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Screening of Maize Rhizosperic Phosphate Solubilizing Isolates for Plant Growth Promoting Characteristics

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A B S T R A C T

Maize forms a major part of cereal crops consumed by man and serve as a source of dietary carbohydrates. It is used for livestock feed and it is the cheapest and palatable livestock feed for animals such as pig, cattle, sheep, poultry and it is also a source of raw materials for the production of corn sugar, corn starch, corn syrup and corn oil. In the present study twenty four (24) phosphate solubilizing bacteria (*i.e.*, sixteen *Bacillus* and eight *Pseudomonas*) isolated from Maize research station and college farm, Rajendranagar, PJTSAU, Telangana and characterised by their Plant Growth Promoting Properties (PGPR) under *in vitro* conditions such as P, Zn and K solubilization. The isolate PSB 6 showed maximum Phosphate solubilization zone of 15.50 mm and the solubilization efficiency (%) is 258.33 %. The isolates both PSB 6 and PSB 19 showed maximum Zinc solubilization zone *i.e.*, 14.00 mm and the solubilization efficiency (%) maximum for PSB 6 i.e., 233.30 %. The isolate PSB 11 showed maximum Potassium solubilization zone of 14.00 mm with the solubilization efficiency (%) of 280.00 %. Apart from these all (24) isolates were screened for IAA production, exopolysacharide production, siderophore production and HCN production also. All the isolates responded positively to the IAA production except PSB 5, PSB 15 and PSB 22 were negative. All the isolates (24) were positive to the exopolysacharide production except PSB 10 and PSB 17. All the isolates (24) were positive to the siderophore production except PSB 2, PSB 9, PSB 13 and PSB 19. All the isolates (24) were positive to the HCN production except PSB 11, PSB 17 and PSB 24.

Introduction

Among the crops corn (*Zea mays*) is an important in temperate climatic region because of the increasing demand for food and livestock feed. Nitrogen and phosphorus are essential nutrients for plant growth and development in corn (Wua *et al.*, 2005). The phosphorus is mostly insoluble form in the soil and it is unavailable to plants. Nitrogen fixing and P solubilizing bacteria are important for plant nutrition by increasing N and P uptake by the plants and playing a significant role as a Plant Growth Promoting Rhizobacteria (PGPR). Nitrogen fixation and P solubilization (Zaidi *et al.*, 2006)

production of antibiotics (Zahir *et al.,* 2004) are the principal mechanism for the PGPR.

In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue.

PGPR are applied to various crops to enhance growth, seed emergence and crop yield and some commercialized.

PGPRs are the potential tools for sustainable agriculture and trend for the future. The present investigation continued on mainly collection of phosphate solubilizing microorganisms from different maize rhizospheric soils and commercial PSB inoculants. Preparing the different types (carrier, liquid and biofilmed) of biofertilizer formulations and test the efficacy and persistence of biofilmed based PSB and other types of biofertilizers with Maize crop.

Beneficial biofilms developed by nitrogen fixing bacteria and P - solubilising fungi *in vitro* conditions and also used as biofertilizers in non - leguminous crops and also observed that the bacteria colonized fungal mycelia to form biofilms. The biofilms showed high rates of biological nitrogen fixation and organic acid production which directly influences the synthesis of indole acetic acid like substances than microbes when used as monocultures. (Seneiviratne *et al.,* 2003)

Materials and Methods

Soil sample collection and Isolation

Samples were collected from Maize Research Station, Hyderabad and College Farm, College of Agriculture, Rajendranagar, Hyderabad. For the isolation of Phosphate solubilizing bacteria Pikovaskya's medium was used.

Morphological characterization of phosphate solubilizing bacterial isolates

All the Phosphate solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction was also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Screening of PSB isolates for Plant Growth Promoting (PGP) properties

Pure isolates were isolated by streaking isolates on respective media and screened for following Plant growth promoting characteristics viz. production of IAA, siderophores, HCN and their ability to solublize phosphorous.

Phosphate solubilization

Sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petriplates and incubated for 24 h. After incubation the Pikovskaya's plates were spot inoculated with isolates and incubated at 28 ± 1°C for 4-5 days. Formation of a clear zone around the colonies was considered as positive result for phosphate solubilization.

PSE (Phosphate Solubilization Efficiency) = Z / C x 100

Z - Clearance zone including bacterial growth

C - Colony diameter

Zinc solubilization

The isolates were inoculated into agar medium containing 0.1% insoluble zinc compounds *viz* ZnO, ZnS and $ZnCO_3$ (Saravanan *et al.,* 2013).Formation of a clear zone around the colonies were considered as

positive result for zinc solubilization. The diameters of the clearing zones around the colonies are measured.

Potassium Solubilization

Bacterial colonies exhibiting clear zone of potassium solubilization on Aleksandrov agar were selected as potassium solubilizers. Secondary screening was carried out on the basis of study of zone activity of the different isolates by using Khandeparkar's selection ratio (Prajapati and Modi, 2012). The diameter of the clearing zone around the colonies were measured.

Exo polysaccharide production (EPS)

Initially prepare TSP broth with -0.30 Mpa osmotic stress with 15% PEG 6000. Inoculated TSP broth with test culture incubate for 3 days. After incubation culture was centrifuged at 20,000 g in refrigerated centrifuge for 25 min. Collect the supernatant and filtrate supernatant 0.45 µm nitro cellulose membrane. These filtrate dialysed against 4^{o}C. The dialysed again centrifuged at 20,000 g for 25 min remove any insoluble material and mixed with 3 volumes ice cold absolute alcohol and kept overnight at 4^{o}C. Precipitated EPS obtained by centrifugation at 10,000 g for 15 min. Suspended in water purification of EPS (Sandhya *et al.,* 2009).

Indole acetic acid production

Indole Acetic acid Production was tested according to Gorden and Weber (1951).The active culture of each test isolate was raised in 5 ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of Orthophosphoric acid was added to 2 ml of supernatant and incubated for 30 min to develop the colour. Development of pink colour considered as positive for IAA production.

Protein estimation

One ml of the sample was taken and cells were pelleted by centrifugation at 10,000 rpm for 8 min. Spectrophotometric measurement of colour development was done by using the method of Lowry *et al.,* (1951). Intensity of blue colour was measured at absorbance maximum of 660 nm.

Siderophore production

Siderophore production was estimated qualitatively. Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987). For the detection of siderophores, each isolate was grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 h on a rotary shaker at room temperature. Chrome Azurol S (CAS) assay was used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar plates and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicated the siderophore production.

Hydrogen Cyanide Production (HCN)

The HCN production was tested by the method of Castric and Castric (1983). First respective media added with glycine plates were prepared separately and incubated for 24 h. After that, 1ml of culture of each test isolate was inoculated on respective media plates separately. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5 % picric acid (w/v) in 1 % sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic

condition. The control plate did not receive the inoculum. The plates were incubated upside down at 28 ± 2°C for 48 - 72 h. Change the colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Broth assay

In vitro biosolubilization of rock phosphate

Phosphate solubilization potential of Phospahte solubilizing microbes were studied *in vitro* by estimation of available phosphorus in Pikovskaya's broth medium with known amount of Tri-calcium phosphate (0.5 g 100 ml^{-1}) as a substrate before sterilization. Each of 5 mm mycelial bit of test fungal culture and 0.5 ml suspension of each bacterial culture was inoculated in 250 ml conical flask containing 100 ml sterilized Pikovskaya's broth medium and make triplicate (biofilm preparation). A control without any PSM was also maintained. The fungal and bacterial isolates were allowed to grow for seven and fourteen days at 28 ± 2^0C in BOD incubator. To know the bios olubilisation of rock phosphate by biofilms, broth was filtered through whatman filter paper No. 42 and centrifuged at 15,000 rpm for 30 min in centrifugation. The clear supernatant was collected in 100 ml volumetric flasks and volume was made up to 100 ml with sterilized distilled water. Thus extract of each test biofilm solution was prepared then the available phosphorus in broth culture was determined (Gaur, 1990).

Results and Discussion

Four soil samples were collected from Maize Research Station, Hyderabad and College Farm, College of Agriculture, Rajendranagar, Hyderabad. Twenty four (24) phosphate solubilizing bacteria (*i.e.,* sixteen *Bacillus* and eight *Pseudomonas*) isolates collected and screen their plant growth promoting characters.

Screening of isolates for their Plant Growth Promoting (PGP) characters

Phosphate Solubilization

Qualitative method

All the sixteen *Bacillus* isolates were able to form clear zone of phosphate solubilization on Pikovaskaya'sagar plate ranged from 15.50- 6.10 mm. Among them PSB 6 of *Bacillus* spp detected the highest solubilization zone (15.50 mm) followed by PSB 5 (14.80 mm) and the lowest solubilization zone was observed with PSB 3 (6.10 mm).

All the eight *Pseudomonas* isolates were able to form clear zone of phosphate solubilization on Pikovaskaya'sagar plate ranged from 12.00 - 6.40 mm. Among them PSB 24 of *Pseudomonas* spp detected as highest solubilization zone 12.00 mm followed by PSB 20 (11.00 mm) and the lowest solubilization was showed by PSB 22 (6.40 mm). The *Aspergillus* spp (Asp1) showed 10.1 mm Phosphate solubilization zone. (Table 1 and Plate 1) *Bacillus* and *Pseudomonas* spp differ in the ability to produce phosphatase enzyme and production of organic acids and hence showed different solubilization efficiency.

Tensingh *et al.,* (2015) identified the selected strains were *Bacillus* and *Pseudomonas*. The isolated strains were characterized under *in vitro* conditions. They showed solubilization zone ranges from 2 - 5 mm at 28 - 30^0C. The highest solubilization was observed with *Pseudomonas putida* (5 mm) followed by *P. flourescens* (4 mm) and the lowest solubilization was observed in *Bacillus megaterium* (2 mm). Similarly Uma and

Sathiyavani (2012) reported phosphate solubilization by *Bacillus* spp from groundnut rhizosphere (*Arachishypogaea L*).

Quantitative estimation of available phosphorus in Pikovaskaya's broth

All the sixteen *Bacillus* isolates were able to solubilize the available phosphorus in Pikovaskaya's broth with known amount of Tri - calcium phosphate as a substrate. Among them PSB 6 recorded the more available phosphorus content of 0.89 mg L^{-1}(pH: 7.10). Second best was showed by different isolates PSB 8 and PSB 10 *i.e.*, 0.82 mg L^{-1}(pH: 7.00and 6.00). The lowest was shown by PSB 16 with 0.57 mg L^{-1}(pH: 7.89).

All the eight *Pseudomonas* isolates were able to solubilize the available phosphorus in Pikovaskaya's broth with known amount of Tri - calcium phosphate as a substrate. Among them PSB 24was recorded the highest available phosphorus content of 0.82 mg L^{-1}(pH: 7.00). Second best was observed by the isolate PSB 18 and PSB 23 *i.e.*, 0.81 mg L^{-1}(pH: 6.80 and 7.00). The lowest was recorded by PSB 21 with 0.54 mg L^{-1} (pH: 6.00) phosphorus solublization. The *Aspergillus* spp (Asp1) showed available phosphorus concentration *i.e.*, 0.78 mg L^{-1}(pH: 6.70) respectively (Table 1).

Similar results were observed by Karpagam and Nagalakshmi. **(**2014) *i.e.*, thirty seven Phosphate solubilizing microbial isolates were isolated on the Pikovskaya's agar medium. Out of 37 microbial isolates eight isolates were showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 3.00mg L^{-1} and they were selected for the qualitative as well as quantitative study. Among these eight potent isolates, 3 strains (PSM 1, PSM 2 and PSM6) showed maximum PSI on agar plates along with high soluble phosphate production of 0.37 mg L^{-1}, 0.30 mg $L^{-1}$and 0.28 mg L^{-1}in Pikovaskaya's broth.

Zinc solubilization

Among sixteen *Bacillus* isolates, thirteen were positive for zinc solubilization on Tris - minimal media supplemented with zinc oxide and ranged from 14.00- 6.40 mm. Among them PSB 6 of *Bacillus* spp recorded the highest solubilization zone (14 mm) followed by PSB 8 (13.00 mm) and the lowest solubilization was observed in PSB 2 (6.40 mm). The zinc solubilization was negative in the isolates PSB 7, PSB 9 and PSB 12.

Among eight *Pseudomonas* isolates, seven isolates recorded to be positive for zinc solubilization on Tris - minimal media supplemented with Zinc oxide and ranged from 14.00 -8.70 mm. Among them PSB 19 of *Pseudomonas* isolate recorded the highest solubilization zone (14.00 mm). Second best was shown by PSB 21 (12.00 mm). The lowest was shown by PSB 24 (8.70 mm). No solubilization was recorded in the isolate PSB 20 whereas fungal isolate *Aspergillus* spp (Asp1) showed 10.00 mm zinc solubilization zone. (Table 1 and Plate 1) Similar results were observed by Goteti *et al.*, (2013) who screened ten strains for zinc solubilization among which P29, P33, and B40 produced 22.0 mm clear halos on solid medium amended with zinc carbonate. Similarly P17 and B40 showed 31.0 mm zone in zinc oxide incorporated medium.

Potassium solubilization

Among sixteen *Bacillus* isolates, fourteen isolates were able to form clear zone of potassium solubilization on modified Aleksandrov media ranged from 14.00 - 8.00 mm. Among them PSB 11 isolate recorded the highest solubilization zone (14.00 mm), followed by PSB 12 (13.90 mm).

Table.1 Phosphorus, Zinc and Potassium solubilisation efficiency of different bacterial isolates

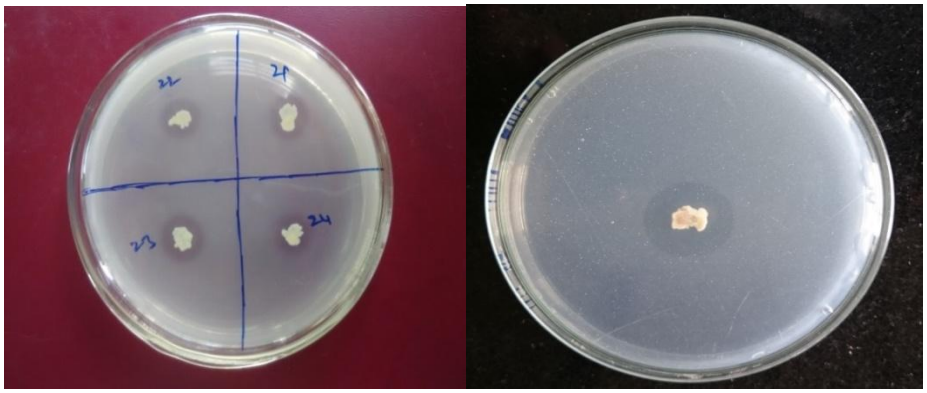
Isolate Code	Zone diameter		Phosphorus Solubilization efficiency (%)	Soluble P concentration (mg L^{-1})	Zinc Solubilization zone (mm)	Culture diameter (mm)	Zinc Solubilization efficiency (%)	Potassium Solubilization zone (mm)	Culture diameter (mm)	Potassium Solubilization efficiency (%)
	Solubilization zone (mm)	Culture diameter (mm)								
PSB1	9.10	5.00	182.00	0.63	12.20	8.00	152.50	9.30	6.00	155.00
PSB2	8.60	6.30	136.50	0.60	**6.40**	**5.80**	**110.34**	0.00	0.00	0.00
PSB3	**6.10**	**5.20**	**117.30**	0.62	7.80	5.90	132.20	**8.00**	**6.30**	**126.98**
PSB4	8.00	4.10	195.10	0.71	8.50	4.50	188.80	11.60	7.20	161.10
PSB5	14.80	6.00	246.60	0.79	9.50	6.00	158.33	13.50	7.30	184.90
PSB6	**15.50**	**6.00**	**258.33**	**0.89**	**14.00**	**6.00**	**233.30**	13.20	7.60	173.60
PSB7	12.30	8.00	153.75	0.76	0.00	0.00	0.00	8.60	6.00	143.30
PSB8	9.10	4.00	227.50	0.82	13.00	6.00	216.60	10.40	6.30	165.00
PSB9	6.60	4.00	165.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00
PSB10	14.50	6.40	226.60	0.82	8.60	6.70	128.30	12.60	6.80	185.20
PSB11	10.40	6.40	162.50	0.75	10.30	8.40	123.80	**14.00**	**5.00**	**280.00**
PSB12	13.50	9.00	150.00	0.78	0.00	0.00	0.00	13.90	6.00	231.60
PSB13	9.30	7.00	132.80	0.66	9.60	7.80	124.30	9.00	6.00	150.00
PSB14	12.00	10.00	120.00	0.77	12.50	6.00	208.33	12.70	7.90	160.70
PSB15	10.80	5.50	196.30	0.60	10.60	5.60	189.20	10.30	5.20	198.00
PSB16	11.40	5.70	200.00	**0.57**	8.00	4.10	195.10	11.60	5.00	232.00
PSB17	8.30	6.40	129.60	0.74	10.80	5.50	196.30	**12.20**	**5.50**	**221.81**
PSB18	9.70	6.00	161.60	0.81	11.00	5.40	203.70	9.40	5.70	164.90
PSB19	8.70	6.90	126.00	0.65	**14.00**	**6.80**	**205.88**	**6.00**	**5.30**	**113.20**
PSB20	11.00	9.20	119.50	0.65	0.00	0.00	0.00	9.80	4.50	217.70
PSB21	8.40	6.70	125.30	**0.54**	12.00	7.00	171.40	0.00	0.00	0.00
PSB22	**6.40**	**5.60**	**114.28**	0.77	9.00	7.00	128.50	9.70	7.00	137.10
PSB23	9.40	6.00	156.60	0.81	9.30	6.00	156.60	10.00	7.00	142.80
PSB24	**12.00**	**6.00**	**200.00**	**0.82**	**8.70**	**7.00**	**124.28**	0.00	0.00	0.00
Asp1	10.10	5.80	174.10	0.78	10.00	6.40	156.20	12.00	8.00	150.00
CD	**0.313**			**0.035**	**0.298**			**0.290**		
SE(d)	**0.155**			**0.018**	**0.147**			**0.143**		
SE(m)	**0.110**			**0.012**	**0.104**			**0.101**		
CV	**1.855**			**2.969**	**1.751**			**1.617**		

Table.2 Evaluation of different isolates for their Plant Growth Promoting (PGP) characters

ISOLATE CODE	PROTEIN ESTIMATION (mg L^{-1})	IAA PRODUCTION (µg mL^{-1})	EXOPLOYSACHARIDE PRODUCTION (EPS)	SIDEROPHORE PRODUCTION	HCN PRODUCTION
PSB1	0.48	39.30	+	++	++
PSB2	**0.30**	41.50	+	-	+
PSB3	0.40	29.50	**+++**	++	++
PSB4	0.44	**50.70**	+	+	+
PSB5	0.43	0.00	+	++	+
PSB6	**0.58**	24.40	++	**+++**	++
PSB7	0.46	33.50	+	+	+
PSB8	0.41	40.60	+	+	+
PSB9	0.43	29.90	+	-	+
PSB10	0.50	25.20	-	++	**+++**
PSB11	**0.30**	39.80	+	+	-
PSB12	0.55	**23.00**	+	+	++
PSB13	0.42	31.50	++	-	+
PSB14	0.35	23.40	+	++	++
PSB15	0.39	0.00	+	+	+
PSB16	0.34	36.70	+	+	++
PSB17	0.43	32.50	-	**+++**	-
PSB18	0.45	**27.70**	+	+	+
PSB19	0.49	31.90	++	-	+
PSB20	**0.50**	**49.80**	**+++**	++	**+++**
PSB21	0.37	28.90	+	+	++
PSB22	**0.35**	0.00	++	**+++**	+
PSB23	0.38	29.40	+	++	+
PSB24	0.40	34.20	**+++**	+	-
Asp1	0.32	36.00	-	+	+
CD	**0.017**	**0.252**			
SE(d)	**0.009**	**0.125**			
SE(m)	**0.006**	**0.088**			
CV	**2.509**	**0.454**			

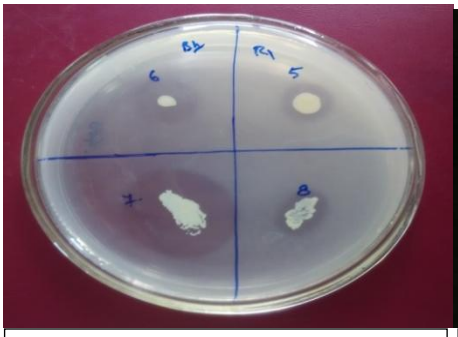
Strong +++ Medium ++ Low + No production –

Plate.1 PGPR characters of PSB isolates



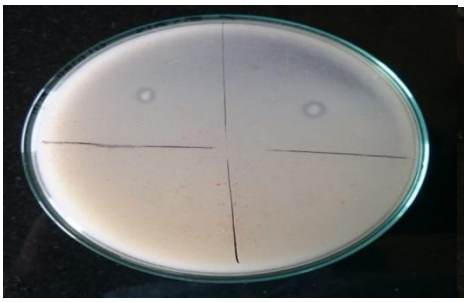
Phosphate solubilization by PSB bacteria

Phosphate solubilization Asp1 fungi



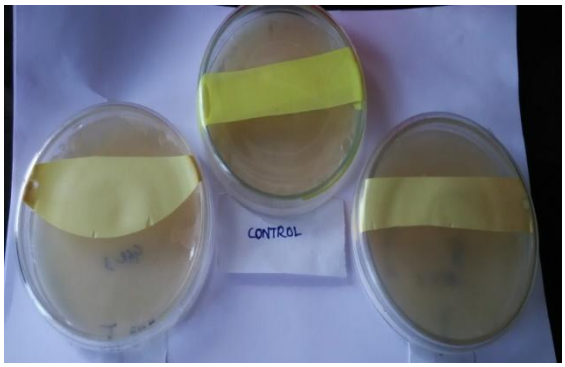
Zinc solubilization by PSB bacteria

Zinc solubilization by Asp1 fungi



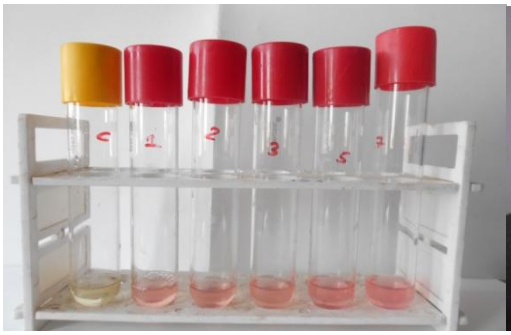
Potassium solubilization by PSB11 bacteria

Potassium solubilization by Asp1 fungi



HCN production

Plate.2 PGP activity of PSB isolates



IAA production

Siderophore production

The lowest was shown by PSB 3 (8.00 mm) and solubilization was not shown by the isolates PSB 2 and PSB 9.

Among eight *Pseudomonas* isolates, six isolates were able to form clear zone of potassium solubilization on modified Aleksandrov's media ranged from 12.20-6.00mm. Among them PSB 17 isolate recorded the highest solubilization zone (12.20 mm) followed by PSB 23 (10.00 mm). Least solubilization recorded in PSB 19 (6.00 mm). No solubilization was observed in PSB 21 and PSB 24, whereas fungal isolate *Aspergillus* spp (Asp1) showed 12 mm Potassium solubilization zone (Table 1 and Plate 1).

The results are agreement with Parmar and Sindhu (2013) who screened one hundred and thirty seven bacterial isolates for the potassium solubilization ability using spot test method on modified Aleksandrov medium plates containing mica powder. They found that out of 137 rhizobacterial isolates tested only 20 formed significant zone of K solubilization on mica powder containing medium. Out of 20 efficient isolates, 15 (*i.e.*, HWP7, HWP28, HWP38, HWP47, HWP57, HWP63, HWP69, WPS3,CPA123, KPM15, GYB106, WPS73, NNY43, PPM115 and CPA 152) showed more solubilization zone on mica incorporated plates. Five bacterial cultures, namelyHWP15, HWP53, HWP61, CP43 and WPS118 showed small solubilization zone. Three bacterial strains *i.e.*, HWP38, NNY43 and WPS73 showed significant K solubilization zone.

Exo polysacharide production (EPS)

Among sixteen *Bacillus*isolates, fifteen isolates were positive for EPS production; out of which PSB 3showed maximum (+++) EPS production followed by PSB 6 and PSB 13 which showed moderate (++) production then remaining were weak (+) producers and no production was shown by the PSB 10 isolate.

Among eight *Pseudomonas* isolates, sevenisolates were positive for EPS production; out of which PSB 20 and PSB 24 were strong (+++)EPS producers followed by PSB 19 and PSB 22 showed moderate (++) producers and the remaining isolates were weak (+) producers. No production was observed by PSB 17 isolate, whereas *Aspergillus* spp (Asp1) was negative for EPS production (Table 2).

Similar results were observed by Ashraf *et al.*, (2006) who isolated and identified the EPS producing bacteria associated with the roots of three wheat lines grown in saline and non-saline soil.

Results indicated the presence of various EPS-producing bacterial genera in unplanted saline and non-saline soil, rhizosphere and rhizoplane of the three wheat lines.

Indole Acetic Acid (IAA) production

Among sixteen *Bacillus* isolates, fourteen isolates were positive for IAA production; out of which maximum was shown by PSB 4 (50.70 µg mL^{-1}) followed by PSB2 (41.50µg mL^{-1}), PSB 8 (40.6 µgmL^{-1}) and the least was recorded in the isolate PSB 12 (23.00 µgmL^{-1}). PSB 5 and PSB 15 were negative for IAA production (Table 2 and Plate 2).

Among eight *Pseudomonas* isolates, seven isolates were positive for IAA production; out of which maximum was shown by PSB 20 (49.80 µgmL^{-1}) followed by PSB 24 (34.20 µgmL^{-1}), PSB 17 (32.50 µg mL^{-1}) and the least was recorded by the isolate PSB 18 (27.70 µgmL^{-1}). PSB 22 was negative for IAA production. *Aspergillus* spp (Asp1) was positive for IAA production it produced (36.00µg mL^{-1}).

Hussain and Srinivas (2013) isolated *Pseudomonas* spp and *Azotobacter* spp each from rhizosphere of *Acacia nilotica* and *Albizialebbeck* and reported that 70% of the isolates were produced IAA.

Verma *et al.,* (2010) evaluated *Rhizobium* spp, *Pseudomonas fluorescens, Bacillus megaterium* and *Azotobacter chroococcum* for plant growth promoting properties. All the bacterial strains were found to be positive for IAA production and phosphate solubilization.

Protein estimation

Among the sixteen *Bacillus* isolates the maximum protein content was recorded in PSB 6 (0.58 $mgmL^{-1}$) followed by PSB 12 (0.55 $mgmL^{-1}$) and lowest was recorded in PSB 2 and PSB 11 (0.30 $mgmL^{-1}$). Among the 8 *Pseudomonas*isolates the maximum protein content was found in PSB 20 (0.50 $mgmL^{-1}$) followed by PSB 19 (0.49 $mgmL^{-1}$) and lowest was recorded in PSB 22 (0.35 $mgmL^{-1}$).Protein content in *Aspergillus* isolate Asp1was 0.32 $mgmL^{-1}$ (Table 2).

Siderophore production

Among sixteen *Bacillus* isolates, thirteen isolates were positive for siderophore production; out of which PSB 6was detected as strong (+++) siderophore producer followed by PSB 1, PSB 3, PSB 5, PSB 10 and PSB 14 showed moderate (++) producers then remaining isolates were weak (+) producers and no production was observed in PSB 2, PSB 9 and PSB 13 isolates.

Among eight *Pseudomonas* isolates, seven isolates were positive for siderophore production; out of which PSB 17 and PSB 22 were strong (+++) siderophore producers followed by PSB 20 and PSB 23 *i.e.*, moderate (++) producers and the remaining were found to be weak (+) producers. No production was observed in PSB 19.*Aspergillus* spp (Asp1) was found to be a weak (+) siderophore producer (Table 2 and Plate 2).

The results are similar to the earlier findings of Sreedevi *et al.,* (2014) isolated ten *Pseudomonas* spp from paddy soil. Among isolated strains three *Pseudomonas* isolates *Pseudomonas* 1, *Pseudomonas* 2 and *Pseudomonas* 3 showed maximum siderophore production on succinic acid medium and chromo azural S (single dye) agar medium. Maximum siderophore production was observed in *Pseudomonas* 1, *Pseudomonas* 2 and *Pseudomonas* 3 with 94, 88 and 83 % respectively.

Hydrogen Cyanide (HCN) production

Among sixteen *Bacillus* isolates, fifteen isolates were positive for Hydrogen Cyanide production; out of which PSB 10was strong (+++) Hydrogen Cyanide producer followed by PSB 1, PSB 3, PSB 6, PSB 12,PSB 14 and PSB 16 were moderate (++) producers remaining isolates were weak (+) producers and no production was observed in PSB 11.

Among eight *Pseudomonas* isolates, six isolates were positive for Hydrogen Cyanide production; out of which PSB 20 was strong (+++) Hydrogen Cyanide producer followed by PSB 21 was moderate (++) producer and the remaining isolates were weak (+) producers. No production was observed in PSB 17 and PSB 24. *Aspergillus* spp (Asp1) was a weak (+) producer for Hydrogen Cyanide production (Table 2 and Plate 1).

The results are similar to the earlier findings of Jha *et al.,* (2009) who reported production of HCN by some new fluorescent Pseudomonad strains. In the present study HCN production by PGPR isolates were in agreement with the earlier reports of Punkuj

and Vishal (2013)on production of plant growth promoting substance by Pseudomonads.

The isolate PSB 6 shows the highest phosphate and zinc solubilisation efficiency (258.33 % and 233.30 %).The highest protein content also was recorded in PSB 6 *i.e.,* 0.58 mg l^{-1}.Highestsiderophore production was observed in PSB 6. Based on the above results we concluded that PSB 6 (*Bacillus* spp) shows more Plant growth promoting characters among 24 isolates.

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