INDIAN STREAMS RESEARCH JOURNAL
ISSN NO : 2230-7850
IMPACT FACTOR : 5.1651 (UIF)
VOLUME - 14 | ISSUE - 8 | SEPTEMBER – 2024

ISOLATION AND SCREENING OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND ITS ROLE IN PLANT GROWTH PROMOTION

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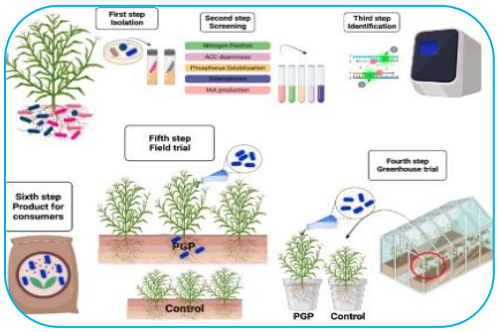
ABSTRACT:-



Plant growth-promoting bacteria are a diverse group of microorganisms that colonize the rhizosphere, the region of soil surrounding plant roots. These bacteria can enhance plant growth and productivity through various mechanisms. They can produce hormones such as indole-3-acetic acid that stimulate root and shoot growth, solubilize nutrients like phosphorus, fix atmospheric nitrogen, and protect plants from diseases and pests. Additionally, plant growth-promoting bacteria can also help plants tolerate abiotic stresses such as drought, salinity, and heavy metal toxicity. These beneficial bacteria have shown great potential in sustainable agriculture and phytoremediation processes, as they can enhance crop yields, reduce the use of synthetic fertilizers and pesticides, and remediate contaminated soils. Plant growth-promoting bacteria are microorganisms that inhabit the rhizosphere of plants and can enhance plant growth and productivity through various mechanisms. Qualitative analysis of indole acetic acid and gibberellic acid production was carried out. Other plant growth mechanisms such as ammonia production and phosphate solubilization are also done. Isolation of microorganisms is carried out from maize and cotton rhizosphere All 4 isolates show distinct characteristics by showing different growth-promoting activities. Subsequently, the effect on plant growth was tested by pot assay. In conclusion, the study suggests that plant growth promoting rhizobacteria and their metabolites as efficient microbial inoculants to promote plant growth.

KEYWORDS: *PGPR, Indole acetic acid, Gibberellic acid, Phosphate solubilization, Rhizobacteria.*

INTRODUCTION

The rhizosphere is a suitable space for microbes. A large number of plant growth-promoting rhizobacteria (PGPR) can be found in the rhizosphere. The bacteria which are present near the root and enhance the plant growth by any mechanism are called as PGPR. Which can be used in the development of bio-inoculants that enhance the growth and yield of crops. The organism found in soil produces some substance that is responsible for plant growth promotion. Phytohormones are known as plant growth regulators and are an organic substance that helps in the growth and development of plants. Plant hormones (phytohormones) are categorized into three classes such as growth promoters, growth inhibitors and growth inhibitors, and growth inhibitors and promoters. The growth promoter is further classified into three subunits Auxins (IAA), Gibberellins, Cytokinins and growth inhibitor is Abscisic acid while the growth inhibitor and promoter is ethylene. (Maheshwari D, *et al.*,2016).

Plant growth-promoting rhizobacteria (PGPR) aid plants in a variety of ways by Direct or Indirect mechanisms including the formation of secondary metabolites such as antibiotics and cyanide, as well as phytohormone-like compounds such as Indole-3-acetic acid (auxin), gibberellin, Cytokinin and ethylene. Synthesis of siderophores for iron immobilization; resistance to soil-borne root diseases; and phosphate solubilization (Kumar A, *et al.,* 2012,Gupta G *et al*; 2015)

Some PGPRs can stimulate plant development by direct mechanisms such as nutrient solubilization, nitrogen fixation, and the generation of growth regulators. The plant growth-promoting rhizobacteria PGPR may be established that inoculation can promote nodulation, nitrogen uptake growth, and yield responses of crop plants using microorganisms as co-culture. The PGPR includes the endophytes and Frankia species both of which can symbiotically fix atmospheric N2 in the higher plant (de Andrade, L.A; 2023).

Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria that colonize the rhizosphere and, when administered to crops, boost plant development. Plant growth-promoting rhizobacteria (PGPR) are commonly used to boost growth in a variety of agricultural crops, including seed germination, plant weight, and harvest yields. PGPR colonization promotes plant growth through bacterial manufacture of plant hormones such as indole-3-acetic acid, cytokinin, and gibberellins, as well as improved mineral and nitrogen availability in the soil. Some of them were also recognized to defend their host plant from harmful bacteria (Igiehon B C; *et al* 2024, Desai S A;2017). A cluster of microorganisms can typically be found either within the rhizosphere or around the root, providing plants with certain advantageous effects.(Leach, J. E., *et al.,*2017). PGPRs are bacteria that are environmentally friendly, residing in the root zone of plants and enhancing their ability to combat pests, ultimately encouraging plant growth. Encouraging the use of PGPRs in farming can decrease the need for artificial fertilizers and emphasizes the use of organic resources found in nature (Andy A.K;2020)

MATERIALS AND METHODS

1. Sample Collection:

Soil samples were collected from the rhizosphere of Cotton and Maize plants at depths of 6 cm to 12 cm growing at different sites at Erendol, Jalgaon, and Maharashtra in India. The intact root system was dug out and the rhizospheric soil. samples were carefully taken in plastic bags and stored at 4^0C. A total of two soil samples were collected for the isolation of rhizosphere bacterial isolates (Chakra P.S, *et al*.,2019)

2. Isolation and Screening of PGPR:

The rhizosphere soil samples were gathered and placed into fresh, unused polythene bags before being taken to the lab. Non-discriminatory media like TSB medium are utilized to isolate rhizosphere bacteria. Roughly 1 gram of soil specimen was mixed in 100 ml of sterile tryptic soy broth. The specimen was kept in an incubator at a temperature of 28°C for a duration of 48 hours. Following incubation, streaking was performed on a TSBA plate and incubated for an additional 24 hours. Mucoid colonies were chosen and streaked onto a new nutrient agar plate in order to isolate a pure culture (Dhruv P,*et al*.;2022)

3. Indole Acetic acid (Qualitative method):

Fifty millilitre of Nutrient broth (NB) containing 0.1% DL-tryptophan was inoculated with 500 µl of 24 h old bacterial cultures and incubated in a refrigerated incubator Shaker at 30±0.1 °C and 180 rpm for 48 h in the dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4 °C. Estimation of indole-3acetic acid (IAA) in the supernatants was done using a colourimetric assay. One millilitre of supernatant was mixed with 4 ml Salkowski reagent and absorbance of the resultant pink colour was read after 30 min at 535 nm in UV/Visible Spectrophotometer. The appearance of pink colour in test tubes indicated IAA production described by Gordon and Weber. (Kumar A, *et al.,* 2012, Chandra S*, et al* 2018)

4. Gibberellic acid (Qualitative method):

This test used nutrient broth media. One ml of bacterial isolates were added to the media and incubated at 37˚C for seven days. The cultures then were centrifuged at 8000 g for 10 min to remove the bacterial cells. Fifteen cultures were added to 5 ml of zinc acetate. Account after 2 minutes, 2 ml of potassium ferrocyanide solution and centrifuged at 8000 g for 10 min. Five ml of the supernatant was added to five ml of 30 percent hydrochloric acid and the mixture was incubated at 270 C for 75 minutes. The blank was prepared with five percent hydrochloric acid. The colour change indicated presence of Gibberelic acid (Kesaulya H *et al.,* 2015).

5. Phosphate solubilization (Qualitative method):

The method outlined by Pikovaskaya was used to test the solubilization of phosphate. Suspension of a bacterial isolate that is 24 hours old and was streaked on solid media containing tricalcium phosphate (Ca3PO4) using the dispersive method. Solubilization activity was indicated by the presence of clear halos surrounding every bacterial colony. After 3 days of incubation, the halos/zones around the colonies displayed phosphate solubilization (Karnwal A, 2019). The halos observed around the colonies post-incubation for 3 days indicated bacterial activity in phosphate solubilization.. (Kesaulya H *et al.,* 2015,Li Land Chen Xu;2023).

6. Nitrogen fixation (Qualitative method):

Isolated bacteria were inoculated on Nitrogen Free Mannitol Broth with composition:- Mannitol: 2 gm K2HPO4 : 0.020gm MgSO4 : 0.020 gm NaCl : 0.020 gm K2SO4 : 0.010gm CaCO3 : 0.500 gm Distilled water : 100ml pH: 7.2 at 37^{o}C for 78hrs . After that bacterial strain was inoculated on a Nitrogen Free Mannitol Agar medium. Incubate at room temperature for 48-96hrs. Nitrogen-free mannitol agar plate incubated at room temperature for 7 days. (Gupta S, *et al.,* 2014).

7. Pot Assay Technique:

Selected Cotton and Maize seeds were collected. The seeds were soaked in Indole acetic acid and Gibberellic acid obtained from centrifugation for 1H. The soaked seed was then sown in sterile soil and incubated for 15 days. Observe the positive results. Germination rate, % increase in shoot length and % increase in root length calculated.

RESULTS

1. Sample collection :

Fig. 1.1: Collection of soil samples from the rhizosphere of cotton and maize plants.

2. Screening of isolates:

All 4 isolates collected from the Department of Erandol rejuvenated successfully on Ashby's Mannitol Agar (Hi-Media).

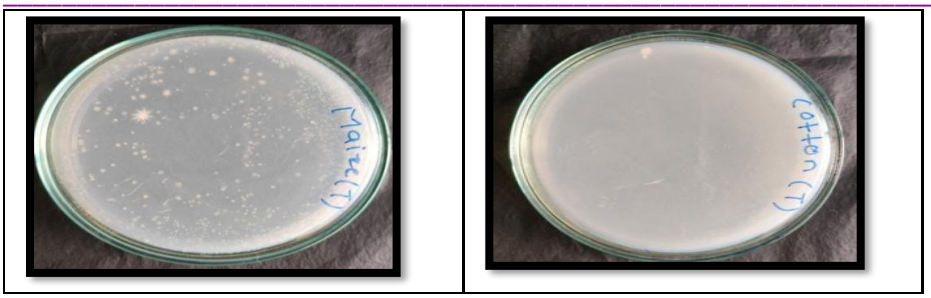


Fig.2.1: Bacterial isolates on Ashby's mannitol agar from cotton plant rhizospheric soil.

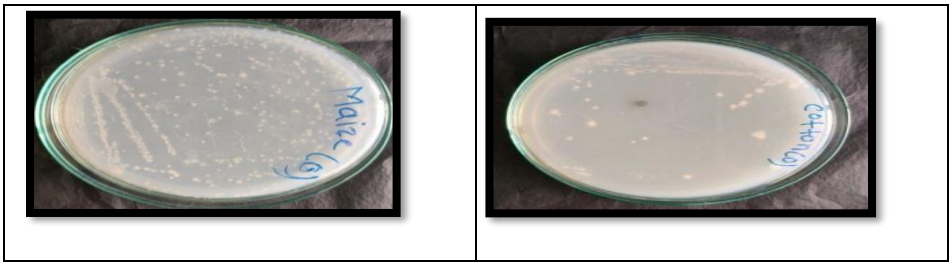


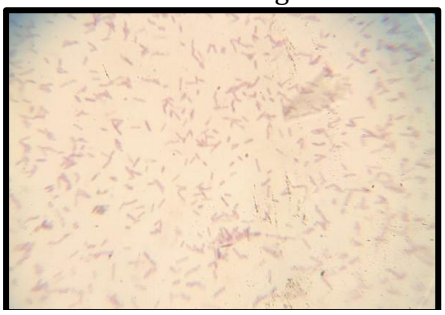
Fig.2.2: Bacterial isolates on Ashby's mannitol agar from maize plant rhizospheric soil.

3. Morphological Characterization:

Rejuvenated isolates were further characterized morphologically. Each isolate indicated almost the same morphological characters showing only a difference in colony size, opacity and other characters.

3.1: Microscopic observation under 100x lens indicating Gram-negative character.

Bacterial isolates namely cotton (1), (2) and Maize (1), (2) were grown on Ashby's Mannitol Agar to study their morphological characters. All isolates showed Whitish colonies and were Mucoid colonies. Gram staining results revealed that all isolates are gram-negative rods.



Gram Staining of the Isolate

Table 1.1: Colony Character of bacterial isolates.

Colony character	Cotton(1)	Cotton (2)	Maize (1)	Maize(2)
Shape	Circular	Circular	Circular	Circular
Size	2mm	1mm	3mm	1mm
Color	Whitish	Whitish	Whitish	Whitish
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Flat	Convex	Flat
Opacity	Opaque	Translucent	Opaque	Translucent
Consistency	Mucoid	Mucoid	Mucoid	Mucoid
Gram character	Gram Negative	Gram Negative	Gram Negative	Gram Negative

4. Indole acetic acid production:

One milliliter of supernatant was mixed with 4 ml Salkowski reagent and incubated in dark for 30 min.

The appearance of pink colour in test tubes indicated IAA production described by Gordon and Weber method.

Fig.4: Appearance of red colour in test tube indicating IAA production.

5. Gibberellic acid production: The appearance of colour change indicates Gibberellic acid production. Both isolates from cotton produced Gibberellic acid.

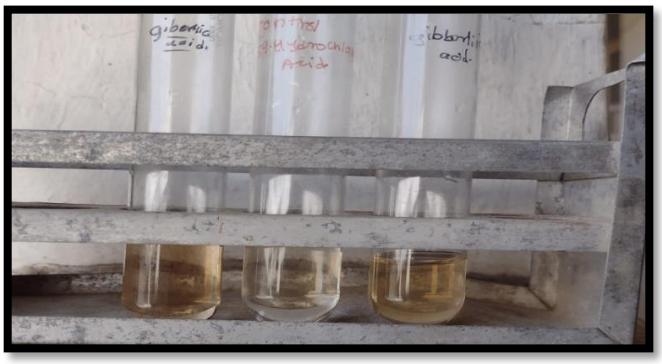


Fig.5: Appearance of colour change indicating production of gibberellic acid.

6. Phosphate Solubilization:

The formation of transparent halos around each bacterial colonies showed solubilization activity. The resulting halos zone around the colonies after incubation for 3 days showed the presence of bacterial activity in solubilization phosphate.

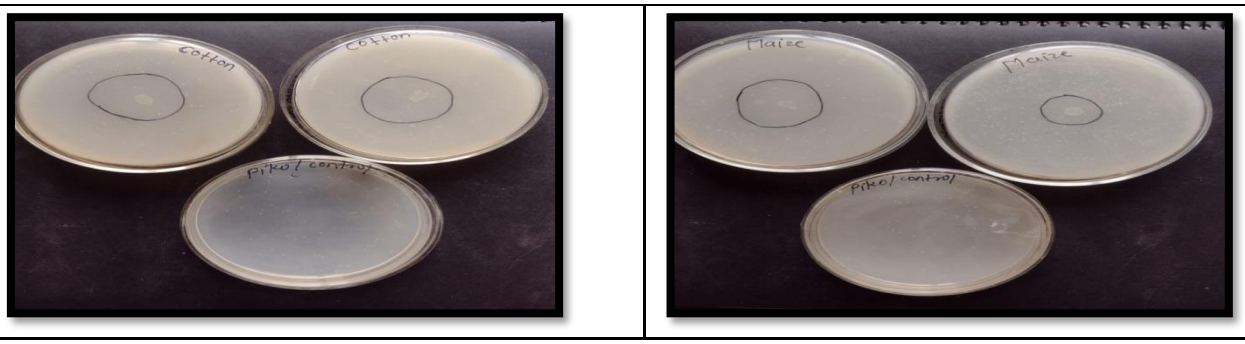
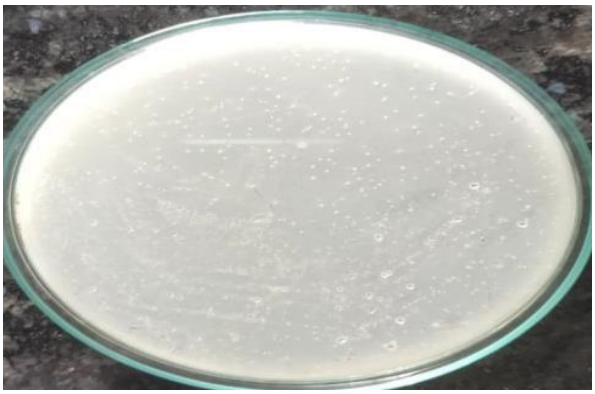
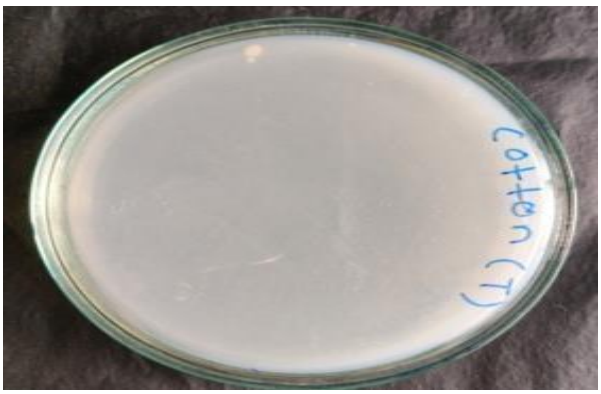


Fig.6: Hellos zone observed after incubation of 3 days on pikovaskaya agar plate showing phosphate solubilization .

7. Nitrogen fixation: After 7day the growth was observed on plates it indicating nitrogen fixation as there is no nitrogen source present in the medium but still organism is still able to grow on nitrogen deficient media suggests that organism possesses ability to fix the nitrogen.





8. Pot assay technique: The seed treatment was given by IAA and GA then the seed showed growth after 15 days of incubation. A significant change in the stem and root growth length occurred.

9.

GERMINATION RATE= (No. of seed germinated / No. of seed sown) X 100

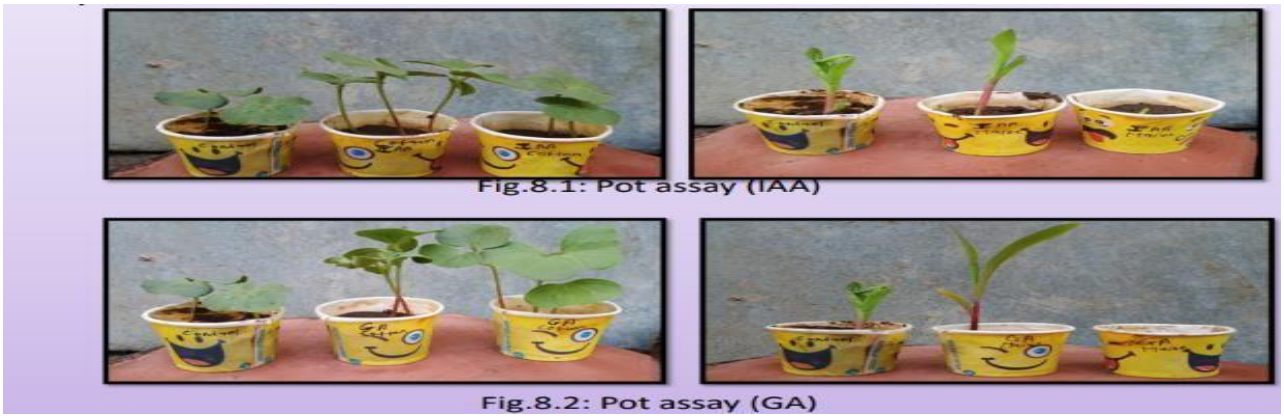


Fig.8.1: Pot assay (IAA)

Fig.8.2: Pot assay (GA)

Fig.8.3: Measured root and shoot growth (seed soaked in IAA)

Fig.8.4: Measured root and shoot growth (GA)

POT ASSAY	No. of seed sown	No. of seed germinated	% germination rate	Average shoot length (cm)	Average root length(cm)
IAA-Control Soil+uncoated cotton seed+D/W	8	2	25%	6cm	2cm
IAA-Test Cottonseed+Sterile soil+liquid IAA	8	6	75%	9cm	5cm
IAA-Control Soil+uncoated maize seed+D/W	4	1	25%	7.5cm	9cm
IAA-Test Maizeseed+Sterile soil+ liquid IAA	4	1	25%	9cm	10cm

Table1.2: Measurement of root and shoot of plants treated with IAA

POT ASSAY	No. of seed	No. of seed germinated	% Germination Rate	Average shoot length (cm)	Average root length(cm)
GA-Control Soil+uncoated cotton seed+D/W	8	2	25%	6cm	2cm
GA-Test Cottonseed+Sterile soil+liquid GA	8	4	50%	9cm	11cm
GA-Control Soil+uncoated maize seed+D/W	4	1	25%	7.5cm	9cm
GA-Test Maizeseed+Sterile soil+ liquid GA	4	1	25%	13cm	8cm

Table.1.3: Measurement of root and shoot of plants treated with GA

DISCUSSION

PGPR colonizes the roots of plants and promotes plant growth and development through a variety of mechanisms. This mechanism such as the production of phytohormones, activation of phosphate solubilization. In the present study, beneficial bacteria were isolated from cotton and maize rhizosphere. Isolated bacteria were screened for different plant growth promotion activities and characterized by morphological characterization current study also indicated that Phytohormones produced by PGPR such as indole acetic acid promote growth of root length in Cotton and Maize and

Gibberellic acid promotes growth in shoot length in both Cotton and Maize. The percentage germination rate increased with the application of phytohormones.All the isolates showed growth on nitrogen free mannitol agar indicates that all isolates have ability to fix nitrogen. All isolates showed growth on Pikovaskaya agar plates it indicate that they have phosphate solubilizing ability which is characteristic of PGPR. . Few bacterial isolates showed more than 10 mm zone of phosphate solubilization. It has been reported that higher concentrations of phosphate-solubilizing bacteria are commonly found in the rhizosphere soil as compared to nonrhizospheric soil. IAA is one of the most important phytohormones and functions as an important signal molecule in the regulation of plant development. In our study, most of the bacterial isolates were positive for IAA production. In the present work, bacterial isolates were positive for Gibberellic acid production. Such type of study is necessary as it shows that the use of PGPR as inoculants or biofertilizers is an efficient approach to replace chemical fertilizers. 2 strains were isolated from plant rhizosphere and are collected from different cultivable land near my village (Moykheda, Jamner, Jalgaon, Maharashtra) Morphological structures and Specific growth promotion tests such as Nitrogen fixation, Phosphate solubilization and IAA production performed by the microbial strains these characteristics were studied.

CONCLUSION

We concluded that phytohormones like Indole-3-acetic acid and Gibberellic acid are produced by most of the rhizobacteria under study which aids plant growth promotion and satisfying hormonal needs. Bacterial isolates showed Phosphate solubilization and Nitrogen fixation which promotes plant growth. PGPR may have practical biological applications in plant growth characteristics which can potentially replace the use of chemical fertilizers. The use and application of such bioformulation in the fields can result in a reduction of the application of harmful chemicals; and protect the environment and biological resources.

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