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# ISOLATION OF ENDOPHYTIC BACTERIA TOLERANT TO HYDROCARBON STRESS AND THEIR IMPACT ON MUNGBEAN GROWTH

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**ABSTRACT** Soil contaminated by petroleum hydrocarbons has posed a serious threat to plants, animals and humans because of its carcinogenicity, toxicity and mutagenicity which has become a serious environmental problem. The present study aims to isolate the hydrocarbon stress-tolerant endophytic bacteria and to assess their plant growth-promoting activities. The endophytic bacteria were isolated and identified. The endophytic strains demonstrated a wide range of plant growth-promoting traits. The strains were able to grow well in the mineral salt medium supplemented with different concentration of petrol and diesel. The hydrocarbon-tolerating strains were evaluated for their ability to promote the growth of Vigna radiata plants under increasing hydrocarbon concentrations. After 20 days of growth, significant increase in the root length and shoot length of the plants inoculated with bacterial strains were observed. The result indicates that the endophytic bacteria exert a positive effect on plant growth under stressful cnditions. Hence, these endophytic bacteria can be added as an inoculant in the development of bio-fertilizers for the increase of plant growth in hydrocarbon-polluted soils.

**KEYWORDS :** Endophytic bacteria, hydrocarbon stress, plant growth, bio-fertilizers.

## INTRODUCTION

Petroleum-derived fuels and chemicals are extensively used by modern society. However, the process of extraction, transport, use, and storage of petroleum and its derivatives are prone to leaking or spilling accidents, resulting in widespread environmental contaminations. The use of the usual physical and chemical methods to cope with petroleum hydrocarbon contamination has shown many limitations [1]. Hence, it is necessary to develop advanced and environmentally friendly technologies to treat those toxic hydrocarbons.

One efficient way to remove petroleum hydrocarbon pollutants from soil is by bioremediation [5]. Microorganisms play a critical role in the biological disposal of pollutants from soil. Currently, based on the mutualistic interactions between plants and their endophytes, a combination of plants and their endophytic bacteria has been proposed to improve the efficiency of remediation of soil polluted by organic pollutants [4]. Endophytic bacteria reside in the plant tissues or organs. They improve plant growth through various innate mechanisms and increase the resistance to stressful environmental conditions. The present study investigated the presence of bacterial endophytes in *Aloe vera* L and *Solanum melongena* L and their ability to tolerate and grow under hydrocarbon stress conditions. The growth of *Vigna radiata* was examined under hydrocarbon stress conditions using these endophytic bacteria as an inoculant.

## MATERIALS AND METHODS

### Isolation Of Endophytic Bacteria

### Collection Of Root And Stem

The healthy and disease-free root and stem were collected from plants like Aloe vera and Brinjal plant from local places of Tiruppur.

### Isolation Of Endophytic Bacteria From Root And Stem

The root and stem were surface sterilized with 0.6% NaCl for 3 min and then washed with 70% ethanol for 1 min. After surface sterilization, they were washed with sterile distilled water and then they were air dried. The root and stem were cut into small pieces (5x5mm) and placed on nutrient agar plates and incubated at 37°C for 48 hrs.

## ANALYSIS OF PLANT GROWTH PROMOTING ACTIVITY (PGP) OF BACTERIA

### Indole Acetic Acid Production

The bacterial strains were inoculated in nutrient broth media amended with 1% L-tryptophan and incubated. The culture supernatant was mixed with the Salkowski reagent. The development of pink color indicated the production of Indole acetic acid. [9]

### Siderophore Production (Ferric chloride test)

1 ml of 2% of ferric chloride solution was added to 1 ml of culture supernatant. The development of orange or red-brown color of the supernatant indicated the presence of siderophore. [8]

### Ammonia Production

The isolates were inoculated in 10 ml of peptone water and incubated. The development of brown to pale yellow color indicated the production of ammonia. [3]

### Hydrogen Cyanide (HCN) Production

The isolates were streaked on nutrient agar containing 4.4g/L of Glycine. Inside the lid of each plate, filter paper soaked in 0.5% picric acid and 2 % sodium carbonate was placed. The presence of an orange-to-red color indicated the production of HCN. [7]

### Phosphate Solubilization

The isolates were inoculated on Pikovskaya's agar medium with tri-calcium phosphate. After incubation, the clear zone around the colony indicated a positive result. [6]

### Analysis Of Hydrolytic Enyzmes

All the bacterial isolates were screened for hydrolytic enzyme production such as amylase, lipase, protease, and cellulase on respective agar plates. The development of a clear zone around the bacterial colonies indicated enzyme production.

## CONFIRMATION OF BACTERIA

### Selective Plate Method

The obtained bacterial culture was inoculated on Bacillus and Cetrimide agar medium and incubated at room temperature for 24 and 48 hours respectively.

### Gram Staining And Biochemical Characteristics

The bacterial culture was identified by Gram staining and biochemical tests using standard procedure.

### Growth Of Bacterial Isolates In Mineral Salt Medium (MSM) With 1%, 2%, And 3% Petrol And Diesel

Bacterial isolates were first grown in half-strength Trypticase soy broth at 28°C for 48 hours with continuous agitation at 150 rpm. Then, cells were collected via centrifugation, washed three times with Phosphate Buffer Saline, and re-suspended in sterile distilled water and 10 µl was used to inoculate MSM amended with 1%, 2%, 3% petrol, and diesel. The experiment was conducted in 125 ml Erlenmeyer flasks with 50 ml of sterile MSM and petrol or diesel as the carbon source. The flasks were continuously agitated at 150 rpm on a rotary shaker while being incubated at 28°C. Cell growth was assessed at 600 nm after a week. [1]

### Testing PGP Straits Under Hydrocarbon Stress Conditions

After the selection of the hydrocarbon stress-tolerant bacterial strains, the PGP traits of the strains were examined in the presence of stress conditions such as 1%, 2%, 3% petrol and diesel.

### POT Trail Experiment

### Collection of seed

The seeds of *Vigna radiata* and the soil were collected from Thindal, Erode district, Tamilnadu.

### Biometric Observations (Growth parameters)

The seeds of *Vigna radiata* were soaked in bacterial suspension and placed in Hoagland's solution with different concentrations of petrol and diesel. After the seed germination, the seeds were sown in soil treated with different concentrations (1%, 2%, and 3%) of petrol and diesel and inoculated with the bacterial suspension. The control soil was treated with petrol and diesel without bacterial inoculation. The plants were left to grow. After 20 days, the plants were collected and growth parameters such as root length, shoot length, and the total plant were measured. [10]

## RESULTS

### Isolation Of Endophytic Bacteria

Totally 4 different strains were isolated from Aloe vera root and Brinjal stem using a nutrient agar medium.

### Analysis Of Hydrolytic Enzymatic Activity And Plant Growth-promoting Activity

All the bacterial isolates showed differential enzymatic activity. All the isolates except isolate 3 exhibited amylase, lipase, and cellulase activity indicated by the zone formation. All the isolates exhibited protease activity. All four isolates showed positive results for the production of indole acetic acid, ammonia, HCN and phosphate solubilization activity. Based on plant growth-promoting activity and hydrolytic enzyme activity from four isolates the best two isolates were selected for further study.

### Identification Of Plant Growth Promoting Bacteria

Based on Gram's staining, isolate 1 was identified as a Gram-positive rod and isolate 4 as Gram-negative rod. Isolate 1 produced white, creamy, flat dry colonies on the Bacillus medium, and isolate 4 produced irregular margins with green pigmented colonies on the Cetrimide medium. The two isolates were subjected to biochemical characterization and identified based on Bergey's Manual of Systematics.

### Invitro Screening Of Plant-growth Promoting Bacteria Under Abiotic Stress Tolerance

The ability of isolates to tolerate the hydrocarbon stress was tested by observing the OD value at 600nm for seven days. The highest value was recorded on 7$^{th}$ day. This showed that *Bacillus* sp., and *Pseudomonas* sp., have the ability to tolerate hydrocarbon stress upto 3% concentration.

**Table 1: Effect Of Hydrocarbon Stress On *Bacillus* Sp.,**

| S. NO | Hydrocarbons | Concentrations | Initial OD | Final OD |
|---|---|---|---|---|
| 1 | PETROL | 1% | 0.39 | 1.38 |
| | | 2% | 0.23 | 1.49 |
| | | 3% | 0.25 | 1.22 |
| 2 | DIESEL | 1% | 0.46 | 1.40 |
| | | 2% | 0.26 | 1.58 |
| | | 3% | 0.32 | 1.29 |

**Table 2: Effect Of Hydrocarbon Stress On *Pseudomonas* sp.,**

| S.NO | Hydrocarbons | Concentrations | Initial OD | Final OD |
|---|---|---|---|---|
| 1 | PETROL | 1% | 0.24 | 1.42 |
| | | 2% | 0.36 | 1.55 |
| | | 3% | 0.35 | 1.31 |
| 2 | DIESEL | 1% | 0.41 | 1.47 |
| | | 2% | 0.43 | 1.70 |
| | | 3% | 0.22 | 1.36 |

### Testing Plant Growth Promoting Bacterial Traits Under Stress Conditions

The investigation illustrated that *Bacillus* sp., grown in different concentrations of hydrocarbon stress produced positive results for Amylase, Ammonia, Siderophore, and HCN production and negative results for Cellulase. *Pseudomonas* sp., produced positive results for Ammonia, Siderophore, and HCN and negative results for Amylase production.

### Biometric Analysis

The root length, shoot length, number of branches, and the total plant length of *Vigna radiata* plants were measured. The plants showed maximum growth at 2% hydrocarbon concentration.

The results revealed that the endophytic bacteria promote the growth of *Vigna radiata* under hydrocarbon stress conditions.

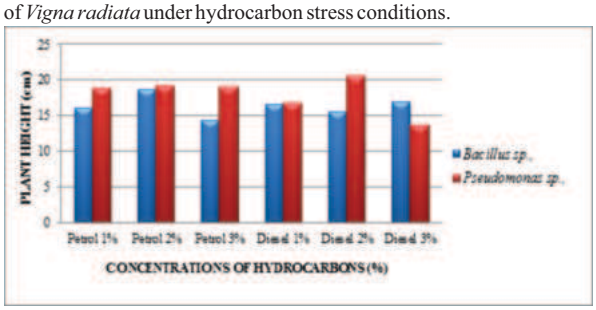


**Figure 1:** Total Length Of The Plants

## CONCLUSION

Plant growth-promoting and hydrocarbon stress tolerant endophytic bacterial strains were isolated from the plant's Aloe vera and Brinjal. The endophytic bacterial species exhibiting multiple plant growth-promoting traits, enhanced the seedling growth (root and shoot lengths) of *Vigna radiata* plants in hydrocarbon-polluted medium. Thus, endophytic bacteria can be used to stabilize hydrocarbon-polluted soil, by providing the essential nutrients required by plants and detoxifying the hydrocarbon, thus enhancing plant growth in such contaminated soil. Therefore, this study suggests that *Bacillus* sp., and *Pseudomonas* sp., can be used as inoculants in the development of bio-fertilizers for increasing the stability and growth of plants in hydrocarbon-polluted soils.

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