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PRODUCTION OF SIDEROPHORE FROM PSEUDOMONAS SPECIES ISOLATED FROM RHIZOSPHERE OF SOYBEAN (GLYCINE MAX).

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ABSTRACT

Iron is a vital micronutrient for both plants and microorganisms, but its availability in soils is often limited due to its insolubility in oxidized forms, especially in alkaline and aerobic conditions. To address this limitation, microorganisms such as Pseudomonas species produce siderophores, which are iron-chelating compounds that help in iron acquisition. This study aimed to isolate and characterize siderophore-producing Pseudomonas species from the rhizosphere of soybean crops in Deulgaon Raja, Maharashtra, and evaluate their potential as biofertilizers. Soil samples were collected, and bacterial isolates were screened for siderophore production using the Chrome Azurol Sulphonate (CAS) assay. The results demonstrated significant siderophore production by all isolates, with the highest decolorization (79.20%) observed in isolate RSVC-44. Further tests revealed that the isolates produced pyoverdine, a water-soluble fluorescent siderophore, and identified both hydroxamate and catecholate-phenolate types using Csaky and Arnow's tests. These findings confirm the ability of Pseudomonas isolates to efficiently sequester iron and promote plant growth. The high siderophore production in RSVC-44 suggests its potential as a biofertilizer to improve iron nutrition in soybean crops, particularly under iron-deficient conditions. This research underscores the significance of siderophore-producing bacteria in sustainable agriculture and provides a foundation for future studies on their application in enhancing crop productivity and reducing chemical fertilizer dependence.

Keywords: Siderophores, Rhizosphere, Pseudomonas, soybean (Glycine max).

1. INTRODUCTION

Iron is an essential micronutrient required for various biological processes in microorganisms and plants, including photosynthesis, respiration, nitrogen fixation, and enzyme activity (Guerinot & Yi, 1994). Despite its abundance in soil, iron is predominantly found in its oxidized ferric form (Fe3+), which is highly insoluble, especially in alkaline and aerobic soils, making it largely unavailable for microbial and plant uptake (Lemanceau et al., 2009). To overcome iron limitation, microorganisms, including plant-associated bacteria, have evolved specialized mechanisms for iron acquisition, with siderophore production being one of the most effective strategies (Neilands, 1995).

Siderophores are low-molecular-weight, high-affinity iron-chelating compounds secreted by microorganisms to scavenge ferric iron from the environment and facilitate its uptake (Hider & Kong, 2010). These molecules play a crucial role in microbial survival and competitiveness in iron-deficient environments, particularly in plant rhizospheres where iron is a limiting factor (Saha et al., 2016). Among rhizosphere-dwelling bacteria, Pseudomonas species are well-known for their ability to produce a diverse range of siderophores, including pyoverdines and pyochelins, which not only help in iron acquisition but also exhibit antimicrobial properties that suppress plant pathogens (Haas & Défago, 2005).

The role of Pseudomonas siderophores in agriculture has been extensively studied, with research indicating their potential as biofertilizers and biocontrol agents (Sharma & Johri, 2003). By improving iron uptake, these bacteria enhance plant growth and yield, particularly in crops like soybean, which are susceptible to iron deficiency-induced chlorosis (Vessey, 2003). Additionally, Pseudomonas species contribute to soil health by promoting beneficial microbial interactions and suppressing soilborne diseases through competitive exclusion and antibiotic production (Compant et al., 2005).

This study focuses on isolating and characterizing siderophore-producing Pseudomonas species from the rhizospheric soil of soybean crops cultivated in Deulgaon Raja, Maharashtra. Furthermore, we evaluate the potential application of these isolates as biofertilizers to enhance soybean growth and productivity under iron-limited conditions.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples:

Rhizospheric soil samples were collected from soybean (Glycine max) fields in Deulgaon Raja, Maharashtra, India. Samples were obtained from a depth of 5-10 cm around the root zone using a sterile spatula. The collected soil was transferred into sterile polyethylene bags and transported to the laboratory for further processing.

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2.2 Isolation of Bacterial Strains:

Soil samples were serially diluted in sterile saline solution (0.85% NaCl) and plated onto Pseudomonas Isolation Agar (PIA) medium using the spread plate technique. Plates were incubated at $28 \pm 2^{\circ}$ C for 24–48 hours. Colonies exhibiting characteristic Pseudomonas morphology, including fluorescence under UV light, were selected and purified by repeated streaking on fresh PIA plates.

2.3 Screening for Siderophore Production

2.3.1 Primary Screening using CAS Assay

Primary screening for siderophore production was performed using the Chrome Azurol Sulphonate (CAS) assay (Schwyn and Neilands, 1987). Bacterial isolates were inoculated into 100 ml of succinate medium (Meyer and Abdallah, 1978) in 250 ml Erlenmeyer flasks and incubated at $28 \pm 2^{\circ}$ C for 48 hours with continuous shaking at 200 rpm on a rotary shaker. After incubation, cultures were centrifuged at 10,000 rpm for 20 minutes to separate bacterial cells from the culture supernatant. The pH of the collected supernatant was adjusted to 7 and subjected to the CAS assay to qualitatively and quantitatively detect siderophore production.

Decolorization of the blue CAS reagent to a wine-red color indicated the presence of siderophores. The intensity of decolorization was measured spectrophotometrically at 630 nm, and the percentage of CAS decolorization was calculated to quantify siderophore production.

2.4 Determination of Siderophore Types

To classify the types of siderophores produced, the following tests were conducted:

- Csaky's Test for Hydroxamate Siderophores: The Csaky test (Csaky, 1948) was performed to detect hydroxamate-type siderophores. A positive reaction was indicated by the formation of a red color upon reaction with ferric chloride.
- Arnow's Test for Catecholate-Phenolate Siderophores: The Arnow's test (Arnow, 1936) was used to identify catecholate-phenolate siderophores. A red color change after addition of the reagent confirmed the presence of catechol-type siderophores.

2.5 Characterization of Fluorescent Pigment Production

The ability of bacterial isolates to produce fluorescent siderophores was assessed by streaking isolates onto PIA plates and incubating them at $28 \pm 2^{\circ}$ C for 24–48 hours. Colonies exhibiting water-soluble fluorescent green pigmentation under UV light were recorded as pyoverdine producers. Representative images were documented in Photo Plate 5.

2.6 Statistical Analysis

All experiments were performed in triplicates. Data were analyzed using descriptive statistics, and results were expressed as mean values. The variations in siderophore production among different isolates were compared using one-way ANOVA (p < 0.05) to determine statistical significance.

This methodology ensures reliable isolation and screening of siderophore-producing Pseudomonas strains and provides insights into their potential application as biofertilizers in soybean cultivation.

3. RESULTS AND DISCUSSION

The bacterial isolates obtained from the rhizospheric soil samples were subjected to primary screening for siderophore production. For this purpose, isolates were inoculated in 100 ml of succinate medium (Meyer and Abdallah, 1978) contained in 250 ml Erlenmeyer flasks and incubated at $28 \pm 2^{\circ}$ C for 48 hours. Incubation was carried out under constant shaking at 200 rpm using a rotary shaker to ensure optimal aeration. After incubation, cultures were centrifuged at 10,000 rpm for 20 minutes to separate bacterial cells from the culture supernatant. The pH of the collected supernatant was adjusted to 7 and subjected to Universal Chemical Assay, i.e., the Chrome Azurol Sulphonate (CAS) assay (Schwyn and Neilands, 1987), for the detection and quantification of siderophores.

The culture supernatants were further analyzed to determine the type of siderophore produced by the isolates. The Csaky test (Csaky, 1948) was performed for detecting hydroxamate-type siderophores, whereas the Arnow's test (Arnow, 1936) was used for identifying catecholate-phenolate siderophores. The results of these tests provided insights into the specific types of siderophores produced by different Pseudomonas isolates, further confirming their potential role in iron acquisition and plant growth promotion.

A total of eight bacterial isolates obtained from Pseudomonas Isolation Agar plates exhibited the production of watersoluble fluorescent green pigment, which is a characteristic feature of Pseudomonas siderophores (Photo Plate 1). This fluorescence is attributed to pyoverdine, a well-known siderophore produced by Pseudomonas species, which plays a crucial role in iron sequestration under iron-deficient conditions.

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3.1 Siderophore Quantification Using CAS Assay

The results of the CAS assay used for detection and quantification of siderophore production are presented in Table 1. The blue-coloured CAS reagent decolorized and turned wine-red in the presence of siderophores, indicating a positive test result for all eight bacterial isolates. The degree of decolorization was measured spectrophotometrically, and the percentage of CAS decolorization was used to quantify siderophore units.

As observed in Table 1, all isolates exhibited significant siderophore production, with percent CAS decolorization ranging from 50.12% to 79.20%. Among all the isolates, RSVC-44 demonstrated the highest siderophore production with 79.20% CAS decolorization, indicating its strong potential for iron acquisition. Other notable isolates included RSVC-1 (66.46%), RSVC-35 (65.51%), and RSVC-24 (62.20%), which also exhibited considerable siderophore production. The lowest siderophore production was observed in isolate RSVC-8 (50.12%).

3.2 Discussion

The results obtained in this study align with previous reports emphasizing the ability of Pseudomonas species to produce siderophores for iron sequestration under iron-limited conditions. The production of water-soluble fluorescent pigments by the isolates further supports their classification as siderophore-producing Pseudomonas species, as pyoverdine production is a hallmark feature of these bacteria (Cornelis, 2010). The variation in siderophore production among different isolates could be attributed to genetic differences, environmental adaptations, and iron availability in the rhizosphere (Radzki et al., 2013).

The high siderophore production observed in isolate RSVC-44 suggests its strong potential as a biofertilizer candidate for promoting plant growth and improving iron nutrition in soybean crops. Similar findings have been reported in other studies, where siderophore-producing Pseudomonas strains were found to enhance plant growth by improving iron uptake and inhibiting the growth of phytopathogens (Sayyed et al., 2005). The identification of hydroxamate and catecholate-phenolate siderophores further indicates the diversity of iron-chelating mechanisms employed by these bacteria.

In conclusion, the siderophore production potential of Pseudomonas isolates from soybean rhizospheric soil highlights their importance in sustainable agriculture. Future research involving greenhouse and field trials will be essential to evaluate their effectiveness as biofertilizers and biocontrol agents in crop production.

Sr. No.	Isolate	% CAS Decolorizing Siderophore Units
1	RSVC-1	66.46
2	RSVC-7	56.30
3	RSVC-8	50.12
4	RSVC-15	51.12
5	RSVC-37	60.02
6	RSVC-24	62.20
7	RSVC-35	65.51
8	RSVC-44	79.20

Table 1: Screening of Bacterial Isolates for Siderophore Production



Photo plate 1: Fluorescent green pigment (Siderophore)

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## 4. CONCLUSION

This study highlights the significant siderophore production potential of Pseudomonas isolates obtained from soybean rhizospheric soil. The isolates exhibited considerable siderophore activity, as evidenced by the CAS assay, with RSVC-44 demonstrating the highest siderophore production. The production of water-soluble fluorescent pigments, particularly pyoverdine, further confirmed the ability of these isolates to function as efficient iron chelators.

The identification of hydroxamate and catecholate-phenolate siderophores through Csaky and Arnow's tests revealed the diversity of iron-chelating mechanisms employed by the Pseudomonas isolates. These findings support the potential application of siderophore-producing bacteria in promoting plant growth, improving iron uptake, and acting as biocontrol agents against phytopathogens.

Given the promising results, future studies should focus on greenhouse and field trials to assess the effectiveness of these isolates as biofertilizers. Further molecular characterization and genetic studies could also provide deeper insights into the mechanisms regulating siderophore production in Pseudomonas species. The integration of these beneficial bacterial strains into sustainable agricultural practices could enhance soil fertility and crop productivity while reducing dependence on chemical fertilizers.

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