International Journal of Biotechnology and Microbiology
Online ISSN: 2664-7680; Print ISSN: 2664-7672; Impact Factor: RJIF 5.46
Received: 08-01-2021; Accepted: 23-01-2021; Published: 18-03-2021
www.biotechnologyjournals.com
Volume 3; Issue 1; 2021; Page No. 32-36

PGPR, best substituent for chemical compounds in cultivation of commercial crops.

Ganchi AY, BS Naphade
Department of Microbiology, Badrinarayan Barwale Mahavidyalaya, Jalna, Maharashtra, India

Abstract
In Indian economy agronomy sector is huge and many other sector is dependent. Food need is increasing rapidly and for these demand many farmers are using chemical fertilizers and pesticides in agriculture activity. Due to use of huge amount of xenobiotics it leads to bio magnifications. Rhizosphere is the centre of intense biological activity due to the food supply provided by the root exudates. Rhizosphere bacteria colonizing the rhizosphere and conferring beneficial effects are called Plant growth promoting rhizo bacteria (PGPR).

Rhizo bacteria were isolated from rhizospheric soil of Jalna, Maharashtra State, India. Out of 35 isolates 10 potential isolates were screened which showed high phosphate solubilization and ability to grow on nitrogen free medium. Phosphate solubilization was studied both quantitatively and qualitatively. Temperature and pH growth optimization parameters for phosphate solubilization were studied. From these ten isolates four showed Siderophore producing activity when grown on deferrated Succinate Minimal medium. Only one isolate showed the capability of producing exopolysaccharide. In the present study antibiotic resistant activity was also studied. The results demonstrated that the use of these PGPR as an inoculants carrier for seed dressing of commercial crop has a promising effect on seed germination.

Keywords: exopolysaccharide, phosphate, siderophore, rhizosphere xenobiotics

Introduction

There is increasing demand of food for the growing population, so the modern agricultural practices have started the use of synthetic chemicals to increase the yield of crop and protect it from diseases. However it is well known that damage can be generated in the environment and human health because of their use (He *et al*., 2005). Bio magnification is usually defined as the transfer of a xenobiotic chemical from food to an organism, resulting in a generally higher concentration within the organism than source, (Connell, 1989, 1990; Rand *et al*., 1995).

PGPR bacteria were isolated from rhizosphere of different commercial crops of wheat, sorghum, cotton, maize, groundnut, pigeon pea, and sugarcane from Jalna District of Maharashtra State, India. For isolation of phosphate solubilizing bacteria Pikovskaya's agar medium was used.

Exopolysaccharides (EPSs) and Siderophores are the most important secondary metabolite isolated from the rhizosphere soil. Microbial polysaccharides are characterized by a considerable diversity in their composition and structure. Most polysaccharides are hetero polysaccharide, this means there is a wide range of possible structures and differences in the properties of EPSs due to the many possible linkages and configurations (Calsteren *et al.*, 2002). EPSs enhances water holding capacity, root aggregates, seed dressing, thickening agents etc.

Siderophores are defined as relatively low molecular weight ferric ion specific chelating agents elaborated by bacteria and fungi growing under low iron stress. Siderophores form high-spin, kinetically labile chelates with ferric ion which are characterized by exceptional thermodynamic stability (Schwarzenbach *et al*., 1963: Raymond and Carrano., 1979) [10, 8].

Materials and Methods

Soil Sampling

The soil sample was collected from different region of the Jalna district of Maharashtra. The rhizospheric soil of wheat, sorghum, maize, cotton, groundnut, pigeon pea and sugarcane.

Isolation of Phosphate Solubilizing Bacteria (PSB)

For isolation of PSB 1.0 gm of soil sample of each was dissolved in 100ml of sterile distilled water. Homogenization was carried out in rotary shaker for 15 minutes at 120 rpm at room temperature. After 15 minutes flasks were kept undisturbed for 10 minutes to settle down soil particles. An aliquot 10 ml was inoculated in sterile 100ml Pikovaskaya's medium and kept at 120 rpm at room temperature for 5 days for enrichment. After 5 days serial dilutions were prepared from enriched medium and were allowed to spread on sterile Pikovaskaya's agar plate of pH 7. Plates were kept at room temperature for 7 days until highest number of colony developed. Amphotericin B 200mg/L added in PKA to avoid fungal growth.

Screening of Potential Phosphate Solubilizing Bacteria

Colonies showing phosphate solubilizing zone around them were considered as PSB. Phosphate solubilization efficiency (PSE) is the ratio of total diameter i.e clearance zone including bacterial growth and the colony diameter. It is also called Solubilization Index (Edi-Prempo *et al*., 1996).

$$\text{Solubilization Index} = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

EPS production
Modified Ashby's medium inoculated with the bacterial culture. It was then incubated at room temperature for 72 hrs at 120 RPM on orbital shaker. Further extraction process was done.

Siderophore Production
For Siderophore production iron free succinic acid medium was used. All the glassware were deferrated. Cultures were grown in a minimal medium at room temperature for 24 hours under shaker conditions (100 rpm) at 48 hours. The cells were removed by centrifugation at 3000 rpm for 15 mins. 0.5 ml of the culture supernatant was then mixed with 0.5 ml CAS solution and 10µl shuttling solution (sulfosalicylic acid). The color obtained was determined using the spectrophotometer at 630 nm after 20 mints of incubation. Necessary blank (minimal medium) & reference solution (minimal medium + CAS dye + shuttle solution) were used during the determination.

Nitrogen Free Medium
Jensen's medium is recommended for detection and cultivation of nitrogen fixing bacteria. Jensen's medium was prepared in agar plates and the PGPR strains were streaked on Jen sense agar (plate $_{P}$H 7) and kept at room temperature for 24 hours.

Antibiotic Sensitivity Test
Himedia Antibiotic octadiscs were used to test the antibiotic sensitivity of the isolated phosphate solubilizing bacteria.

Germination Test
The influence of PGPR isolate 9 which produce exopolysaccharide was checked on the germination of groundnut seed by plate assay method.

Result and Discussion
Phosphate Solubilizing Bacteria
The rhizospheric soil sample isolated from commercial crops of Jalna district of Maharashtra was evaluated for isolation of phosphate solubilizing bacteria. Total 35 isolate were obtained from different soil sample of which 10 important isolate 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were isolated as shown in table 1. The isolate 1 showed maximum phosphate solubilization index of 2.9, were as isolate 2 and 6 showed 2.5 and 2.83. Due to viscous nature of isolate 9 it was grown on modified ashbh y medium.

Exopolysaccharide and Siderophore
The wet weight and dry weight of the fractionated exopolysaccharide of isolate 9 was 39.23gm/100ml and 0.365gm /100ml respectively. The phosphate solubilizing bacteria isolates 3, 4, 5 and 7 showed positive CAS test which shows the ability for the production of the Siderophore. Further results are shown in table 2.

Other Test
Results of Starch degradation, Gelatin degradation, Casein degradation test and growth of PGPR isolates on Nitrogen free Jensen's media are expressed in table 2.

Antibiotic Sensitivity Test
Result of antibiotic sensitivity test are shown in table 3 and photo 2. From the present study it is revealed that rhizospheric soil of the commercial crops contains various plant growth promoting rhizobacteria (PGPR). The phosphate solubilizing bacteria are very much important for conversion of insoluble phosphate to soluble form phosphates to the plants. The PGPR also have the multiple role like production of Siderophores, exopolysaccharide and ability to grow in nitrogen free medium**.** The germination percentage of the groundnut seed was increased as compared to the untreated groundnut seed, this indicates that the rhizospheric bacteria have important role in seed germination. The antibiotic sensitivity test is also very much important for the preliminary detection of the antibiotic sensitive bacteria. Thus the use important plant growth promoting bacteria is beneficial for plant growth and sustainable agriculture.

Table 1: Phosphate solubilizing activity of the PGPR isolates on Pikovaskaya's agar plate.

S.No.	PGPR isolates	Colony Measurement(cm)	Zone Measurement(cm)	Solubulization Index (SI)
1	Isolate 1	1.0	1.9	2.9
2	Isolate 2	0.8	1.2	2.5
3	Isolate 3	1.0	1.3	2.3
4	Isolate 4	0.8	1.2	2.5
5	Isolate 5	0.7	1.0	2.42
6	Isolate 6	0.6	1.1	2.83
7	Isolate 7	2.0	2.4	2.2
8	Isolate 8	1.2	1.6	2.33
9	Isolate 9	0.8	1.1	2.37
10.	Isolate 10	0.8	1.0	2.25

Table 2: Different test performed on PGPR isolates.

PGPR Isolates	Different Test Performed				
	Starch Degradation	Gelatin Degradation	Casein Degradation	Growth Jensen's medium	Siderophore Production
Isolate 1	+	---	+	+	---

Isolate 2	+	---	---	+	---
Isolate 3	+	---	---	+	+
Isolate 4	--	+	---	+	+
Isolate 5	+	---	+	+	+
Isolate 6	+	---	---	+	---
Isolate 7	--	---	---	+	+
Isolate 8	+	---	---	+	---
Isolate 9	+	---	+	---	---
Isolate 10	---	+	---	---	---

Table 3: Antibiotic sensitivity test of the PGPR isolates.

Antibiotics	Isolates									
	1	2	3	4	5	6	7	8	9	10
AMX(10mcg)	----	+	---	+	+	---	+	+	+	+
COX (5 mcg)	----	----	---	+	+	---	+	+	+	+
E (15 mcg)	----	+	---	+	+	---	+	+	+	+
TE (10mcg)	----	+	+	+	+	+	+	+	+	+
P (2 units)	----	----	+	+	+	+	+	+	+	+
COT(25 mcg)	----	+	+	+	+	+	+	+	+	+
PV (3 mcg)	----	-----	---	+	+	---	+	+	---	---
CN (30mcg)	----	+	+	+	+	---	+	+	+	+

(AMX- Amoxycillin, COX-Cloxacillin, E- Erythromycin, TE- Tetracycline, P- Penicillin, COT- CO-Trimoxazole, PV- Penicillin V and CN- Cefalexin)

Table 4: Plate assay of germination of groundnut seed.

Treatment	Hours	Germination
Control	48	Negative
Direct culture (isolate 9)	48	Positive
Supernatent (isolate 9)	48	Positive

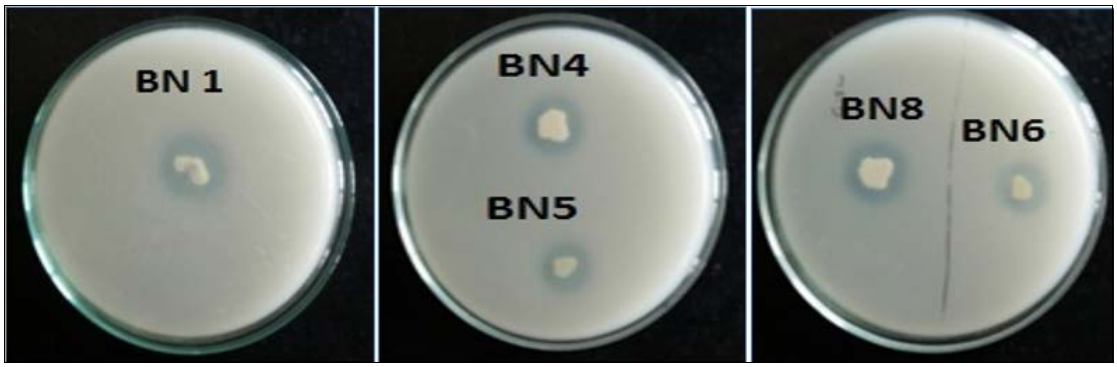

Fig 1: Halozone formation around colony of the PGPR isolates.

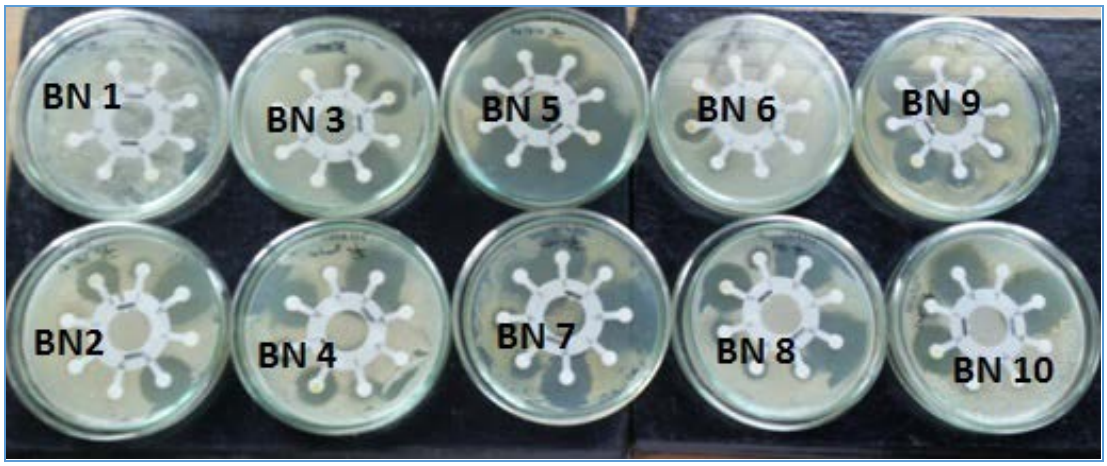

Fig 2: Antibiotic sensitivity checking of the PGPR isolates.

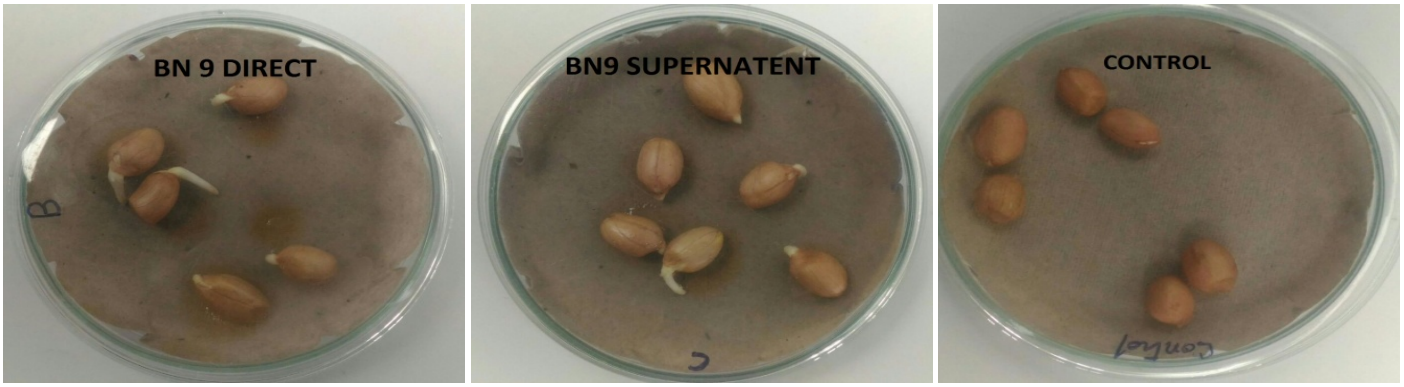

Fig 3: Plate assay of germination of groundnut seed.

Fig 4: Exopolysaccharide production of BN9 isolate on modified Ashby's medium.

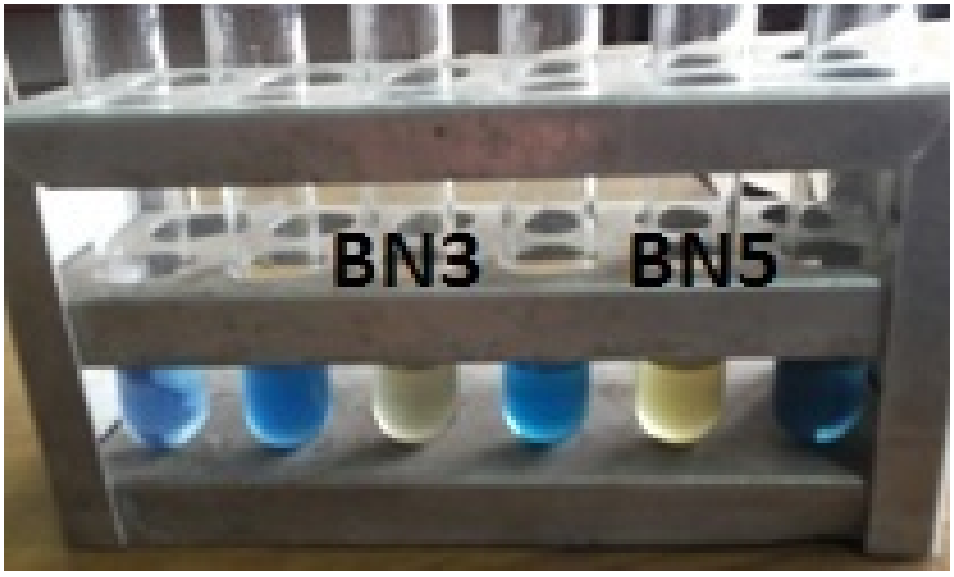

Fig 5: CAS test of the PGPR Isolates.

References

1. Calsterin MV, Pau-Roblot C, BE A, Roy G, Roy D. Structure determination of the exopolysaccharide produced by *Lactobacillus rhamnosus* strains RW-9595M and R. biochem. J. 2002; (363):7-7.
2. Edi-Premono MA, Moawad, Vleck PLG. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the Rhizosphere. Indonasian J. Crop Sci. 1996; (11):13-23.
3. Maheshwar UA, Sathiyavani G. Solubilization of phosphate by *Bacillus sps*, from groundnut Rhizosphere (Arachishypogaea L). J. Chem. Pharm. Res. 2012; 4(8):4007-4011.
4. Neilands JB. Microbial Iron Metabolism, New York:

Academic Press, 1974.
5. Park J, Bolen N, Megharaj M, Naidu R. Isolation of phosphate solubilizing bacteria and characterization of their effects on lead immobilization. J. Hazard. Mater. 2010; 185(2-3):829-836.
6. Patel D, Parmar P. Isolation and screening of phosphate solubilizing bacteria from sunflower Rhizosphere. G.J.B.B. 2013; 2(3):438-441.
7. Pikovaskaya R. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Mikrobiologiya. 1948; 17:362-370.
8. Raymond KN, Carrano CJ. Accts. Chem. Res. 1979; (12):183-190.
9. Reyes I, Bernier L, Simard R, Antoun H. Effect of nitrogen source on solubilization of different organic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiol. Ecol. 1999; (28):281-290.
10. Schwarzenbach G, Schwarzenbach K. Helv. Chim. Acta. 1963; (46):1390-1399.
11. Shankarrao O. Isolation and characterization of phosphate solubilizing bacteria from rhizospheric soil samples. Online International Interdisciplinary Research Journal, (Bio-monthly), ISSN 2249-9598, 2012; 2(4):28-39.
12. Vijay abaskar P, Babinastarlin S, Shankar T, Siva kumar T, Anandapandian KTK. "Quantification and Characterization of Exopolysaccharides from *Bacillus subtilis (MTCC 121*)". Advances in Biological Research. 2011; 5(2):71-76.