomonas sp. S3* for Biosurfactant characterization, PGP trait evaluation, remediation of petroleum-contaminated soil and enhancement of plant growth parameters.

### Materials and Methods:

#### 1. Isolation and Screening:

Petroleum-contaminated rhizospheric soil samples were collected and enriched in mineral

salt medium (MSM) supplemented with 1% petroleum oil. Isolates were screened for biosurfactant production and PGP traits.

## 2 Screening of Plant Growth Parameters (PGP) Activities of S3:

- Phosphate solubilization (Pikovskaya agar)
- Siderophore production (CAS assay)
- IAA production (Salkowski method)

## 3 Biosurfactant Screening- Biosurfactant Characterization:

- Drop collapse test
- Oil displacement test
- Emulsification index (E24)
- Foam test
- Surface tension measurement

## 4 Pot Experiment:

Petroleum-contaminated soils (1%, 3%, 5%) were treated under different experimental sets (EX1–EX5). Plant growth parameters were recorded after 72 days.

## Results:

### 1 Screening of Isolates:

Among screened isolates (S1–S5), strain S3 showed maximum biosurfactant and PGP potential.

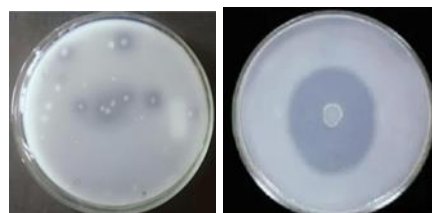
**Figure 1.** Isolated Strain *Pseudomonas* sp. S3 and its Biosurfactant Activity



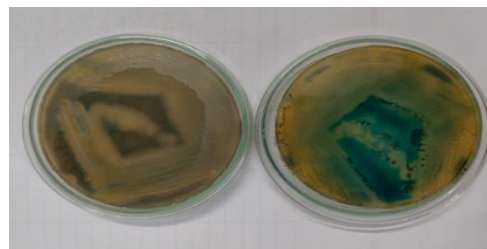
Figure: *Pseudomonas* sp. S3

## 2 Plant Growth Parameters (PGP) Activities of S3:

The quantitative evaluation of plant growth-promoting (PGP) traits revealed that strain *Pseudomonas* sp. S3 possesses strong growth-promoting potential. The strain exhibited significant indole-3-acetic acid (IAA) production, which plays a crucial role in root elongation and overall plant development. It also demonstrated efficient phosphate solubilization capacity, thereby enhancing nutrient availability in the rhizosphere. Additionally, siderophore production by strain S3 suggests its ability to improve iron acquisition and suppress phytopathogens, contributing to improved plant health and vigor. Collectively, these attributes confirm the multifunctional PGP efficiency of strain S3 under stressed soil conditions.



Phosphate Solubilization



Siderophore Production

**Figure 2.** Potentiality of Isolated Strain *Pseudomonas* sp. S3 Plant Growth Parameters (PGP) Activities

Activity	Result
Phosphate solubilization	94.46 µg/mL
Siderophore production	64.41% SU
IAA production	80.41 µg/mL

**Table 1.** Quantitative PGP Traits of S3

Strain S3 exhibited high phosphate solubilization activity (94.46  $\mu\text{g/mL}$ ), indicating its ability to convert insoluble phosphate into bioavailable forms, thereby enhancing phosphorus availability in soil.

The isolate also demonstrated significant siderophore production (64.41% SU). Siderophores chelate iron from the soil environment, making it more accessible to plants while simultaneously limiting iron availability to phytopathogens. This contributes to improved plant nutrition and biocontrol potential and produced a substantial amount of indole-3-acetic acid (IAA) (80.41  $\mu\text{g/mL}$ ), a key phytohormone involved in root elongation and cell differentiation.

The strong expression of phosphate solubilization, siderophore production, and IAA synthesis confirms that strain S3 possesses efficient PGP characteristics, which contribute to enhanced plant growth and improved phytoremediation performance in petroleum-contaminated soils.

In addition to these primary traits, the synergistic interaction among phosphate solubilization, siderophore production, and IAA synthesis likely enhances root architecture and rhizosphere colonization efficiency. Improved root development increases the plant's capacity to access water and nutrients under hydrocarbon

stress conditions. Enhanced iron acquisition and nutrient mobilization further support metabolic activities essential for stress tolerance. These combined mechanisms may also stimulate microbial activity in the rhizosphere, accelerating hydrocarbon degradation. The multifunctional PGP profile of strain S3 therefore strengthens its suitability as a bioinoculant for integrated bioremediation strategies. Overall, the quantitative results presented in Table 1 clearly demonstrate the robust plant growth-promoting potential of strain S3 in contaminated soils.

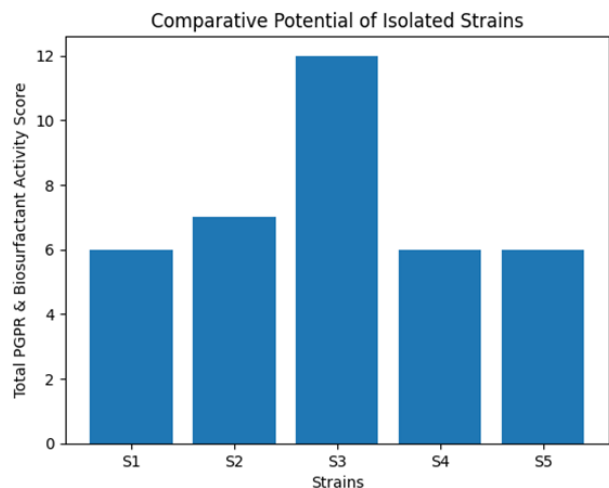
Parameter	Result
Emulsification index (E24)	42%
Surface tension reduction	32.5 mN/m
Oil displacement diameter	2.1 cm
Drop collapse test	-
Foam test	-

**Table 2.** Characterization of Biosurfactant Produced by isolated strain *Pseudomonas* sp S3

Strain	Gram Reaction	Emulsification	Oil Displacement	IAA	Phosphate Solubilization	Siderophore	Oil Degradation
S1	-	+	+	+	+	+	+
S2	-	++	+	+	+	+	+
S3	-	++	++	++	++	++	++
S4	-	+	+	+	+	+	+
S5	-	+	+	++	+	+	+

**Table 3.:** Potentiality of Isolated Strains Based on PGP and Biosurfactant Activity

(++ = strong; + = positive; - = negative).

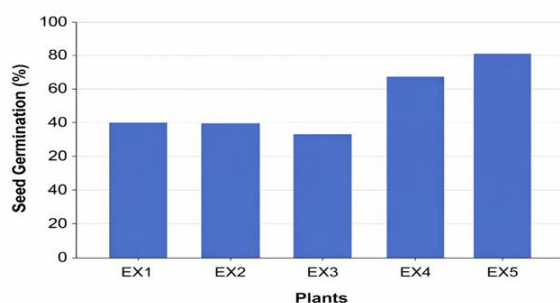
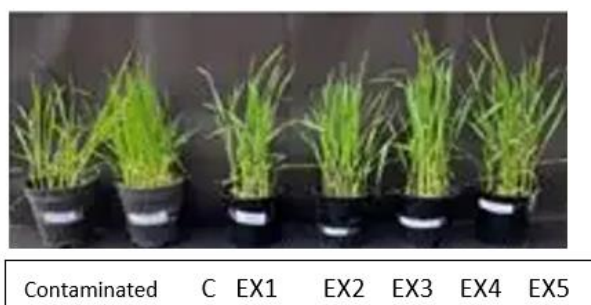


**Figure 3:** A comparative bar graph showing the total PGP and biosurfactant activity score of strains S1–S5 (where – = 0, + = 1, ++ = 2).

#### 4 Effect on Seed Germination and Plant Growth Promotion:

Petroleum contamination reduced germination in untreated soil. Treatments containing S3 and biosurfactant significantly improved germination rates.

Root and shoot lengths were significantly enhanced in treatments EX1–EX5 compared to contaminated controls.



**Figure 5.** Effect of Treatments on Seed Germination (%) and on Root and Shoot Length

#### Discussion:

Petroleum hydrocarbons negatively influence plant growth by altering soil physicochemical properties, reducing nutrient availability, creating oxygen-limited conditions, and inducing oxidative stress. These adverse effects result in poor seed germination, stunted root and shoot development, and reduced biomass accumulation.

In the present study, *Pseudomonas* sp. S3 demonstrated strong biosurfactant production, as evidenced by significant emulsification activity and reduction in surface tension. Biosurfactants increase hydrocarbon bioavailability by reducing surface and interfacial tension, thereby enhancing microbial access to hydrophobic petroleum compounds and accelerating biodegradation. This mechanism plays a crucial role in mitigating petroleum toxicity in soil.

Strain S3 also exhibited multiple plant growth-promoting traits. High phosphate solubilization enhances phosphorus availability in contaminated soils where nutrient mobility is restricted. Siderophore production improves iron acquisition while limiting pathogen growth. IAA production stimulates root elongation and branching, facilitating better nutrient and water uptake. The combined expression of these traits significantly contributed to improved plant growth parameters in contaminated soil.

Pot experiment results demonstrated that petroleum contamination reduced seed germination and plant growth in untreated controls. However, inoculation with strain S3, particularly in combination with crude biosurfactant (EX5), significantly improved germination percentage, root and shoot length, biomass accumulation, and photosynthetic pigment content. The superior performance of EX5 indicates a synergistic interaction between bacterial inoculum and biosurfactant, enhancing

both hydrocarbon degradation and plant growth stimulation.

Overall, the integrated action of biosurfactant production and PGP activities enabled strain S3 to alleviate petroleum-induced phytotoxicity and promote plant development, highlighting its strong phytoremediation potential.

### Conclusion:

The present investigation demonstrates that *Pseudomonas* sp. S3 is an efficient biosurfactant-producing plant growth-promoting capable of reducing petroleum hydrocarbon toxicity in soil, enhancing hydrocarbon bioavailability and biodegradation, nutrient availability through phosphate solubilization and siderophore production and stimulating plant growth via IAA production and other PGP mechanisms. Application of strain S3 in combination with crude biosurfactant significantly improved seed germination, plant biomass, and overall growth performance in petroleum-contaminated soil, with EX5 treatment showing maximum enhancement (10–15%). Thus, *Pseudomonas* sp. S3 represents a sustainable, eco-friendly, and cost-effective approach for bioremediation and restoration of petroleum-contaminated agricultural soils and supporting their safe reuse for crop production. Furthermore, the synergistic interaction between biosurfactant production and plant growth-promoting traits highlights the dual functional potential of strain S3 in integrated remediation strategies. The results suggest that its application can accelerate soil recovery while maintaining soil fertility and microbial balance. Therefore, large-scale field validation of strain S3 is recommended to establish its practical applicability in sustainable environmental management programs.

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