Isolation and Characterization of PGPR from Rhizospheric Soil

Pratibha Sharma and D. K. Shrivastava

Abstract— PGPR are naturally occurring soil bacteria which actively colonize plant root and benefit plants by providing growth promotion. They help in providing free living nitrogen fixing bacteria, increase supply of other nutrients, produce plant hormones, enhance other beneficial bacteria or fungi, control bacterial and fungal diseases and help in controlling insect pest. The present work was carried out to determine the diversity of bacterial populations in the rhizospheric soils of various vegetable crop fields located nearby Bilaspur city in Chhattisgarh, India. Ten bacterial strains out of several isolates were characterized as PGPR. These bacteria are not simply additional elements to biological diversity in the rhizosphere but are essential components to the survival of other microorganisms and plants. Bacterium provides benefits to the plant, resulting in plant growth stimulation, plant protection as well as production of antibiotics, geochemical cycling of minerals and plant colonization.

Index Terms— Bacterial diversity, Bilaspur, Chhattisgarh, PGPR, Plant Growth, Rhizosphere, Soil,.

1 Introduction

The soil layer which is influenced by the plant root is called rhizosphere and in the rhizospheric soil the density of Rhizospheric bacteria is much higher rather than the surrounding soil because different metabolites secreted by plant roots are used as nutrient by these organisms [1]. Rhizospheric bacteria which play an important role in plant growth promotion are termed as PGPRs. They not only promote plant growth but also help in sustainable agricultural development and protecting the environment. During last few decades a large no. of bacteria including species of Pseudomonas, Azotobacter, Azospirillum, Bacillus, Rhizobium etc. have reported to enhance plant growth [2], [3], [4]. PGPR are beneficial soil bacteria which help the plant in both direct and indirect way. Increase in plant productivity occurs through different mechanism such as a symbiotic nitrogen fixation [5] solublization of mineral phosphate and other nutrient [6] production of plant hormone [7], [8] and control of phyto pathogenic microorganisms[9].

The variance in the performance of PGPR may be due to various environmental factors that may affect their growth and so there effect on the plant. To achieve the maximum growth promoting interaction between PGPR and nursery seedling it is necessary to know how the Rhizospheric bacteria exert their effect on plants and whether the effects are attend by various environmental factors including the presence of other microorganism [10]. Therefore, it is necessary to develop efficient strain in field conditions so to reduce the negative impact of chemical fertilizers on the environment. Keeping in view such constrains the present investigation was designed to isolate and screen certain rhizobacterial isolate belonging to Pseudomonas, Bacillus, Rhizobium, Azotobacter etc.

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2 Materials and Mathods

2.1 Collection and characteristics of soil of samples

Soil and root samples were collected aseptically in sterile plastic bags from the agricultural field of Bilaspur district of Chhattisgarh periodically and brought to the laboratory and prior to their processing kept at 40C. Rhizospheric soil samples were collected mostly from roots of vegetable crops. Various physical characteristics of soil viz temperature, pH and moisture content were studied applying common laboratory methods.

2.2 Isolation of PGPR

Rhizospheric soil samples were separated from roots of vegetables crop by brushing gently in a Petridis. 10 gram of rhizospheric soil was taken into a 250 ml. of conical flask containing 90 ml of sterile distilled water and the flask was shaken for 10 minutes. Serial dilution technique was employed up to 105 dilutions. An aliquot (0.1 ml) of diluted suspension was spread over the plates of Nutrient Agar Medium. Plates were incubated for three days at 28 ± 20C to observe the colony of bacteria. Typical bacterial colonies were observed over the streak in culture plates. The technique was repeated thrice and cultures were made single colony type and bacterial colonies for various strains developed from single colony were isolated and stored for characterization.

2.3 Characterization of Isolates

Morphological characteristics of the colony of each isolate were examined on suitable and specific media. All the isolates were streaked on petriplates containing suitable media. After three days of incubation, different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation and gram reaction were recorded.

2.4 Biochemical characterization

The various biochemical characteristics viz Oxidase test, IM-

ViC test, Urease test, Catalase test, and Nitrate reduction test were carried out according to [11]

2.5 Functional characterization

The functional diversity amongst recovered isolates was studied by qualitative screening of their ability to Solubilizing phosphorus and Sederophore production.

2.6 Phosphorus Solubilization

Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crop plants. Ironically soils may have large reserves of total phosphorus but the amount available to plants is usually a tiny proportion of this total [12]. Many soil microorganisms are able to solubilizing unavailable form of bound phosphorus [13]. The plates were prepared with Pikovaskya's medium. The isolates were spot inoculated on the plates and incubated in an incubator at 28^0C for 3 to 5 days. Formation of clear zone around the microbial colonies indicated phosphate Solubilization.

2.7 IAA Production

IAA production was detected by the modified method as described [14]. Diverse soil microorganisms including bacteria, fungi, and algae are also capable of producing physiologically active quantities of auxin. The entire cultures were incubated in the peptone broth enriched with tryptophan broth to check for production of indole acetic acid a precursor of auxin which is an important plant hormone. The quantitative estimation of IAA is performed by using Salkwouski method by using the reagent (50 ml, 35% of per Chloric acid, 1 ml of 0.5 M $FeCl_3$ solution .mixtures were incubated at room temperature for 30 min, and 90 min and observe for pink colour production and read calorimetrically at OD 530 nm.

2.8 Siderophores production

Bacterial isolates were assayed for Siderophore production on the Chrome azurole S agar medium (Sigma, Ltd.) Described [15]. The culture isolates were streaked on the surface of CAS agar medium and incubated at 28± 10C for 48 to 78 hours. Sideraphore production was indicated by orange halos around the colonies was considered as positive for Siderophore production.

3 Result and Discussion

The physical parameter viz temperature, pH and moisture content of soil were studied at different time interval (Table – 1). The temperature varied from 15 ± 0.30^0 C (0d) to 35± 0.10^0 C (120d). pH of the field varied significantly that has been found from slightly acidic i.e. 6.00 ±0.03(0d) to 7.00 ±0.02. Moisture content of the field varied from 65 ±0.25 (0d) to 35 ±0.20 (120d).

TABLE 1
PHYSICAL PARAMETERS OF SOIL SAMPLES

Characteristics	Time period of sampling(days)				
	0	25	50	75	100
Temperature (^{o}C)	15.00 ±0.30	18.00 ±0.40	22.00 ±0.45	28.00 ±0.20	35.00 ±0.10
pH	6.00 ±0.03	6.00 ±0.03	6.00 ±0.03	6.00 ±0.03	6.00 ±0.03
Moisture content	65.00 ±0.25	58.00 ±0.40	45.00 ±0.28	40.00 ±0.18	35.00 ±0.20

3.1 Isolation and Characterization of PGPR

Ten bacterial strains were successfully isolated in the rhizospheric soil of agro economic field from different areas in Bilaspur. The present study characterized the rhizo-bacterial associated with the vegetable crop plant. Culture and morphological characterization has been done (Table -2). The distribution and dominance pattern of rhizo-bacterial is usually influenced by the crop. Optimization of temperature for growth of isolates was observed and finds that 30 – 37 degree centigrade is optimum temperature for luxuriant growth (Table–3). The result of biochemical characterization of all ten isolates have mentioned in Table – 4 and shown in Plate –1.

Phosphate is essential major nutrient required by plant and most of it is present in insoluble form. The ability of bacteria to solubilizing mineral phosphate and make it available to plant as it enhances the plant growth is a topic of interest to agricultural microbiologist Phosphate solublization ability of bacteria can be detected by using agar plate method (Table – 5). All the isolate showing clear zone around the colony were able to solubilizing phosphate. IAA production was found to be a common trait in all isolates. All isolate were positive for IAA production that shows pink color formation after addition of Salkowski reagent in 48hrs grown broth (Table – 5). Another important trait of PGPR is Ammonia production that indirectly influences the plant growth. This was also common traits observed in all isolates. . It has been reported that IAA production by PGPR can vary among different species and strains and it is also influence by culture condition growth state and substrate availability [16].

Iron is essential requirement of plant and microorganism. Siderophore producing bacteria make it available to plant and also these bacteria compete for iron with soil borne pathogen and play a role as bio control agent [17]. Out of ten isolates, seven isolates were positive for siderophore production that shows yellow orange zone around the colony (Table -5). Siderophore are low molecular weight iron chelating compounds which place an important role in plant growth promotion [18],[19] it is one of the bio control mechanisms belonging to PGPR group under iron limiting conditions. Isolates from rhizosphere are more efficient auxin producer than isolates from bulk soil [20].

TABLE 2

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF 2 DAYS OLD COLONY OF PGPR ISOLATES

Isolates Code	Cultural characteristics						Morphological properties			
	Colour	Size	Shape	Margin	Elevation	Opacity	Gram staining	Shape	Mot-ility	Pigme-ntation
B1	Whitish	0.5mm	Circular	Smooth	Convex	Opaque	-	SR	M	None
B2	Milky	0.4mm	Circular	Smooth	Flat	Opaque	-	SR	M	None
B3	Milky	0.9mm	Irregular	Rough	Raised	Transluscent	-	SR	M	None
B4	Yellowish	1.2mm	Circular	Rough	Raised	Translucent	-	SR	M	None
B5	Off-white	0.3mm	Irregular	Rough	Flat	Translucent	-	SR	M	None
B6	Brownish	1.4mm	Circular	Smooth	Convex	Translucent	-	SR	M	None
B7	Whitish	0.8mm	Circular	Smooth	Flat	Translucent	-	SR	M	None
B8	Off-white	0.4mm	Irregular	Smooth	Convex	Opaque	-	SR	M	None
B9	Milky	0.3mm	Circular	Smooth	Flat	Opaque	-	SR	M	None
B10	Yellowish	0.3mm	Circular	Smooth	Convex	Opaque	-	SR	M	None

M-Motile, - Negative

TABLE 3

OPTIMIZATION OF TEMPERATURE FOR GROWTH OF ISOLATES

Isolates	20° C	30°C	37°C	45°C
B1	++	++	+	+
B2	++	++	+	-
B3	++	+	+	-
B4	+	+	++	+
B5	+	+	++	+
B6	+	++	++	-
B7	++	++	+	-
B8	++	++	+	-
B9	+	+	+	-
B10	+	+	+	-

Where: (++) = Luxuriant growth, (+) = Moderate growth, (-) = No growth.

TABLE 4

BIOCHEMICAL PROPERTIES OF ISOLATED CULTURES

Culture Code	Biochemical Properties									
	Catalase	Urease	Sucrose	Glucose	Indole Production	V.P. Taste	Methyl Red	Citrate Utilization	Starch Hydrolysis	Nitrate Reduction
B1	+	-	+	+	+	+	-	+	+	-
B2	+	-	+	+	+	-	+	+	+	-
B3	+	-	+	+	+	-	+	+	+	-
B4	+	-	+	+	+	-	+	-	+	+
B5	+	-	+	+	+	+	-	-	-	+
B6	+	+	+	+	+	-	+	+	+	-
B7	+	-	+	+	+	-	-	+	-	+
B8	+	-	+	+	+	+	+	+	+	-
B9	+	+	+	+	+	-	+	-	-	-
B10	+	-	+	+	+	-	+	+	+	-

PLATE 1

FIGURE SHOWING MORPHOLOGICAL AND BIOCHEMICAL RESULTS OF ISOLATES (PGPR)

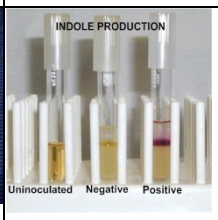
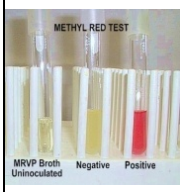
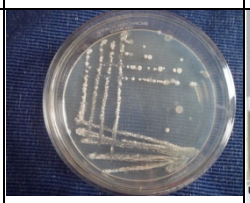
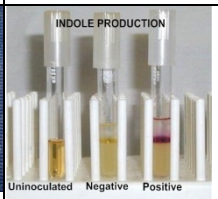
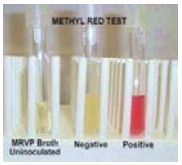
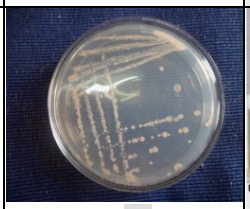
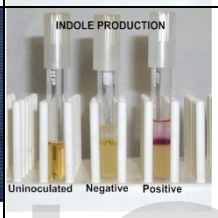
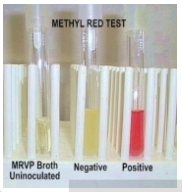
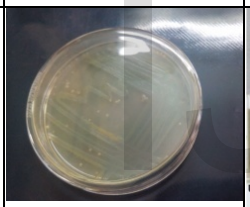
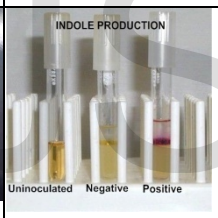
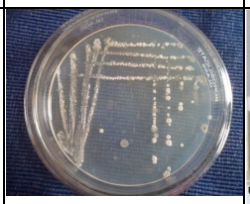
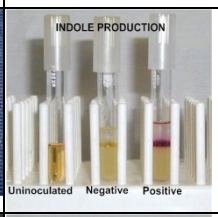
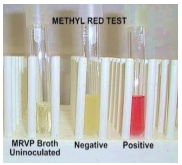
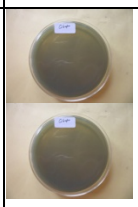
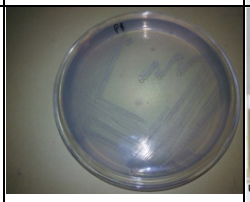
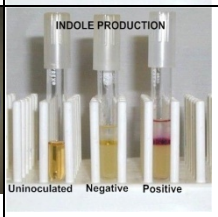
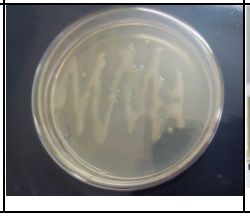
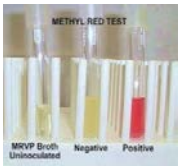
Sl. No	Culture Code	Culture Plates	Indole Test	MR- Test	Citrate Test	Catalase Test	Sucrose Test
1	B1						
2	B2						
3	B3						
4	B4						
5	B5						
6	B6						
7	B7						

TABLE 5
SCREENING OF SOIL BACTERIA FOR PGPR ACTIVITY.

Bacterial isolates	Phosphate solublization	Siderophore production	IAA production
B1	+	+	+
B2	+	+	+
B3	+	+	+
B4	+	-	+
B5	+	+	+
B6	+	-	+
B7	+	+	+
B8	+	+	+
B9	+	-	+
B10	+	+	+

Key : + = Positive, - = Negative

Plant rhizosphere is known to be preferred ecological niche for various types of soil microorganisms due to rich nutrient availability. PGPR colonize plant roots and development by a wide variety of mechanisms. To be effective PGPR bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population diversities sufficient to produce the beneficial effect. The rhizosphere harbors bacterial flora whose diversity is mainly expressed in term of functions adapted to the root presents and in particulars to favor the plant growth. Thus in term is beneficial to the whole rhizosphere micro biota through the highly nutritive and energetically rhizo-deposition. Plant growth promoting and development can be facilitated both directly and indirectly. Cultures were isolated from the Rhizospheric region of the soil collected from different regions of Chhattisgarh. In present study ten cultures were selected and screened for plant growth promoting activity and all the cultures showed the maximum plant growth promoting traits.

4 CONCLUSION

Present study illustrates the significance of rhizobacteria under in vitro conditions for multiple PGPR traits. It can be concluded from the above discussion that PGPR enhance the plant growth due to the production of IAA, Phosphate solublization, Siderophore production. Such investigation is necessary as it advocates that use of PGPR as bio-inoculants is an efficient approach to replace chemical fertilizers and these PGPR isolates may also be used as bio fertilizers to enhance the growth and productivity for commercially grown plants under local agro-climatic conditions of C.G. Simultaneous screening of PGPR from field is a good tool to select effective PGPR for bio fertilizer development technology. Hence, it is concluded that the isolated bacteria can be efficiently used in agricultural soils and have the potential in future to be exploited in bio fertilizer formulations.

5 ACKNOWLEDGMENT

Authors are thankful to the Principal, Govt. E. Raghavendra Rao Postgraduate Science College, Bilaspur for his kind support and encouragement during the course of present investigation.

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