



ISOLATION AND EVALUATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND THEIR EFFECT ON THE GROWTH OF LADY'S FINGER (*Abelmoschus esculentus*)

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ABSTRACT :

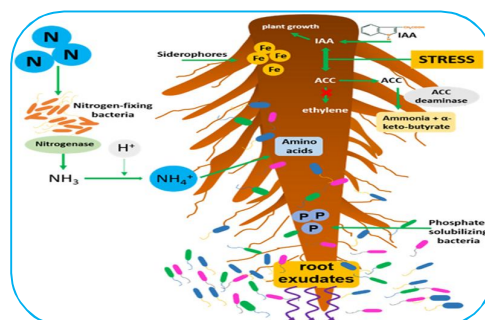
Plant growth-promoting rhizobacteria (PGPR) are beneficial soil microorganisms that may enhance plant growth through nutrient mobilization, biological nitrogen fixation, and the production of plant growth regulators. The present study investigated the isolation, identification, and plant growth-promoting properties of four rhizobacterial groups: *Azotobacter*, *Azospirillum*, *Rhizobium*, and *Pseudomonas fluorescens*. Rhizosphere soil samples were collected from paddy and groundnut fields and processed using serial dilution and selective culture media. The bacterial isolates were examined using morphological, staining, biochemical, sugar fermentation, and phosphate-solubilization tests. Extracellular protein concentration was estimated using the Lowry method, while indole-3-acetic acid (IAA) production was investigated using the Salkowski colorimetric method and thin-layer chromatography (TLC). The effects of bacterial inoculation on lady's finger growth were evaluated using root length, root number, plant height, fresh weight, and dry weight.

Among the four isolates, *Rhizobium* showed the highest recorded extracellular protein concentration (210 µg/mL). All four isolates produced a pink reaction in the qualitative IAA assay. The highest reported absorbance values in the quantitative IAA assay were recorded for *Azospirillum* at 30 minutes (0.42) and 120 minutes (0.36). In the plant-growth experiment, *Azospirillum*-treated plants exhibited the highest recorded root length (12.5 cm), root number (35), plant height (47 cm), fresh weight (8.6 g), and dry weight (2.6 g). These findings suggest that the tested rhizobacteria, particularly *Azospirillum*, have potential as plant growth-promoting inoculants. Further replicated experiments and direct measurements of nitrogen fixation are necessary to validate their agricultural effectiveness.

KEYWORDS: Plant growth-promoting rhizobacteria, PGPR, *Azotobacter*, *Azospirillum*, *Rhizobium*, *Pseudomonas fluorescens*, indole-3-acetic acid, nitrogen fixation, biofertilizer, lady's finger.

1. INTRODUCTION

Agriculture plays a vital role in the economy and food security of India. Increasing agricultural productivity has traditionally involved the application of chemical fertilizers to supply essential nutrients required for plant growth. However, excessive or inappropriate fertilizer use may contribute to soil degradation, nutrient imbalances, and environmental pollution. Therefore, sustainable



agricultural practices that improve soil fertility while reducing environmental impacts are increasingly important.

Biofertilizers containing beneficial microorganisms offer one approach to improving nutrient availability and plant development. Plant growth-promoting rhizobacteria are microorganisms that colonize the rhizosphere, the soil region influenced by plant roots, and may promote plant growth through several mechanisms. These mechanisms include atmospheric nitrogen fixation, phosphate solubilization, production of plant growth regulators, and suppression of certain soilborne pathogens.

Among the commonly investigated PGPR groups, *Azotobacter* is known for its free-living nitrogen-fixing ability and potential production of plant growth regulators. *Azospirillum* is associated with plant roots and may promote root development and nutrient acquisition. *Rhizobium* is well known for its symbiotic nitrogen-fixing relationship with compatible leguminous plants. Certain strains of *Pseudomonas fluorescens* contribute to plant health through nutrient competition, siderophore production, and other beneficial interactions. Indole-3-acetic acid is an auxin involved in root initiation, cell elongation, and root-system development. Bacterial production of IAA may contribute to changes in root architecture and improve the capacity of plants to acquire water and nutrients. Phosphate solubilization is another important PGPR trait because it can increase the availability of phosphorus in soils where a portion of this nutrient occurs in poorly soluble forms.

The present investigation examined four rhizobacterial groups isolated from paddy and groundnut rhizosphere soils. Their morphological and biochemical characteristics, phosphate-solubilizing activity, extracellular protein concentration, and IAA-producing ability were evaluated. A pot experiment was also conducted to compare the growth of lady's finger plants inoculated with the bacterial isolates against an uninoculated control.

2. OBJECTIVES OF THE STUDY

1. To isolate and identify rhizobacteria from paddy and groundnut rhizosphere soils.
2. To evaluate the phosphate-solubilizing properties and selected biochemical characteristics of the bacterial isolates.
3. To estimate extracellular protein concentration as an indirect indicator associated with nitrogen-related activity.
4. To investigate IAA production using qualitative, colorimetric, and chromatographic techniques.
5. To assess the effects of four rhizobacterial isolates on the growth of lady's finger plants under pot conditions.
6. To identify promising bacterial isolates for further evaluation as potential biofertilizer candidates.

3. MATERIALS AND METHODS

3.1 Collection of soil samples

Rhizosphere soil samples were collected from agricultural fields growing paddy and groundnut. Samples from different locations were pooled as described in the project. Soil was collected in sterile polythene bags, transported to the laboratory, and stored at 2–8°C until processing.

3.2 Isolation of rhizobacteria

Serial dilution was used to isolate bacteria from the collected soil samples. Approximately 1 g of soil was mixed thoroughly with 9 mL of sterile distilled water. Serial dilutions ranging from 10^{-2} to 10^{-9} were prepared, and suitable dilutions were plated on selective culture media.

The project describes the following media for isolation:

- *Azotobacter*: Ashby's agar.
- *Azospirillum*: Pikovskaya's medium, as recorded in the original project.
- *Rhizobium*: Yeast extract mannitol agar.
- *Pseudomonas fluorescens*: King's B medium.

The inoculated plates were incubated at approximately 28°C for up to one week. Colonies showing characteristic growth were selected and subcultured to obtain individual cultures. The isolates were maintained on appropriate culture slants under refrigerated conditions.

3.3 Morphological and biochemical identification

The bacterial isolates were examined using colony morphology, Gram staining, motility observation, biochemical reactions, and sugar fermentation tests. Gram staining was performed using crystal violet, Gram's iodine, a decolorizing agent, and safranin. Motility was assessed using the hanging-drop technique. The biochemical tests described in the project included indole production, methyl red, Voges-Proskauer, citrate utilization, carbohydrate fermentation, catalase, and oxidase tests. Sugar fermentation was evaluated using lactose, glucose, sucrose, and mannose. The recorded positive and negative reactions were used to compare the isolates.

3.4 Phosphate-solubilization assay

Phosphate-solubilizing activity was investigated using agar medium containing precipitated tricalcium phosphate. Bacterial cultures were inoculated onto the medium, and the development of a clear zone around colonies was recorded as evidence of qualitative phosphate-solubilizing activity.

3.5 Soil characterization

Soil pH was measured using a pH meter. Organic carbon was estimated using the modified Walkley-Black method, as described in the original project.

3.6 Estimation of extracellular protein

The Lowry method was used to estimate extracellular protein concentration in bacterial culture samples. The procedure involved preparation of protein standards, addition of alkaline copper reagent and Folin-Ciocalteu reagent, incubation, and measurement of absorbance at 660 nm. The recorded protein concentrations were used to compare the isolates.

Methodological clarification: The Lowry assay measures protein concentration; it does not directly quantify the amount of atmospheric nitrogen fixed. Therefore, the results are reported as extracellular protein concentrations rather than definitive nitrogen-fixation rates.

3.7 Qualitative detection of IAA

The isolates were screened for IAA production using the Van Urk-Salkowski reagent method. Bacterial cultures were grown on tryptophan-amended medium and examined for a pink-to-red colour reaction. The colour and associated colony measurements were recorded.

3.8 Quantitative estimation of IAA

Bacterial isolates were cultured in liquid medium and the culture supernatants were separated by centrifugation. The supernatants were mixed with Salkowski reagent, and absorbance was measured at 530 nm after the specified reaction periods. IAA standards ranging from 5 to 30 ppm were used to prepare a calibration series. The original project reports absorbance values at 30 and 120 minutes. Because the numerical concentration values calculated from the calibration curve were not established in the results reproduced here, the recorded absorbance values are presented without converting them into IAA concentrations.

3.9 Extraction and chromatographic analysis of IAA

The original project describes acidification of culture supernatants, extraction using ethyl acetate, and subsequent thin-layer chromatographic analysis. A chloroform-methanol-water solvent system in the ratio 85:14:1 was used.

The retention factor (Rf) was calculated as:

$$\text{Rf} = \text{Distance travelled by the sample} / \text{Distance travelled by the solvent front}$$

Chromatographic values were compared with the standard IAA value recorded in the project.

3.10 Pot experiment using lady's finger

Lady's finger seeds were treated with the four bacterial inoculants using the seed-coating procedure described in the project. An untreated group was maintained as the control.

The recorded plant-growth parameters were:

- Root length (cm)
- Root number
- Fresh weight (g)
- Dry weight (g)
- Plant height (cm)

4. RESULTS

4.1 Isolation and enumeration of rhizobacteria

Four rhizobacterial groups were isolated from the rhizosphere samples using selective media and serial dilution.

Table 1. Colony counts recorded at different soil dilution levels

Rhizobacterial group	10 ⁻³ dilution	10 ⁻⁴ dilution	10 ⁻⁵ dilution
<i>Azotobacter</i>	8	2	–
<i>Azospirillum</i>	7	1	–
<i>Rhizobium</i>	8	2	1
<i>Pseudomonas fluorescens</i>	10	3	1

The highest recorded colony count at the 10⁻³ dilution was observed for *Pseudomonas fluorescens* (10 colonies). Colony counts decreased at higher dilution levels. Since plated volumes, replicate counts, and the full calculation details were not consistently documented, these values are presented as recorded colony counts rather than calculated CFU per gram.

4.2 Colony morphology:

Table 2. Colony characteristics reported for the isolates

Isolate	Culture medium reported	Observed colony characteristics
<i>Azotobacter</i>	Ashby's agar	Milky-white colonies; some developed pigmentation later
<i>Azospirillum</i>	Pikovskaya's medium	Light-yellow to pink colonies; clearing zone reported
<i>Rhizobium</i>	Yeast extract mannitol agar	Small, white, pinpoint colonies
<i>Pseudomonas fluorescens</i>	King's B medium	Light-yellow to green colonies with fluorescence under UV light

The colony characteristics provided preliminary support for the selection of the four bacterial groups. Definitive species-level identification would require additional confirmatory testing.

4.3 Sugar fermentation characteristics:

Table 3. Sugar fermentation reactions

Isolate	Lactose	Glucose	Sucrose	Mannose
<i>Azotobacter</i>	+	–	+	+
<i>Azospirillum</i>	+	+	–	+
<i>Rhizobium</i>	+	–	–	+
<i>Pseudomonas fluorescens</i>	–	–	–	–

Note: + indicates a positive reaction; – indicates a negative reaction.

The isolates showed different carbohydrate reaction patterns. These patterns were used in the original project as part of the identification process.

4.4 Phosphate-solubilization activity:

Table 4. Qualitative phosphate-solubilization results

Isolate	Recorded activity
<i>Azotobacter</i>	Slightly positive
<i>Azospirillum</i>	Positive
<i>Rhizobium</i>	Positive
<i>Pseudomonas fluorescens</i>	Positive

Three isolates showed positive phosphate-solubilization activity, while *Azotobacter* showed slightly positive activity. The qualitative results suggest that the isolates may differ in their ability to mobilize phosphate under the test conditions.

4.5 Soil characteristics:

Table 5. Soil pH and organic carbon values

Soil sample	pH	Organic carbon value reported
Paddy field	8.34	9.8
Groundnut field	7.92	9.5

Both soil samples had pH values above 7, indicating alkaline conditions.

4.6 Extracellular protein concentration:

Table 6. Extracellular protein concentration estimated using the Lowry method

Isolate	Optical density	Extracellular protein ($\mu\text{g/mL}$)
<i>Azotobacter</i>	0.11	100
<i>Azospirillum</i>	0.17	130
<i>Rhizobium</i>	0.23	210
<i>Pseudomonas fluorescens</i>	0.19	170

Among the four isolates, *Rhizobium* showed the highest recorded extracellular protein concentration (210 $\mu\text{g/mL}$), followed by *Pseudomonas fluorescens* (170 $\mu\text{g/mL}$), *Azospirillum* (130 $\mu\text{g/mL}$), and *Azotobacter* (100 $\mu\text{g/mL}$).

4.7 Qualitative IAA production:

Table 7. Qualitative IAA screening results

Isolate	Pink-to-red reaction	Recorded measurement (mm, as interpreted from the project)	Colour
<i>Azotobacter</i>	Present	1.83	Pink
<i>Azospirillum</i>	Present	1.66	Pink
<i>Rhizobium</i>	Present	1.00	Pink
<i>Pseudomonas fluorescens</i>	Present	1.33	Pink

All four isolates produced the reported pink reaction. *Azotobacter* showed the highest recorded measurement in this qualitative assay, followed by *Azospirillum*.

4.8 IAA standard curve and quantitative assay:**Table 8. Absorbance of IAA standards**

IAA concentration (ppm)	Absorbance at 30 minutes	Absorbance at 120 minutes
5	0.13	0.10
10	0.19	0.15
15	0.29	0.21
20	0.32	0.28
25	0.39	0.31
30	0.46	0.38

The recorded absorbance generally increased with IAA standard concentration at both observation times. This trend provides the basis for a calibration curve.

Table 9. Absorbance values of rhizobacterial culture samples

Isolate	Absorbance at 30 minutes	Absorbance at 120 minutes
<i>Azotobacter</i>	0.39	0.31
<i>Azospirillum</i>	0.42	0.36
<i>Rhizobium</i>	0.16	0.15
<i>Pseudomonas fluorescens</i>	0.18	0.14

The highest absorbance values at both time points were recorded for *Azospirillum* (0.42 and 0.36). *Azotobacter* showed the second-highest values (0.39 and 0.31). These results indicate stronger colourimetric responses for these two isolates under the reported conditions.

4.9 Thin-layer chromatography:**Table 10. TLC retention factors of the extracted samples**

Isolate	Sample distance travelled	Solvent-front distance	Recorded Rf
<i>Azotobacter</i>	2.8	6.33	0.44
<i>Azospirillum</i>	3.0	6.33	0.47
<i>Rhizobium</i>	2.6	6.33	0.41
<i>Pseudomonas fluorescens</i>	2.5	6.33	0.39

The project reported an Rf value of approximately 0.47 for standard IAA. The value reported for *Azospirillum* was also 0.47, suggesting a chromatographic spot at a similar position under the solvent conditions used..

4.10 Effect of rhizobacteria on lady's finger growth:**Table 11. Growth parameters of inoculated lady's finger plants**

Treatment	Root length (cm)	Root number	Dry weight (g)	Fresh weight (g)	Plant height (cm)
<i>Azotobacter</i>	8.7	30	2.5	7.4	36
<i>Azospirillum</i>	12.5	35	2.6	8.6	47
<i>Rhizobium</i>	6.7	34	1.9	6.5	32
<i>Pseudomonas fluorescens</i>	6.9	27	1.7	5.6	25
Uninoculated control	6.5	23	1.2	5.5	30

The growth observations showed differences among the inoculated treatments.

- **Root length:** *Azospirillum* recorded the highest root length (12.5 cm).
- **Root number:** *Azospirillum* recorded 35 roots, compared with 23 in the control.

- **Fresh weight:** The highest recorded fresh weight was 8.6 g in the *Azospirillum* treatment.
- **Dry weight:** The highest recorded dry weight was 2.6 g in the *Azospirillum* treatment.
- **Plant height:** *Azospirillum* produced the tallest recorded plants (47 cm).

The results indicate that *Azospirillum* performed best across all five reported growth parameters. *Azotobacter* also showed higher recorded root length, root number, fresh weight, dry weight, and plant height than the control. *Rhizobium* and *Pseudomonas fluorescens* showed mixed responses across the measured parameters.

5. DISCUSSION

The study demonstrated that four rhizobacterial groups could be isolated from agricultural rhizosphere soils and screened for selected plant growth-promoting properties. Their different colony characteristics, sugar fermentation patterns, and phosphate-solubilization reactions indicate variation among the isolates. The phosphate-solubilization results were positive for *Azospirillum*, *Rhizobium*, and *Pseudomonas fluorescens*, while *Azotobacter* showed a slightly positive response. Such activity may be useful for improving phosphorus availability, although quantitative phosphate-solubilization measurements and soil-based validation would be required to determine its practical importance.

The Lowry assay showed that *Rhizobium* had the highest extracellular protein concentration among the tested cultures. This finding should be interpreted cautiously because protein estimation is not a direct measurement of nitrogenase activity or atmospheric nitrogen fixation. Direct nitrogen-fixation assays would be needed to establish the nitrogen-fixing efficiency of each isolate.

IAA screening revealed pink colour reactions for all four isolates. The quantitative colourimetric results showed higher absorbance for *Azospirillum* and *Azotobacter* than for the other two isolates. The TLC results further showed that the *Azospirillum* sample had a reported R_f value similar to that of the standard IAA. These observations support further investigation of the isolates' potential to produce IAA, but chemical confirmation and calibrated concentration measurements are necessary for stronger conclusions. The pot experiment provided the clearest comparative evidence of plant response. *Azospirillum*-inoculated plants had the highest root length, root number, fresh weight, dry weight, and height among the reported treatments. Greater root development may help plants explore a larger soil volume and improve access to water and nutrients. The growth response could reflect multiple mechanisms, including phytohormone production, nutrient mobilization, or other interactions between bacteria and roots. The present experiment does not establish which mechanism caused the observed differences.

The findings are consistent with the general concept that beneficial rhizobacteria may contribute to plant growth. Nevertheless, the lack of reported replication details, error estimates, and statistical testing limits the strength of the conclusions. Further controlled studies using standardized inoculum concentrations, replicated treatments, and field validation are required before recommending these isolates for agricultural application.

6. FIGURES FOR THE RESEARCH PAPER

PLATE I: *RHIZOBIUM*



PLATE II: *PSEUDOMONAS FLOURESENCE*

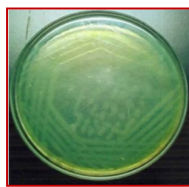
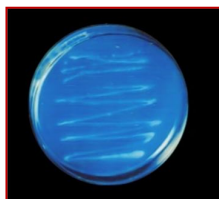


PLATE III: AZOSPIRILLUM**PLATE IV: AZOTOBACTER****7. LIMITATIONS OF THE STUDY**

1. The available project does not clearly report the number of replicates or the variability of the plant-growth measurements.
2. The Lowry method estimates protein concentration and does not directly measure atmospheric nitrogen fixation.
3. The IAA absorbance values are available, but validated regression equations and calculated concentrations are not established in the presented results.
4. TLC Rf similarity provides preliminary evidence and is not definitive chemical identification.
5. The species identities should be confirmed using additional biochemical or molecular methods where possible.
6. The pot experiment requires replicated testing and statistical analysis before the results can be generalized to field conditions.

8. CONCLUSION

The present study evaluated four rhizobacterial groups isolated from paddy and groundnut rhizosphere soils and investigated their potential plant growth-promoting properties. The isolates showed different biochemical characteristics and phosphate-solubilization responses. All four isolates produced a positive colour reaction in the qualitative IAA assay, while *Azospirillum* recorded the highest absorbance in the quantitative assay at both observation times. The reported TLC Rf value for *Azospirillum* was similar to that of standard IAA.

In the lady's finger pot experiment, *Azospirillum*-treated plants recorded the highest values for root length, root number, fresh weight, dry weight, and plant height. *Azotobacter* also showed promising growth responses compared with the untreated control. These observations identify *Azospirillum* as a promising candidate for further investigation as a plant growth-promoting inoculant under the conditions studied.

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