International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **8(2),** 1-5, February **(2019)** *Int. Res. J. Biological Sci.*

Screening of plant growth hormone producing microorganisms

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Available online at: www.isca.in, www.isca.me
Received 29th August 2018, revised 30th December 2018, accepted 25th January 2019

Abstract

A study was conducted to determine the Plant growth promoting rhizobacteria (PGPR). This study was focusedto isolate the Plant growth promoting bacteria (PGPB)such as Azotobacter spp, Pseudomonas spp, Rhizobium spp, and Phosphate solublizing bacteria from various samples. In this study, microorganisms were isolated from rhizospheric soil, non-rhizospheric soil and from the root nodules of leguminous plants.From the present study we can conclude that rhizosphere, non-rhizosphere and root nodular bacteria are able to produce Indole Acetic Acid (IAA), Gibberellic Acid (GA), Ammonia and Siderophore. All the four species of microorganisms were capable of producing phytohormones such as IAA, GA and growth promoting substances like Siderophore and Ammonia are able solublize phosphate in varied concentrations.

Keywords: Phosphate solublizing bacteria, PGPR, phytohormones, siderophore, *Ammonia,* leguminous plants.

Introduction

Plants are considered to be the complex micro-ecosystems wherein different habitats are exploited by a wide variety of bacteria[1]. Plant rhizospheric region were considered as the most versatile and dynamic environment for this interaction[2]. Numerous bacterial species called rhizobacteria exist in the rhizospheric region[3]. Some of this rhizobacteria are known to produce plant growth promoting hormones or phytohormones[4]. The most important PGPR strains include different nitrogen fixing bacteria such as Pseudomonas spp, Azotobacterspp, Rhizobium spp and Phosphate solubilizing bacteria. The present study is an attempt to isolate PGPR organisms from rhizospheric samples and to analyze the production of phytohormones.

Materials and methods

Study area: Samples were collected from TIES campus and local areas of Kottayam, Kerala. The soil samples were serially diluted up to 10-5 and plated with King's B (10-5), Jensons (10-5), and Pikovaskaya (10-5) mediums using pour plate technique. Then the samples incubated at 37°C for 24-48 hours in inverted position.

Isolation of Rhizobium Species: The leguminous plants were carefully uprooted and then washed thoroughly with running water. The mature root nodules were collected from each root and washed under tap water and then surface sterilized with 0.2% Mercuric Chloride ($MgCl_2$) followed by washing for five to six times with sterile distilled water. It was then crushed gently with sterile mortar and pestle to get a suspension. Then 1 ml of suspension were transferred into 9ml of sterile water blank and then serially diluted up to 10-5 dilution. Shake well for 30 seconds and then plated with Yeast Extract Mannitol Agar. Then it is incubated at 37°C for 24-48 hours in inverted position.

Indole Acetic Acid (IAA) Production: The bacterial strains were inoculated into test tubes containing Sucrose Minimal Salts (SMS) medium (7.0) added with 0.05mg/ml tryptophan. After the incubation at 37°C for 48hours, 2ml of culture was centrifuged at 12,000 rpm for 5 minutes. 1.5ml of supernatant was added to 3ml of Salkowski's reagent. Then incubate the mixture in dark for 20-30 minutes. The absorbance of pink colour developed after incubation was read at 530nm using UV-VIS Spectrophotometer.

Gibberellic Acid (GA) Production: The bacterial strains were inoculated into Nutrient agar broth and incubated at 35°C for 48 hours. After incubation cultures were filtered and 2ml of zinc acetate solution was added to 15ml of filtrate. After 2minutes, 2 ml of potassium ferrocyanide solution was added and centrifuged at 8000rpm for 10minutes. Then 5ml of supernatant was added to 5ml of 30% hydrochloric acid and mixture was incubated at 27°C for 75minutes. The blank was prepared with 5% HCl. Absorbances were measured at 350nm using UV-VIS spectrophotometer.

Siderophore Production: The isolates were inoculated into test tubes containing King's B broth and incubated at room temperature for 24hours. Subsequently, centrifuged at 1000 rpm for 10 minutes. Then 2ml of supernatant was mixed with 2ml of aqueous ferric chloride ($FeCl_3$) solution and observed for the color change[7].

Ammonia production: The isolates were inoculated into a test tube containing peptone water and incubated at room temperature for 4days. After incubation, 2ml of Nessler's reagent was added.

Results and discussion

In this study, microorganisms were isolated from rhizospheric soil, non-rhizospheric soil and from root nodules of leguminous plants. Total population of microbes present in soil and root nodule samples are estimated by counting number of colonies on each culture plates. The colonies are counted and estimated after 48 hours of incubation at 37^{o}C.

Total number of microorganism: From the serial dilution, the root nodules contain highest population count (223.5×10^{5}cfu) and rhizospheric soil contains more population than non-rhizospheric soil. In rhizospheric soil Pseudomonas has highest population (32.9×10^{5}cfu). In non-rhizospheric soil also Pseudomonas has highest population (20.41×10^{5}cfu). The results were tabulated in Table-1.

Table-1: Total microbial count on soil and nodule.

	Rhizosphere Soil (cfu)	Non-rhizosphere Soil (cfu)	Root Nodules
King's B Medium (Pseudomonas)	32.9×105	20.41×105	0
Jenson's Medium (Azotobacter)	0.155×105	0.155×105	0
Pikovaskaya's Medium(PSB)	1.24×105	0.46×105	0
Yeast Extract Mannitol Agar Medium (Rhizobium)	0	0	223.5× 105

Isolation of microorganism: Morphologically different *Rhizobium, Pseudomonas, Azotobacter* and Phosphate solublizing bacteria isolates were selected and pure cultures of organisms were obtained by transferring to petridishes containing Nutrient Agar Medium. After incubation, the colonies were stored forfurther studies.

Detection of P-solublization: The isolates that are able to produce clear zone around the colonies were known as phosphatesolublizers. All isolates were produced clear zones around the colonies.

The solublization efficiency was calculated by the following formula: Solublization Efficiency (SE) = Solublization diameter /Growth diameter. Phosphate solubilizing bacteria showed high solublization efficiency and *Azotobacter*spp showed the lowest solublization. Results were showed in Table-2 and Figure-1.

Table-2: P-solubilizing efficiency of isolates.

Isolates	Colony Growth (cm)	Colony Growth (cm)	Solublizing Efficiency
Rhizobium	1.6	1.9	118.75
Azotobacter	1.3	1.4	107.69
Pseudomonas	1.4	1.6	114.28
PSB	1.2	1.5	125

Estimation of Indole Acetic Acid (IAA) Production: All the isolates were able to produce IAA. Maximum amount was produced by *Pseudomonas,* medium amount was produced *Azotobacter* and lowest amount was produced by Phosphate solublizing bacteria. The results were showed in Table-3 and Figure-2.

Table-3: Indole Acetic Acid (IAA) Production.

Isolates	Amount of IAA Production	OD at 530nm
Pseudomonas	++++	1.538
Azotobacter	+++	0.737
Rhizobium	++	0.561
PSB	+	0.354

Estimation of Gibberllic Acid (GA) Production: All the isolates are able to produce gibberllic acid. *Pseudomonas* spp produces more amount of GA as compared to other species of microorganisms. The amount of GA produced by each isolate was measured using UV-VIS spectrophotometer at 350nm.The results were shown in Table-4 and Figure-3.

Table-4: Gibberllic Acid (GA) production.

Isolate	High	Moderate	Low	OD at 350nm
Pseudomonas spp	++++			1.206
Azotobacter spp		+++		0.921
PSB		++		0.831
Rhizobium spp			+	0.553

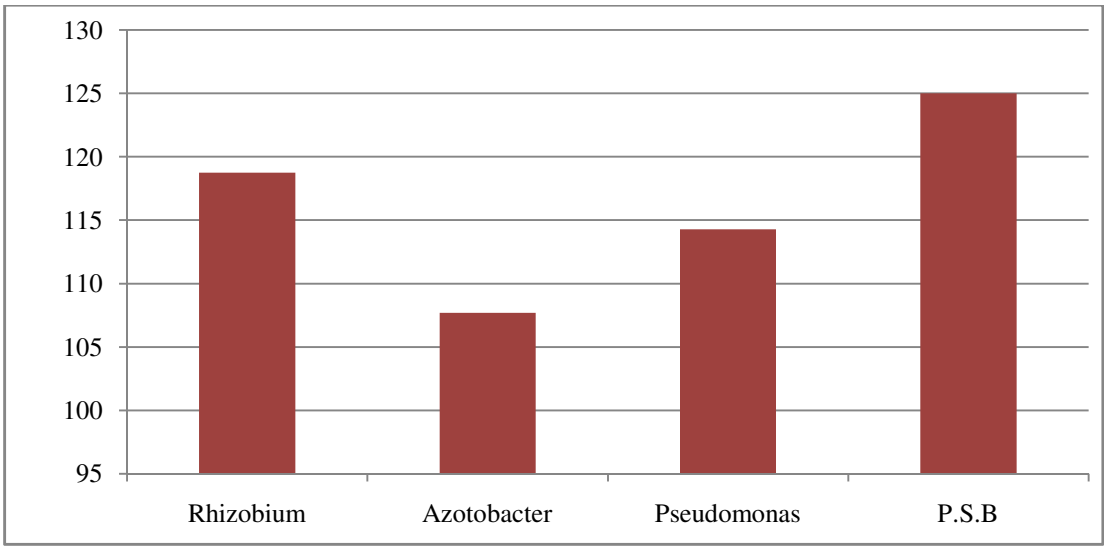

Figure-1: P-solubilizing efficiency of isolates.

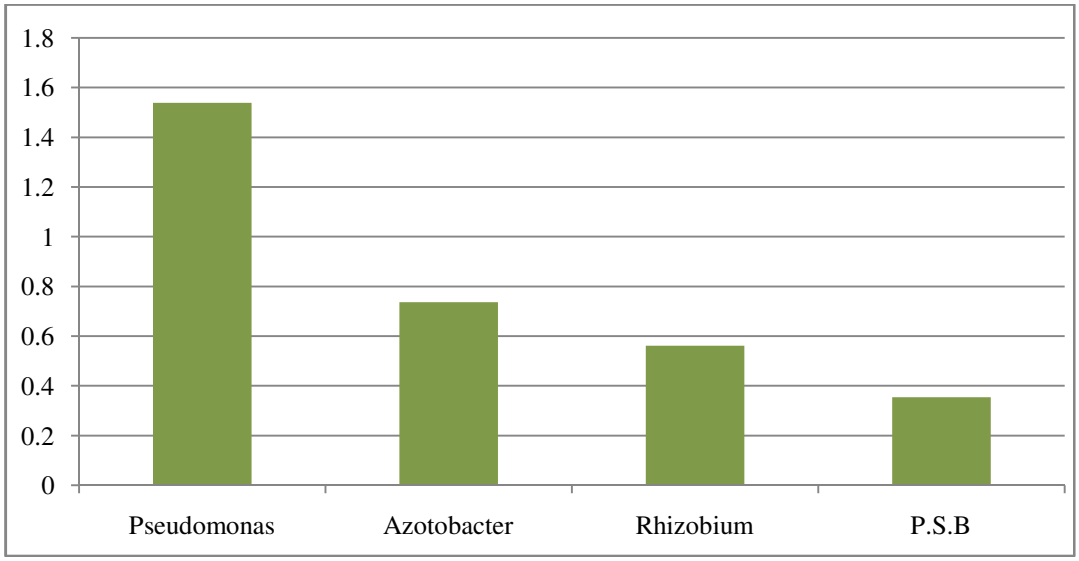

Figure-2: Indole Acetic Acid (IAA) Production.

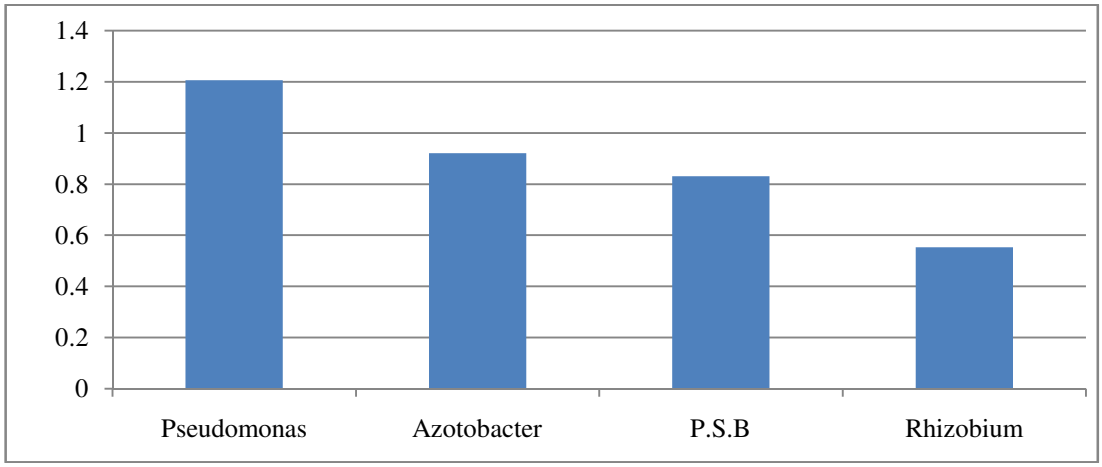

Figure-3: Gibberllic Acid (GA) production by isolates.

Detection of Siderophore Production: All the isolates showed a reddish brown color which means that organisms are capable of producing Siderophores. *Pseudomonas* produces highest amount of siderophore and medium amount is produced by PSB and *Azotobacter* and lowest amount by *Rhizobium.* The results were shown in Table-5.

Table-5: Siderophore Production.

Isolates	High	Moderate	Low	OD at 440nm
Pseudomonas	++++			1.794
Azotobacter		+++		1.512
PSB		++		1.521
Rhizobium			+	1.457

Detection of Ammonia Production: All the isolates showed yellow to orange reaction, this shows that all isolates are capable of producing ammonia. *Pseudomonas produced* maximum amount of ammonia. *Azotobacter* and PSB produced medium amount of ammonia and *Rhizobium* produces lowest amount of ammonia. The results were shown in Table-6.

Table-6: Ammonia Production.

Isolates	Amount of Ammonia
Pseudomonas	++++
Azotobacter	+++
PSB	+++
Rhizobium	++

In this study, the isolated microorganisms were able to produce the growth promoting substances such as IAA, GA, Siderophore, Ammonia and capable of solubilizing phosphate. Plant Growth Promoting Rhizobacteria (PGPRB) has the potentiality to contribute sustainable plant growth promotion[4,6-10]. Hayat et al. conducted a study on effect of microorganism's inoculation on different plants[5]. The inoculated plant gave significantly much higher nodule number, shoot weight and seed yield when compared to the non-inoculated plants[9].

Conclusion

From the present study we can conclude that rhizosphere, non-rhizosphere and root nodular bacteria were capable to produce plant growth promoting substances such as Indole Acetic Acid (IAA), Gibberellic Acid (GA), Ammonia and Siderophore. All the four species of microorganisms were capable of producing phytohormones such as IAA, GA, Siderophore, Ammonia and are able to solubilize phosphate in varied concentrations. *Pseudomonas* species are important PGPR as they produce these plant growth promoting substances in large amounts as compared to other isolates. Therefore, some of these strains are used as Biofertilizers, and are available to host plants. The host plants are expected to grow quickly and luxuriantly and later increases soil fertility through addition of nitrogenous biomass thereby reduces the application of chemical fertilizers.

Acknowledgements

We sincerely thank to Tropical Institute of Ecological Sciences, for successful completion of the project.

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