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Isolation of Plant Growth Promoting Microbes from the Rhizosphere of Ocimum Sanctum on Okra Crop

Nagarajan[1*], Rajkumar Thamarai[2], Kalidass S[3], Kanakaraj K[4], Petchimuthu K[5], Jasline Pavina[6]

[1]Assistant Professor, Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

[2]Post Doctoral Fellow, Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

[3]Associate Professor, Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

[4]Research Scholar, Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

[5]Assistant Professor, Dept. of Botany, Manonmaniam Sundaranar University, Sri KGS College of Arts and Science, Srivaikundam, Tamilnadu, India.

[6]Research Scholar, Dept. of Botany, Manonmaniam Sundaranar University, Sri KGS College of Arts and Science, Srivaikundam, Tamilnadu, India.

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*Corresponding Author: nagabp@gmail.com

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Abstract

The present study investigates the potential of plant growth-promoting rhizobacteria (PGPR) isolated from the rhizosphere of Ocimum sanctum to enhance the growth and yield of okra (Abelmoschus esculentus). A total of 10 bacterial strains were identified, comprising proteolytic and amylolytic types, with four key isolates (S1, S2, S3, and S4) selected for field trials. These isolates were applied at three critical stages of okra growth: germination, flowering, and fruiting. Significant differences were observed between treated and control plots. Plants treated with isolates S3 and S4 exhibited superior growth, with S3-treated plants producing dark green fruits with an average length of 17 cm and an overall yield of 15 kg per plant. In contrast, control plants yielded malformed, curved fruits with an average length of 10 cm and were more susceptible to pest infestations and defoliation. The study highlights the role of PGPR in enhancing crop health, productivity, and resistance to pests. The proteolytic activity of S3 and S4 isolates proved effective in improving morphological parameters such as canopy density and fruit quality while maintaining soil fertility. These findings underscore the importance of microbial inoculants as eco-friendly, sustainable alternatives to chemical fertilizers and pesticides. The successful application of these isolates supports their potential use in vegetable cultivation for improved yield and quality without compromising environmental health. In conclusion, isolates S3 and S4 demonstrate remarkable potential as plant growth promoters and pest resistance agents in okra cultivation. Their application can significantly contribute to sustainable agricultural practices and address the challenges posed by chemical inputs. Further research is recommended to explore their scalability and impact on other crops.

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Keywords: *Plant Growth Promoter, Ocimum Sanctum, Okra, Rhizobacteria, Rhizosphere..*

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1. INTRODUCTION

Beneficial microbes from the soil, water, and air mediums attract scientists due to their extensive application in medicine, agriculture, industry, and bioremediation. These microbes, however, can also act as pathogens to humans, animals, and plants. The rhizosphere, a unique microenvironment around plant roots, is a critical area for studying microbial interactions. It is highly dynamic, facilitating various biological processes under the influence of the plant's roots **(Morgan et al., 2005)**. Plant growth-promoting rhizobacteria (PGPR) significantly enhance plant development through multiple mechanisms, including increased nutrient availability in the soil and root zone **(Ahmad et al., 2008)**.

Research highlights the role of PGPR in improving forest ecosystems by enhancing productivity, particularly in species like Pinus, Tsuga, Pseudotsuga, and Eucalyptus **(Chanway, 1996; Mafia et al., 2009)**. Microbes associated with plants influence chemical communication, stimulate development, and support sustainable agricultural practices **(Chebotar et al., 2015)**. These microbial interactions can significantly alter plant hormone levels through the production or metabolism of phytohormones **(Bacon & White, 2000)**.

Chemical fertilizers, while effective in enhancing yields, often pose risks to ecosystem balance, soil health, and the food chain. Alternative approaches, such as ecological methods using PGPR, are therefore imperative for maintaining soil fertility and meeting global agricultural demands without chemical residues **(Tilman et al., 2002)**. Introducing beneficial microflora like PGPR into agricultural systems is an effective strategy for increasing soil health and plant immunity **(García de Salamone, 2011; Verma et al., 2010)**.

Plant roots harbor diverse microorganisms, including mycorrhizal fungi, rhizobia, and endophytic fungi, which promote sustainable crop production **(Gupta et al., 2020)**. Rhizobacteria specifically contribute to drought tolerance, pest resistance, and nutrient cycling in plants **(Jeffries et al., 2003; Rosas et al., 2009)**. These bacteria establish symbiotic relationships with plants, aiding in nutrient uptake, growth, and immunity **(Reinhold-Hurek & Hurek, 2011)**.

Plants like Ocimum sanctum (Holy Basil) hold a prominent place in traditional medicine for their myriad health benefits, including anti-inflammatory, antifungal, and antioxidant properties. However, research on their role in plant growth promotion is limited. This study explores the potential of PGPR isolated from the rhizosphere of Ocimum sanctum and evaluates their efficacy on Okra plants, considering parameters such as leaf count, flowering, and fruit yield. The findings aim to advance sustainable agricultural practices and enhance productivity.

2. MATERIALS AND METHODS

2.1. Rhizosphere Collection

The study was conducted using the herb *Ocimum sanctum*, which was collected from Thiruvettanallur, Tamil Nadu, India (Latitude: 9.1371° N, Longitude: 77.4092° E, Altitude: 154 MSL). The plant was identified and authenticated at the Department of Plant Science, Manonmaniam Sundaranar University, where a specimen was cataloged under the identifier OCIS2023. Rhizosphere soil samples were collected following the method described by **McPherson et al. (2018)**. The sampling involved excavating the root zone up to 30 cm using a spade and preserving the root-soil mass in plastic bags to maintain moisture and prevent desiccation during transport. Samples were stored under refrigeration until further analysis.

2.2. Isolation of Endophytic Probiotics

The roots of *Ocimum sanctum* were processed for isolation of endophytic bacteria. After washing off debris under running water, roots were surface-sterilized using 70% ethanol for 30 seconds, followed by treatment with 2% sodium hypochlorite for five minutes. Sterile deionized water was used for two rinses post-sterilization **(Elbeltagy et al., 2000)**. A gram of sterilized roots was homogenized with 1 mL of double-distilled water using a mortar and pestle. The filtrate from this homogenate, obtained using Whatman No. 1 filter paper, was stored in sterile

containers and used for culturing on nutrient agar medium.

2.3. Screening of Enzyme Activity

- **Amylase Activity:** The amylase activity of crude extracts was evaluated by determining the reducing sugars released from soluble starch using the dinitrosalicylic acid (DNS) method, as described by Ettalibi and Barratti (1988).
- **Proteolytic Activity:** Protease production was screened using a skim milk agar assay. Bacterial isolates were inoculated on skim milk agar plates and incubated at room temperature for 24 hours. The formation of clear zones around colonies indicated protease activity.

2.4. Field Evaluation

Ten bacterial isolates (designated S1 to S10) were obtained from the rhizosphere of *Ocimum sanctum*. Four isolates (S1, S2, S3, and S4) with significant enzymatic activity were selected for field trials on Okra (*Abelmoschus esculentus*) crops. The experimental field in Thiruvettanallur was divided into five plots, each containing 25 plants, totaling 125 plants. One plot was maintained as a control, devoid of chemical or microbial treatment.

The selected isolates were applied to their respective plots at a concentration of 10^5 spores/mL. Applications were conducted at three critical growth stages:

1. Germination Stage (10 days post-germination)
2. Flowering Stage
3. Fruiting Stage

Growth parameters, including the number of leaves, flowers, and healthy fruits, were recorded every five days until harvest. The experiment monitored pest incidence, fruit yield, and morphological changes in treated and untreated plots.

2.4. Morphological Characterization of Isolates

Isolated bacterial colonies were characterized based on color and shape. Observations recorded the morphology of strains as follows:

- S1: White, round
- S2: Colorless, round
- S3: White, filamentous
- S4: Pink, irregular

2.5. Statistical Analysis

Data from field trials were statistically analyzed to determine significant differences in growth parameters and fruit yield between treated and control groups. Yield data and pest infestation levels were compared using standard deviation and analysis of variance (ANOVA).

2.6. Yield Analysis

Fruit yield was assessed by measuring fruit length and total yield per plant across multiple harvests. Healthy, dark green fruits were observed in treated plots, while control plots exhibited malformed and pest-damaged fruits.

The study highlighted the superior performance of proteolytic isolates S1, S3, and S4 in terms of plant growth, yield, and resistance to pest infestation. The final harvest recorded a yield of over 15 kg per plant in S3-treated plots compared to 3 kg in the control.

The methods employed in this study provide a replicable framework for isolating and evaluating plant growth-promoting rhizobacteria from medicinal plants like *Ocimum sanctum*. The use of native isolates demonstrates potential for sustainable agricultural practices by enhancing crop yield and resilience to biotic stressors.

3. RESULTS

The present study focused on assessing the diversity and effectiveness of rhizobacterial populations isolated from the rhizosphere of *Ocimum sanctum* and their influence on the growth of Okra (*Abelmoschus esculentus*). The results revealed critical insights into the enzymatic activities, morphological characteristics, and growth-promoting capabilities of these bacterial isolates under field conditions.

3.1. Isolation and Screening of Rhizobacteria

The rhizosphere of *Ocimum sanctum* yielded 10 bacterial isolates labeled S1 through S10. Among these, four isolates (S1, S2, S3, and S4) exhibited significant enzymatic activities, including proteolytic and amylolytic functions. Table 1 summarizes the morphological characteristics of the selected isolates, indicating their diverse appearances, which suggest varying biological activities.

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Table-1. Morphological Characters of Suspected Colonies from the Rhizosphere of *Ocimum sanctum*

Isolate	Color and Shape
S1	White, round
S2	Colorless, round
S3	White, filamentous
S4	Pink, irregular

The diversity in colony morphology highlights the variability in functional roles of these isolates in the plant rhizosphere.

3.2. Screening for Extracellular Enzyme Activity

The isolates were further screened for amylase and protease activity. The results, shown in Table 2, demonstrate the enzymatic capabilities of the bacterial strains.

Table-2. Screening of Extracellular Enzyme Activity of Rhizobacterial Colonies

Isolate	Amylase Activity	Protease Activity
S1	ACZ	ACZ
S2	ACZ	DCZ
S3	ACZ	ACZ
S4	ACZ	DCZ

Note: *ACZ = Appearance of Clear Zone; DCZ = Disappearance of Clear Zone.*

The isolates S1, S3, and S4 exhibited both amylase and protease activity, making them strong candidates for plant growth promotion under field conditions. These enzymatic functions are crucial for nutrient mobilization and improved plant nutrient uptake.

3.3. Field Trials and Growth Parameters

Field trials with Okra plants demonstrated significant differences between treated and control plots. Growth parameters, including leaf count, flower formation, and fruit length, were measured across treatments. The untreated control plots exhibited stunted growth, yellowish leaves, and malformed fruits, while the treated plots displayed vigorous growth and higher yields.

3.4. Yield Analysis and Morphological Changes

The yield analysis revealed stark contrasts between treated and untreated groups. Table 3 shows the fruit lengths recorded across multiple harvests.

Table-3. Average Length (in cm) of Fruits in Treated and Control Plots

Type of Plot	H1	H2	H3	H4
Control	12.0	11.5	10.0	10.0
S1 Treated	15.4	16.1	16.8	16.9
S2 Treated	14.0	14.6	15.0	15.3
S3 Treated	15.0	16.0	16.7	17.0
S4 Treated	14.9	15.2	15.8	16.0

Note: H = Harvest Number.

Fruits from S3-treated plots consistently measured longer and appeared healthier compared to control plots, which showed reduced fruit size and pest damage. The maximum yield was observed in S3-treated plots, reaching over 15 kg per plant by the final harvest, while the control yielded only 3 kg per plant.

3.5. Pest Infestation and Resistance

Pest infestation was notably higher in control plots, where leaves and fruits showed visible damage. In contrast, S1, S3, and S4 treatments exhibited significantly reduced pest incidence. This suggests that the proteolytic isolates contributed to enhanced pest resistance, possibly through the production of metabolites that deter pest activity.

3.6. Role of Proteolytic Bacteria in Plant Growth

Proteolytic bacteria play a vital role in nutrient mobilization and plant development. In this study, isolates S1, S3, and S4 promoted healthy foliage, flower formation, and robust fruiting. These isolates likely enhanced nitrogen availability, leading to improved overall plant vigor.

The field trials confirmed the potential of these isolates as plant growth promoters, particularly for Okra crops. Their application resulted in taller plants, darker green leaves, and a higher number of flowers compared to untreated plants.

4. DISCUSSION

The effectiveness of PGPR in this study aligns with findings from previous research. Proteolytic and amylolytic enzymes produced by rhizobacteria are known to facilitate the breakdown of organic matter, releasing essential nutrients into the soil **(Ahmad et al., 2008)**.

Enhanced nutrient availability likely contributed to the superior growth observed in treated plots. Moreover, the role of microbial inoculants in mitigating pest infestations is well-documented **(Jeffries et al., 2003)**. The reduced pest incidence in treated plots may be attributed to the production of secondary metabolites or induced systemic resistance in plants **(Hallmann et al., 1997)**.

The morphological diversity of isolates observed in this study is consistent with previous reports on rhizobacterial communities. Native isolates are particularly advantageous for agricultural applications due to their adaptability to local environmental conditions **(Chen et al., 2006)**.

4.1. Implications for Sustainable Agriculture

This study highlights the potential of PGPR as a sustainable alternative to chemical fertilizers and pesticides. The use of native rhizobacteria not only enhances plant growth but also improves soil health, ensuring long-term agricultural productivity. By reducing dependence on chemical inputs, PGPR-based interventions align with global goals for sustainable farming practices **(Tilman et al., 2002)**. The findings demonstrate the significant role of rhizobacterial isolates, particularly S1, S3, and S4, in promoting plant growth, improving yield, and enhancing pest resistance in Okra crops. These isolates offer a viable solution for sustainable agriculture, fostering high productivity while preserving environmental integrity.

5. CONCLUSION

The present study explored the potential of rhizobacterial isolates from the rhizosphere of Ocimum sanctum as plant growth-promoting agents. Among the ten isolates identified, S1, S3, and S4 showed significant proteolytic and amylolytic activities, making them effective in enhancing plant growth and yield under field conditions. Field trials on Okra (Abelmoschus esculentus) revealed that these isolates contributed to better plant health, increased fruit length, reduced pest infestations, and higher overall yields compared to untreated control plots. The S3-treated plots particularly stood out, demonstrating superior growth parameters, pest resistance, and productivity, with over 15 kg of fruit per plant at the final harvest, compared to 3 kg in the control. The use of PGPR isolates offers a sustainable alternative to chemical fertilizers and pesticides, aligning with the principles of ecological farming and soil health conservation. These findings underscore the importance of leveraging native microbial populations for enhancing agricultural productivity while minimizing environmental impact. In conclusion, the isolates S1, S3, and S4 show significant potential as biofertilizers and biocontrol agents. They can be recommended for field application in vegetable crops like Okra, contributing to sustainable agricultural practices and meeting the growing global demand for chemical-free, high-yield farming solutions. Further research may focus on large-scale field evaluations and formulation development for commercial use.

6. DECLARATION OF COMPETING INTEREST

The authors declare that they have no known financial or personal conflicts that could have appeared to influence the work reported in this study. All data and methodologies presented are original and unbiased, ensuring the scientific integrity of the research.

7. DATA AVAILABILITY

Data will be made available on reasonable request.

8. ACKNOWLEDGEMENTS

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