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Study of Plant Growth Promoting Abilities of *Rhizobium* Strains Isolated from Mungbean Root Nodules

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Plant Growth Promoting Rhizobacteria (PGPR) are a type of bacteria that use biofertilizers and other plant growth-promoting agents to increase plant output and growth. In the last several decades, there has been a global surge in the usage of beneficial soil microorganisms like PGPR for safe and sustainable agriculture due to the detrimental effects of artificial fertilizers on

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the environment and their rising prices. Plant-beneficial rhizobacteria have the potential to reduce the world's reliance on dangerous agricultural chemicals that upset agro-ecosystems. Today a large part of yield is lost due to the various stress mechanisms of plants. It could be classified into biotic and abiotic stress. These are generally a group of microorganisms that is found either in the rhizosphere or in the root nodules of plants. PGPR has beneficial impact, they colonize in the plant roots and improve crop yields and agricultural productivity and also produce some useful growth promoting growth hormones *viz.,* IAA, Auxins, Cytokinin and Gibberellins.

Methods: The bacterial isolates were screened for plant growth promoting traits such as phosphate solubilization, IAA production and siderophore production was tested qualitatively by using pikovskayas media, TSB (Tryptone Soy Broth) agar and Chrome Azurol Sulphate (CAS) blue agar respectively.

Results: In present study out of the eight rhizobial isolates, five exhibited the phosphate solubilization zones, three shows negative result. However out of eight isolates five isolates produced IAA and three showed negative result. Among all isolates seven isolates were able to produce siderophore, while one showed negative result.

Keywords: IAA production; phosphate solubilization; plant growth promoting rhizobacteria; rhizosphere; siderophore production; sustainable agriculture.

1. INTRODUCTION

Legume plants have the unusual capacity to form symbiotic relationships with Rhizobia, which fix nitrogen. Most legume crops yield more when *Rhizobium* inoculants are used, and they can also minimize the usage of synthetic fertilizers, which are costly and deplete soil fertility. Rhizobia not only fix nitrogen symbiotically but also create PGRs, or plant growth regulators. "The term PGPR was first used by Kloepper in [1]. It is also referred to as Nodule Promoting Rhizobacteria (NPR) and Plant Health Promoting Rhizobacteria (PHPR) when referring to rhizosphere-found PHPR and NPR. Plant Growth Promoting Rhizobacteria (PGPR) comprise a several type of bacteria that provide growth benefits to plants through several mechanisms. It regulates the growth of plants either through direct or indirect mechanism. It may also include the addition of compounds which are related to the microbe metabolism" [2,3].

"The rhizosphere of plant tends to contain PGPR that are capable of releasing protons to solubilize soil-bound phosphorus for plant use, and are usually referred as Phosphorus Solubilizing Bacteria (PSB). Furthermore, due to excessive use of the plant protection chemicals, rhizosphere microflora gets diminished in a negative way by learning from associative favorable microbes to detrimental ones. A more sustainable alternative is to use Plant Growth Promoting Rhizobacteria (PGPR). PGPR based inoculants are widely being accepted globally as an alternative for chemical fertilizers in view of agricultural sustainability. Rhizobacteria increases plant growth by production of plant growth hormones, those enhance the uptake of other nutrients, induce root exudation and suppresses phytopathogens are termed as plant growth promoting rhizobacteria" [2].

"IAA is the main auxin in plant regulating growth and developmental processes such as cell division, cell elongation, tissue differentiation, apical dominance and roots are most sensitive to fluctuations in IAA level. Phosphate- solubilizing bacteria play an important role in plant nutrition through the increase in P uptake by the plant and their use as PGPR is an important contribution to biofertilization of agricultural crops. The phosphate solubilizing microorganisms (PSM) are the important contributors to soil P pools which constitute 0.4% to 2.4% of total P in arable soils. They also decompose the organic residue by immobilization and mineralization thus maintaining equilibrium with soil solution P pools. Siderophores have application in microbial ecology to enhance the growth of several unculturable microorganisms and thus can alter the microbial communities. In the field of agriculture, different types of siderophores promote the growth of several plant species and increase the crop yield" [3].

"Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria which increases the plant growth and yield and also various plant growth promoting substances such as biofertilizers. The use of beneficial soil microorganism such as PGPR for sustainable and safe agriculture has

enhance globally during the last couple of decades. These are the group of bacteria that could be seen in the rhizosphere and are known as the promoter of plant growth. It colonizes the part of root and soil environment called rhizosphere. Rhizosphere shows the maximal activity of microbes with the confined environment consisting of many essential micro and macro nutrients. Taking in account the above points, a study of Plant Growth Promoting abilities of *Rhizobium* Strains isolated from mungbean rootnodules was conducted" [2].

"PGPR is known to improve crop yield and plant protection. Studies on PGPR have attracted increasing scientific interest in recent decades. Through direct or indirect mechanisms, the PGPR stimulates the growth and development of plants" [4]. Plant growth promoting rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays.

2. MATERIALS AND METHODS

Total eight isolates of rhizobia were successfully obtained from root nodules of green gram plants collected from different location of Jalna district. All isolates exhibited maximum growth on YEMA selective medium. The colonies isolated on YEMA medium displayed characteristics such as being circular, mucoid, white, translucent, and possessing a sticky appearance. All isolates were gram-negative and had a rod-shaped morphology. Colonies of bacteria become visible after 24 hours of inoculation. Isolates were designated as RDB, RBJ, RRB, RDM, RKP, RMG, RSA, and RKJ.

2.1 Phosphate Solubilizing Test

Phosphate solubilizing activity was tested qualitatively using pikovskayas media, containing 10g glucose, 5g $MgCl_2.6H_2O$, 0.25g $MgSO_4.7H_2O$, 0.2g KCL, 0.1g $(NH_4)_2SO_4$, 15g agar, 1L distilled water and 5g tricalcium phosphate {TCP, $Ca_3(PO_4)_2$} as sole P source for selecting PSB isolates [5]. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121℃) for 15 min. After sterilization of culture media, pour into the sterilized petri plates, allow it to solidify, then bacterial isolates were streaked on petri plates containing the Pikovskayas media, covered it with the aluminium foil and incubate for 7 days at 30℃ temperature. Observed the colonies after 2-7 days.

2.2 Indole Acetic Acid (IAA) Production

The IAA production activity was tested by using TSB (Tryptone Soy Broth) agar. Gordon and Weber [6] were the first to provide a colorimetric assay using Salkowski reagent for the detection of IAA. This method is commonly used for detection of IAA from microorganisms. Salkowski reagent is a mixture of 0.5 M ferric chloride ($FeCl_3$) and 35% perchloric acid ($HClO_4$). Approximately 1 ml of salkowski solution was dropped on top of the growing colonies and then incubated in a dark condition for 1 hour.

2.3 Siderophores Production

Siderophore production was assayed qualitatively using Chrome Azurol Sulphate (CAS) blue agar as described by the, method of Schwyn and Neilands [7]. Autoclaved CAS media was plated and incubated for 24 hrs at 30℃ for the detection of contamination. Later the isolates were spot inoculated to CAS agar plates and incubated at 30℃ for 72 hours. Observations were taken after 3-4 days.

3. RESULTS AND DISCUSSION

3.1 Plant Growth Promoting Traits of the Test Isolates

The bacterial isolates were screened for multiple plant growth promoting activities, which are summarized in Table 1. The bacterial isolates were screened for plant growth promoting traits such as phosphate solubilization, IAA production and siderophore production.

3.2 Phosphate Solubilizing Test

Out of the eight rhizobial isolates, five were able to solubilise phosphate on Pikovaskayas media containing Tri calcium phosphate. Further out of five isolates Rh_3 and Rh_5 exhibited the highest (+++) solubilization zone, while Rh_1 and Rh_6 showed moderate (++) solubilization zone, Rh_2 showed slight (+) solubilization zone. In contrast, Rh_4, Rh_7 and Rh_8 displayed no solubilization activity. Results are in line with previous findings of Gyaneshwar et al. [8] who reported that the

development of red colouration around the bacterial colonies on TRP agar medium indicated rock phosphate (RP) solubilisation. Gupta et al. [9] similarly reported that phosphate solubilizing activity was tested qualitatively using pikosvkaya media and incubated at 27-28^{o}c for 2-7 days. A positive result was indicated by a halo zone production by a colony, indicating that the bacteria can solubilize phosphate (Table 1, Fig. 1).

Results obtained were in similar line with previous findings of Nautiyal et al. [5] who suggested that further, the zone of clearance around the colony was observed for phosphate solubilization. Mehta and Nautiyal [10] similarly suggested that the formation of halo zones around colonies was considered as positive for phosphate solubilisation.

3.3 IAA Production

The bacterial isolates were screened for plant growth promoting traits IAA i.e. indole -3- acetic acid. Out of the eight rhizobial isolates, five were able to produce IAA. Rh_3 and Rh_6 displayed a high intensity (+++) of pink color, Rh_1 and Rh_8 exhibited moderate intensity (++) of pink color, and Rh_7 showed a slight (+) intensity of pink color. However, Rh_2, Rh_4, and Rh_5showed negative activity for IAA production (Table 1, Fig. 2).

Results obtained were in similar line with previous findings of Williams and Singer [11] and Myron and Williams [12]. They suggested that development of pink colour indicated IAA production. Bano and Musarrat [13] similarly reported that the appearance of the red colour indicates the presence of IAA production by the bacteria. Gravel et al. [14] reported that the IAA production activity was tested using TSB (Tryptone Soy Broth) agar and positive result was noted by a colour change to pink indicating these isolates were able produce IAA.

3.4 Siderophore Production

Siderophore production was assayed qualitatively using Chrome AzurolSulphate (CAS) blue agar as described by method of Schwyn and Neilands [7]. Out of eight rhizobial isolates, seven isolates were able to produce siderophores. Further out of seven isolates specifically, Rh_2, Rh_3 and Rh_5 exhibited strong (+++) siderophore production, Rh_6 and Rh_8 displayed moderate (++) activity, while Rh_1 and Rh_4 exhibited slightly (+) siderophore production. In contrast Rh_7 showed no activity in siderophore production (Table 1, Fig. 3).

“CAS agar plates were spot inoculated with each of the bacterial strain and development of an orange halo zone around the colonies were observed as siderophore production. Results obtained were in similar line with previous findings” of Schwyn and Neilands [7], Dhul et al. [15], Joshi et al. [16] and Harshitha et al. [17]. Louden et al. [18] similarly reported that the formation of orange zone around the bacterial colonies indicates production of siderophore. Similarly, Milagres et al. [19] Perez Miranda et al. [20] and Ahmad et al. [21] reported that the isolates producing orange colour with of halo zone around the colonies were considered as siderophore producers.

Table 1. *In vitro* screening of root nodule bacteria for PGPR traits

Sr. No.	Isolate No.	Phosphate solubilization	IAA production	Siderophore production
1	Rh_1	**++**	**++**	**+**
2	Rh_2	**+**	**-**	**+++**
3	Rh_3	**+++**	**+++**	**+++**
4	Rh_4	**-**	**-**	**+**
5	Rh_5	**+++**	**-**	**+++**
6	Rh_6	**++**	**+++**	**++**
7	Rh_7	**-**	**+**	**-**
8	Rh_8	**-**	**++**	**++**

- Negative result
+ Slightly positive
++ Moderately positive
+++ Highly positive

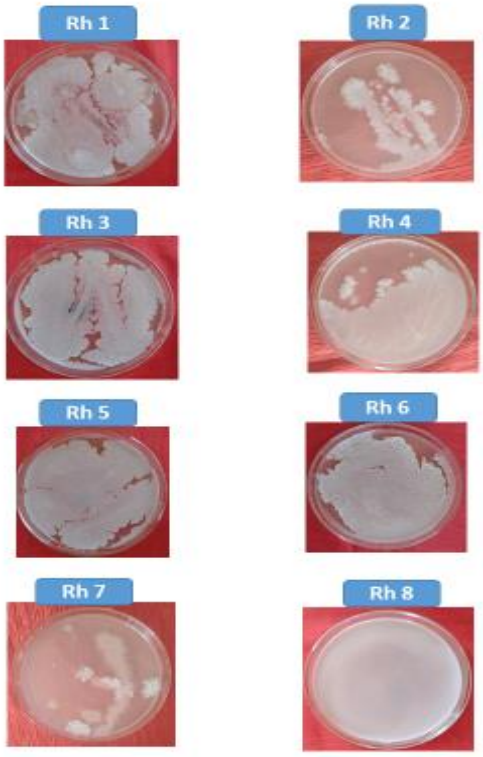

Fig.1. Rhizobium isolates showing Phosphate solibilization activity

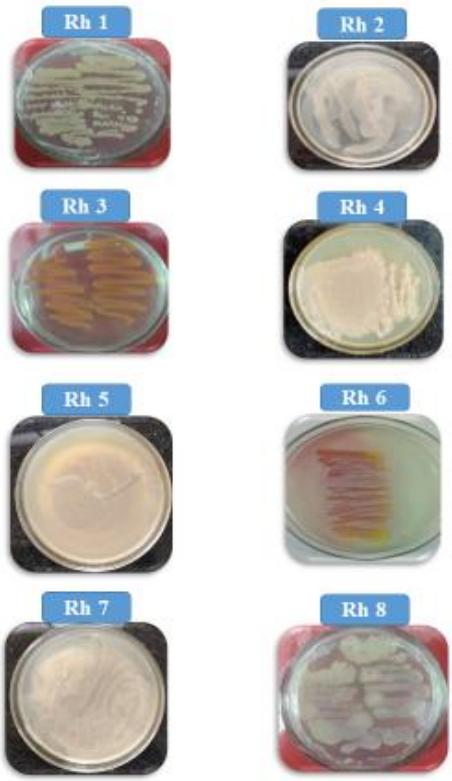

Fig. 2. Rhizobium isolates showing IAA production activity

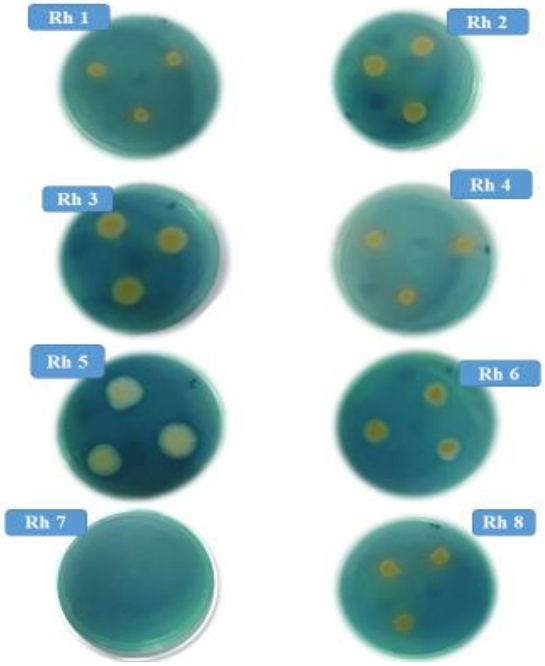

Fig. 3. Rhizobium isolates showing siderophore production activity

4. CONCLUSION

Out of the eight rhizobial isolates, two exhibited the highest phosphate solubilization zones, while two showed slightly solubilization zones, oneshowed moderate solubilization zones. In contrast, three displayed no solubilization activity. Out of the eight rhizobial isolates, twodisplayed a high intensity of pink color, twoexhibited moderate intensities of pink color, and one showed a slight intensity of pink color. However, three isolates did not exhibit IAA production. The three isolates exhibited strong siderophore production, two displayed moderate activity, while two exhibited slightly siderophore production. In contrast one showed no activity in siderophore production.

Plant growth-promoting rhizobacteria (PGPR) have been shown to have a significant role in the field of sustainable agriculture. These days, one of the biggest challenges is meeting the growing need for food output while using less synthetic chemical fertilizers and pesticides.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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