



“Isolation, Screening And Characterization Of PGPR From Different Agricultural Rhizospheric Soil.”

Krishna Varma, Guru Nikam, Dr. Anupriya Chudiwal

Student at MGM University, Student at MGM University, Assistant Professor and project guide.

Department of Biotechnology MGM University,
MGM University, Chh. Sambhaji Nagar 431001, India.

Abstract: The PGPR Plant growth promoting rhizobacteria are most beneficial strain of bacteria that enhance the plant growth and biocontrol by direct and indirect mechanism. The PGPR identified as the efficient microbe as bio fertilizer for plant growth. Hence the present investigation was carried out to isolate and characterize the PGPR from rhizosphere soil. A total ten in number isolates were isolated from soil sample and were characterize by different characterization techniques. In this present study the out of ten isolates two 10^{-5} F1 and 10^{-5} F3 shown high IAA production, displayed phosphate solubilizing activity in Pikovskaya agar, almost all isolates were positive for catalase test and showed antifungal activity against *Aspergillus* species.

Index Terms - Rhizobacteria, phosphate solubilizing, antifungal activity.

I. INTRODUCTION

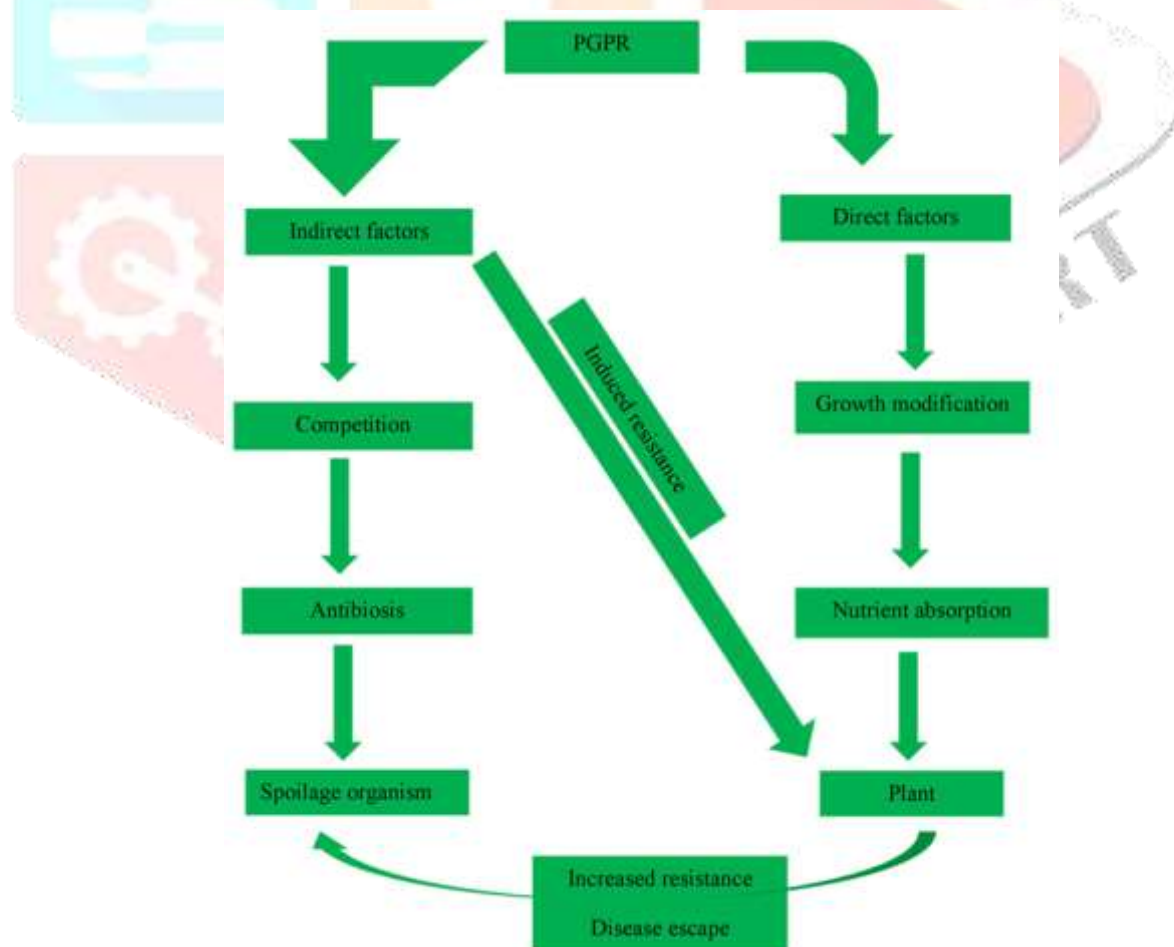
The Plant growth promoting rhizobacteria (PGPR) are the group of beneficial bacteria that enhance plant growth and bio control by wide variety of mechanisms (Kloepper et al., 1978). In the increasing of the world concern for the food and safety and environmental quality, the utilization of PGPR of minimizing. (Tiwari et al 2016) the chemical inputs in agricultural practices in a potential important issue (Verma et al., 2015). The modern agriculture relies on excessive use of chemical fertilizers and pesticides to increase crop production and variety which cause severe effect on the crop and soil health and environment (Aktar et al., 2009). However, the PGPR in agricultural in order to enhance the growth of plant via providing the nutrients in the soil is an ecofriendly strategy to minimize the use of synthetic fertilizers as much as possible (Saharan et al., 2011).

PGPRs can be defined as beneficial bacterial strains that colonize the roots of plant for growth stimulation and biocontrol potential for the growth of plants (Suslow. et al., 1979). They can affect plant growth by promoting plant microbe symbiosis, competition for colonization space and nutrients and decreasing the activity of plant pathogen (Glick 1995). The PGPR stimulate plant growth and control by various direct and indirect mechanisms. Direct mechanism of PGPR includes facilitating resource acquisition i.e. solubilization of phosphate, nitrogen fixation (Patten et al., 1996). Indirect mechanism of PGPR includes fungal, bacterial and nematode pathogens by the production of various enzymes and compounds likewise the chitinase protease cellulase HCN and ammonia (Nehra et al., 2011).

Plant roots with bacteria have a beneficial impact on the development and production of crop plant (Fausi et al., 2021). The syndicate of bacterial species is the PGPR that lives in the soil found around the plant root, influencing plant development and is profitable health – related. They are agricultural biological resources that induce the plant's growth and fruitfulness. They also motivate resistance in plants, i.e., a wide range of vegetation of fruits, vegetables, and various forest trees, to different phytopathogens (Zia et al., 2020).

In the recent decades, the importance of reducing chemical inputs into horticulture system has been growing and governments and growers are aiming to decrease them. The chemical contamination of underground water and rivers and the salinization of soils caused by horticulture practices have risen in recent years (Phogat et al., 2014). Therefore, solution to maintain crop productivity simultaneously reduces chemical inputs in terms of chemical fertilizers and pesticides. Another resource that limits plant productivity is water. According to global-change or dictions several areas of the globe could become arid or semi-arid regions due to a reduction in precipitation (Sivakumar 2011). Therefore, any strategy that reduces that amount of water added to crops without a loss in yield is the desirable.

The Plant always have been symbiotic relationship between with the several form of soil microbes for their growth and development and other requirements. The symbiotic microorganisms inhabiting the rhizosphere of many plant species have diverse beneficial effects on the host plant. These beneficial free-living soil bacteria are usually referred to as PGPR. This plant growth promoting bacteria help as bio-fertilizer for their growth and development. It is generally achieved through direct or indirect approaches. In the direct approach, a bacterium directly provides the plant with essential compounds that can promote plant growth and can be achieved through techniques such as bio-fertilization, rhizo-remediation and plant stress control. The ability of absorbing water, temperature and other nutrients are some of the environmental factors that strongly restrict terrestrial plant's growth. The introduction of PGPR improves the plant growth by increasing its water accessibility and uptake of nutrients from a limited nutrient pool in the soil through the process of bio-fertilization. It also involves rhizo-remediation, which is the interaction of soil and microbes with the plant to enhance plant growth. Plant stress neutralization is another important factor of PGPR where it basically neutralizes two types of stress, that is biotic and abiotic stress. Therefore, approaches that can be adapted for the implementation of PGPR as tools to combat plant diseases and enhance agricultural productivity (Sushanto gouda 2018).



II. METHODOLOGY

2.1 Collection of soil sample:

Rhizosphere soil samples of different plants were collected from different agricultural areas of Chh. Sambhajinagar. The soil samples were carefully collected and transferred in sterile polythene bags and labelled properly.

2.2 Isolation of bacterial Isolates:

Bacteria were collected from the rhizospheric soil samples by serial dilution technique on nutrient agar (NA) plates and incubated at 28°C for 72 hours. After incubation period, NA plates were observed for morphological appearances and number of bacterial colonies. Bacterial isolates having different morphological appearance on agar plates were selected and maintained on nutrient agar slant and 50% glycerol and stored in refrigerator.

2.3 Characterization of bacterial isolate for different plant growth promoting activities:

2.3.1 IAA production:

Indole acetic acid (IAA) production was quantitatively estimated by Salkowski method. Bacterial cultures were grown on Luria broth liquid medium at 37°C. Fifty millilitre of Luria Bertani (LB) broth containing 0.1% DL tryptophan was inoculated with 500 µl of 24 h old bacterial culture and incubated in refrigerated incubator shaker at 30°C at 180 rpm for 48 h in dark. Fully grown bacterial culture was centrifuged at 10,000 rpm for 10 min at 4°C. 1 ml of supernatant was mixed with 100 µl of 10mM orthophosphoric acid and 2 ml of the Salkowski reagent (1 ml of 0.5 M FeCl₃ in 50 ml of 35% HClO₄) at 28°C for 30 min. Development of orange colour in test tubes at the end of the incubation indicated IAA production. (Brick and Gordon et al. 1991).

2.3.2 Phosphate solubilization:

Loops full of fresh bacterial cultures was streaked on the centre of agar plates modified with Pikovskaya agar with insoluble tricalcium phosphate (TCP) and were incubated for 120 h at 28°C. The presence of halo zone around the bacterial colonies indicated positive phosphate solubilization ability. (Pikovskaya et al. 1948)

2.3.3 Catalase activity:

Bacterial cultures were grown in nutrient agar medium for 48 h at 28°C. 48 hr old bacterial colonies were added with 2-3 drops of hydrogen peroxide (3%) on a clean glass slide and mixed using a sterile tooth pick. The evolution of oxygen as effervescence indicated catalase activity. (Rorth et al. 1967).

2.3.4 Gram staining:

The isolated bacteria were identified based on the characteristics including microscopic characteristics using compound microscope with the help of gram staining.

2.3.5 Antifungal activity:

The antifungal activity of the PGPR isolates was evaluated by agar well diffusion method against *Aspergillus* sp. Sterile Mueller Hinton agar plates were made using aseptic methods. The plates were then inoculated with 6 days old *Aspergillus* sp. broth culture using spread plate method. After which using a sterile well borer a well was made, one in each plate. This was done for each PGPR isolate separately and plates were labeled accordingly. After which the 24 hours viable broth cultures of the PGPR isolates were centrifuged at 10,000 rpm for 10 mins. The resulting supernatant was collected in sterile tubes. In each well, 20 µl supernatant of the respective isolate was added aseptically. The plates were then incubated at

28°C for 7 days. After incubation period was complete the plates were examined for zone or clearance/inhibition (Balouiri et al. 2016).

III. RESULTS AND DISCUSSION

3.1. Collection of samples:

Rhizospheric soil samples were collected from agricultural areas of Chh. Sambhajinagar. Soil samples were collected in the sterile polythene bags.

3.2. Isolation of bacterial isolate:

The collected soil samples were immediately shifted to laboratory in sterile polythene bags and then the further process for isolation of bacteria was carried out where we were done the serial dilution and isolates the bacteria where we used the Nutrient Agar for their growth and provided them the optimum temperature 37°C for their growth in the Incubator.

3.3. Isolation of Bacteria and Fungi:

The PGPR which are colonized on roots of plants where they have beneficial effect on the plant growth and development of plant in the very effective manner. In the present study we have isolated the PGPR from the plants rhizosphere which were grown in agricultural field and isolated the bacterial colonies through the process of serial dilution where we used the 1gm of soil and serially diluted it. The bacterial colonies were isolated on the Nutrient Agar Plates and total 10 pure bacterial colonies were isolated by subculturing the bacterial colonies. (10^{-5} F3, 10^{-6} F2, 10^{-4} F1, 10^{-5} E4, 10^{-4} F4, 10^{-5} F1, 10^{-6} F1 10^{-1} B1, 10^{-5} F3, 10^{-6} E5). Then further the characterizations of the Bacterial isolates were carried out.

3.4 IAA production and phosphate solubilization:

IAA is usually considered to be most vital phytochrome that functions as important signal for the plant growth and development. In the present study the isolates were induced the production of IAA in the presence of tryptophan among the all isolates the 10^{-5} F1, and the 10^{-5} F3 were found to produce the high IAA production rather than the other isolates. However, the IAA production varies from the different strains and species.

3.5 Phosphate solubilization:

Phosphorus is the second most important nutrient used by plants after ammonia for their growth and development. The most of the phosphorus present in the soil cannot be used by the plants. In this study the isolates were found to be clear zone on the pikovskaya agar containing insoluble phosphate as tri - calcium phosphate.

3.5 Antifungal Activity:

The Antifungal activities of isolates were tested against the *Aspergillus* sp. on the Mueller Hinton agar using the well diffusion technique. The maximum inhibition was shown by the 10^{-5} F1. The zone of inhibition was recorded by the F1 isolate was 10 mm.

3.6 Catalase Test:

The catalase test was also performed; the all isolates were positive for the catalase test these show the rhizobacterial isolates showing the catalase activity were highly resistant to the chemical and environmental stress.

3.7 Gram staining:

The microscopic observation such as gram staining was performed the shape of bacterial isolate was observed the bacteria was gram positive cocci shaped. (fig. 5 and 6)



Figure 1- Bacterial Isolates

Table 1 IAA PRODUCTION

Isolates	Colour Change	O.D at 540nm
10 ⁻⁵ F1	++	0.27
10 ⁻⁵ F3	+	0.24

Table 2. Catalase test

Isolates	Results
10 ⁻⁵ F1	Positive
10 ⁻⁵ F3	Positive

Table 3. Antifungal test

Isolates	Zone of Inhibition
10 ⁻⁵ F1	10mm
10 ⁻⁵ F3	-

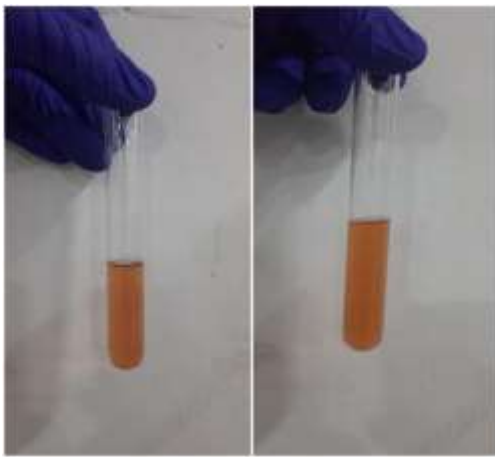


Figure 2- IAA production



Figure 3- Catalase test

Zone of inhibition recorded

Zone of inhibition recorded

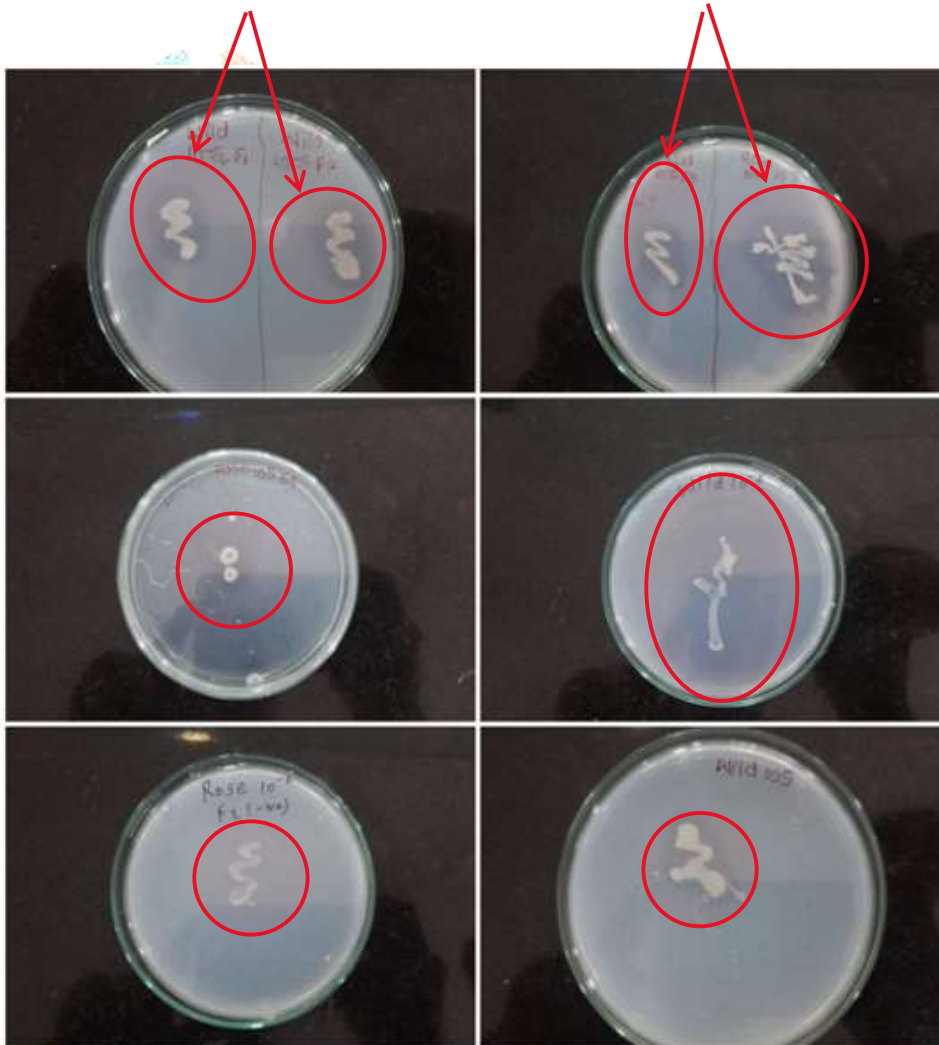


Figure 4- Phosphate Solubilization zone of Inhibition around the Bacterial Isolates

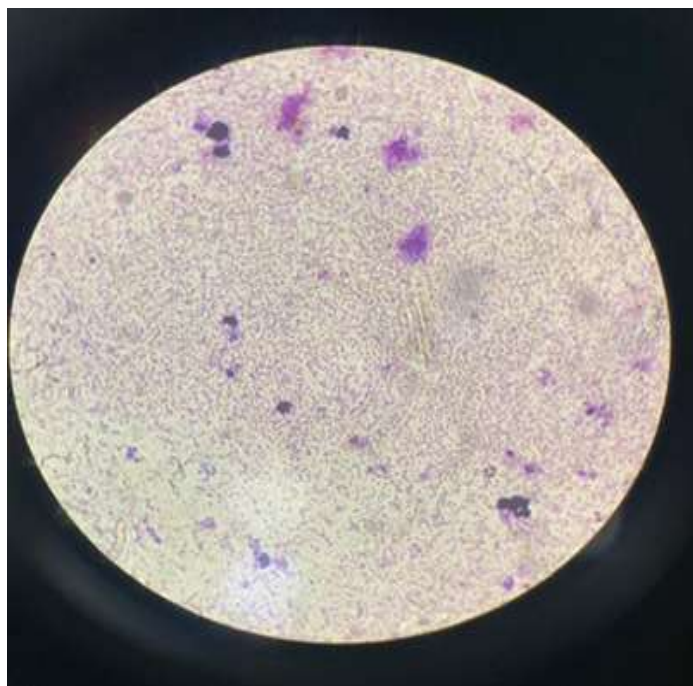


Figure 5 – Gram staining of Bacterial Isolate 10⁻⁵ F1

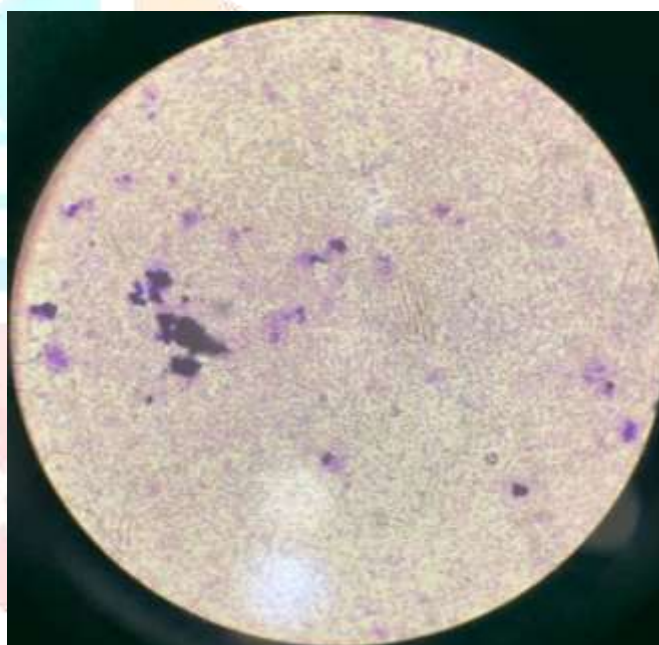


Figure 6 - Gram Stanning of Bacterial Isolate 10⁻⁵F3

In recent years, there has been a growing interest in endophytic rizophoric bacteria and PGPR which are beneficial to the plant growth and development, Studies have shown that rizophoric bacteria and PGPR, have a wide range of beneficial effects on their host plants, including growth promotion, disease resistance, and stress tolerance. In this discussion, we will be focusing on a specific study that investigation on the isolation and characterization of PGPR and study it.

In this study we had collected the soil samples and isolated the bacteria using previously established methods. The isolated bacteria were identified using gram staining and characterization using the IAA production, catalase test, antifungal test, and phosphate solubilization. To determine the antifungal activity, the zone of inhibition was measured using established methods.

In this present study we found that the soil samples collected from the agricultural field had the bacteria and the PGPR were present. However the isolates obtained from the sample were tested positive for the antifungal activity, the isolates were tested positive for the IAA production there was the sudden color change was observed and all the isolates tested positive for the catalase test, where the phosphate solubilization was also tested positive were we got the halo zone appearance in the plates and derived that the bacteria were phosphate solubilizing. However, the PGPR in agricultural in order to enhance the growth of plant via providing the

nutrients in the soil is an ecofriendly strategy to minimize the use of synthetic fertilizers as much as possible (Saharan B.S. et al., 2011).

In conclusion the results states that all the isolates having the plant growth promoting activities and were found to be producing, IAA and insoluble phosphate, catalase, and also showed antifungal activity against the *Aspergillus* sp.

Conclusion

The study involved the collection of soil samples from the agricultural fields, from which bacteria were isolated and identified using Gram staining and characterization methods such as catalase test, IAA production, Antifungal test, Phosphate solubilization for bacteria and. The antibacterial and antifungal activity of the isolated bacteria was also determined by measuring the zone of inhibition. The study found that rhizosphere bacteria and PGPR can promote plant growth and enhance their resistance to various pathogens and environmental stresses. Additionally, rhizosphere and PGPR bacteria have diverse applications in agriculture, medicine, and other industries.

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