

## Isolation and Screening of Multifunctional Plant Growth Promoting Rhizobacteria (PGPR) from Onion Rhizosphere (*Allium cepa*)

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Received date: July 30, 2018; Accepted date: August 07, 2018; Published date: August 12, 2018

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### Abstract

Plant Growth Promoting Rhizobacteria (PGPR) can enhance the growth and productivity of plants through direct and indirect mechanisms. In this study, rhizobacteria were isolated from onion rhizosphere and their plant growth promoting activities were studied. The investigations of activities include: nitrogen fixation, phosphate solubilization, zinc mobilization, hydrogen cyanide and indole-3-acetic acid production abilities. Plant growth promoting rhizobacteria (PGPR) influence plant health and productivity by various ways. It can be summarized that the isolated multifunctional PGPR strains may have potential to be successful bio-inoculants for onion plant.

**Keywords:** Rhizobacteria; Rhizosphere; PGPR; Phosphate solubilization; Zinc mobilization; Nitrogen fixation

### Introduction

Plant roots in the soil show profuse activity relative to the bulk soil. pH, nutrients and exudates gradients changing as distance from the root increases [1]. This region of gradients in chemical and physical factors strongly influenced by the presence of plant roots and characterized by high rates of microbial population and activity is increasingly referred to as the rhizosphere. In fact, in 1904, Hiltner first defined the rhizosphere as that zone of soil in which the micro flora is influenced by plant roots [2].

Microorganisms are essential for maintenance of sustainable ecosystems and bio-diversity. Colonization of the plant root system is the very first step in nearly all interactions between plants and soil-borne microbes. Plant growth promoting rhizobacteria (PGPR) influences plant health and productivity by various ways. A good selection of a PGPR strain requires understanding of bacterial communities colonizing the rhizosphere. To date, only limited information exists on microbial diversity and dynamic of population in agricultural soil [3]. The properties related to the PGP character are auxin production, nitrogen fixation, phytopathogen antagonism, cyanogenesis, phosphate solubilization and amino cyclopropane carboxylic acid (ACC) deaminase activity [4]. Indian economy is agricultural based. But the use of chemical fertilizer has led to a considerable increase in agricultural productivity. The use of PGPR gives better way to replace chemical fertilizer, pesticides. PGPR help in the disease control in plants. PGPR especially if they are inoculated on the seed before planting, can establish themselves on the crop roots this is possible with onion crop as it is re-planted. Beneficial soil microbes are studied and exploited for the development of sustainable agriculture. The purpose of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth. It has shown considerable promise in laboratory and greenhouse studies. The important environmental benefits of this approach, leading to a reduction in the use of agricultural chemicals

and the fit with sustainable management practices, are driving force of this project.

The objectives of this study are to isolate bacteria from onion root rhizosphere, to isolate plant growth promoting rhizobacteria with multifunctional ability and to evaluate and analyze growth promoting ability under in-vitro conditions.

### Materials and Methods

#### Sampling

The sample used to isolate Rhizobacteria is rhizosphere soil sample around Onion roots. Onion root washed with water to remove adhering soil particles. Roots washed in saline suspension taken in 5 sterile tubes one after the another aseptically. Washing collected and mixed thoroughly with the help of Vortex mixture.

#### Isolation

10% Nutrient Agar, Pikovskaya medium, Solid Extract Agar, Norris broth were used for isolation. Loop full of each washing aseptically spread on the sterile 10% Nutrient agar plates i.e., 10% Salt added to Nutrient Agar and on 10% Soil Extract Agar plates. Incubated these plates for 48 hours at Room temperature. Growth observed in the form of number of different colonies on both the medium (Figure 1). Then transferred those colonies on Nutrient Agar slant with appropriate label and stored them in refrigerator for further tests.

#### Screening

Phosphate solubilizing capacity of each isolate checked by making grids on the Pikovskaya's agar medium (Table 1), the activity was checked by spot inoculating the isolates. Zone of clearance taken to be the positive test (Figure 2).

Pikovskaya's Agar is suspend 31.3 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

| Ingredients           | Gms/Litre |
|-----------------------|-----------|
| Yeast Extract         | 0.5       |
| Dextrose              | 10.0      |
| Tri-Calcium Phosphate | 5.0       |
| Ammonium sulphate     | 0.5       |
| Potassium chloride    | 0.2       |
| Magnesium sulphate    | 0.1       |
| Manganese sulphate    | 0.0001    |
| Ferrous sulphate      | 0.0001    |
| Agar                  | 15.0      |

**Table 1:** Pikovskaya's Agar Composition.

Zn solubilizing property of isolates checked by adding zinc oxide in Nutrient Agar as per above procedure (Figure 3).

Test for nitrogen fixation was done by inoculating isolates in Nitrogen free medium (Table 2) that is Norris broth. Turbidity after incubation was the positive test. Norris Glucose Nitrogen free Broth is suspend 12.5 grams in 1000 ml distilled water. Heat just to boiling. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

| Ingredients           | Gms/Litre |
|-----------------------|-----------|
| Glucose               | 10.0      |
| Dipotassium phosphate | 1.0       |
| Magnesium sulphate    | 0.2       |
| Calcium carbonate     | 1.0       |
| Sodium chloride       | 0.25      |
| Sodium molybdate      | 0.005     |
| Ferrous sulphate      | 0.1       |
| Final pH              | 7.0 ± 0.2 |

**Table 2:** Norris Glucose Nitrogen free Broth Composition.

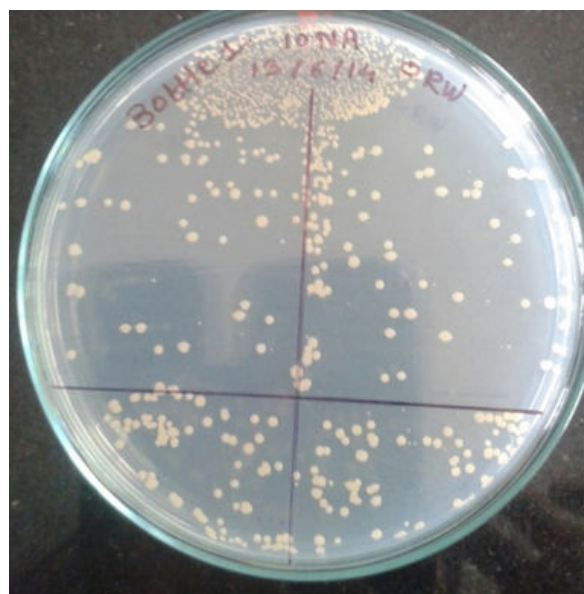
Then isolate was tested for Indole Acetic Acid Production. IAA or Indole acetic acid is plant growth promoting hormone. For the experiment, each isolate was inoculated in Tryptone broth. After incubation 1 ml of Broth was taken in Eppendorf tube and allowed to centrifuge for 2 min at about 15000 rpm. The supernatant was then taken and to it Salkowaskys Reagent was added. The reaction between reagent and IAA produced by isolate produced the characteristic red color which is taken as confirm Positive test.

The last test performed was to detect whether the Isolate can produce HCN gas or not. For the test the organism was grown on Glycerin containing medium and then on the upper part of test tube the Picric acid paper was placed. The tubes were then incubated at Room Temperature for 48 hours. The reaction of HCN Gas with picric acid imparts red color to the Paper. This was taken as positive test (Figure 4).

## Results and Discussion

The isolates 1, 3, 5, 30, 31, 38, 45, 46 are of interest and show good plant growth promoting activity (Table 3).

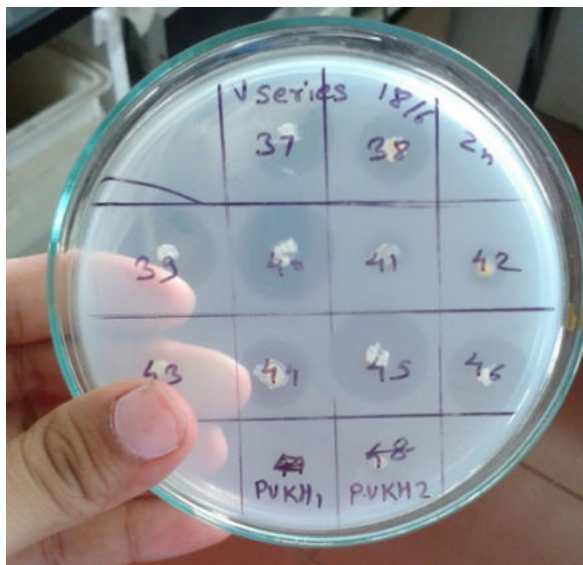
However, the cultures 14, 15, 22, 26, 27, 44 are of no use because they show property of production of toxic HCN gas which can inhibit the growth of beneficial organisms in the vicinity.



**Figure 1:** Growth of organisms after their enrichment on 10% Nutrient Agar.



**Figure 2:** Isolates showing phosphate mobilization.



**Figure 3:** Isolates showing Zinc mobilization.



**Figure 4:** Isolates showing production of HCN gas.

| Isolate No. | Phosphate mobilization | Zinc mobilization | Nitrogen fixation | Indole Acetic Acid Production | HCN gas Production |
|-------------|------------------------|-------------------|-------------------|-------------------------------|--------------------|
| Isolate 1   | +                      | +                 | +                 | +                             | -                  |
| Isolate 3   | +                      | +                 | +                 | -                             | -                  |
| Isolate 5   | +                      | +                 | +                 | +                             | -                  |
| Isolate 14  | -                      | +                 | -                 | -                             | +                  |
| Isolate 15  | +                      | +                 | +                 | +                             | +                  |
| Isolate 22  | -                      | +                 | -                 | -                             | +                  |
| Isolate 26  | -                      | +                 | -                 | +                             | +                  |
| Isolate 27  | +                      | +                 | +                 | -                             | +                  |
| Isolate 30  | -                      | +                 | +                 | -                             | -                  |
| Isolate 31  | +                      | +                 | +                 | +                             | -                  |
| Isolate 38  | +                      | +                 | +                 | -                             | -                  |
| Isolate 44  | -                      | +                 | +                 | -                             | +                  |
| Isolate 45  | +                      | +                 | -                 | -                             | -                  |
| Isolate 46  | +                      | +                 | -                 | -                             | -                  |

**Table 3:** Screening of isolates for Phosphate mobilization, Zinc mobilization, Nitrogen fixation, Indole Acetic Acid Production and HCN gas Production.

## Conclusion

Undoubtedly there are numerous and complicated interaction that occur in the rhizosphere. High degrees of activity by both the plant roots and the microbial community are often difficult to conclusively

characterize. Does the microbial community drive the structure and function of the plant community, or vice versa? Is there a constant co-evolution and simultaneous succession of both communities? Further research is clearly necessary.

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## References

1. Marschner H (1995) Mineral nutrition of higher plants. 2nd edn. Academic Press, United States.
2. Hartmann A, Rothballer M, Schmid M (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 312: 7-14.
3. Normander B, Prosser JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66: 4372-4377.
4. Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for Plant Growth-Promoting Rhizobacteria to Promote Early Soybean Growth. Soil Sci Soc Am J 63: 1670-1680.