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Salinity tolerant phosphorous solubilising bacteria from saline soils of Telangana

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Abstract
Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigour, crop yield and many soils throughout the world are P deficient, keeping both these points in view we isolated sixteen phosphorous solubilizing bacteria (PSB) from saline soils of Telangana, which tolerated 15% NaCl. The isolates were also characterized for morphological, cultural and biochemical characteristics. Of all the 16 strains isolated, 5 were tentatively identified as *Bacillus* sp., 8 as *Pseudomonas* sp., 2 as *Azotobacter* sp. and 1 as *Rhizobium* sp. according to the Bergey's manual of systemic bacteriology. The isolates were further screened for plant growth promoting (PGP) attributes like siderophore, HCN, IAA production and also for ACC deaminase activity, EPS production. Siderophore was produced by 37.5% isolates, HCN was produced by 75% isolates and IAA was produced by 50% isolates respectively. Only four isolates used ACC as a sole nitrogen source and EPS was produced by only 13 isolates.

Keywords: Salinity, PSB and characterization

Introduction
Salinity is one of the most important abiotic stresses that cause a reduction in plant growth and yield in many parts of the world (Kaya *et al*, 2007) [21, 22]. Twenty per cent of the cultivable lands in the world are adversely affected by high salt concentration which inhibit the plant growth and yield (Razmjoo *et al.* 2008) [37]. An excess of soluble salts in the soil leads to osmotic stress, specific ion toxicity and ionic imbalances (Munns, 2003) [28] and the consequences of these can be plant death or yield losses (Rout and Shaw, 2001) [38]. However, salt tolerance capacity differs in crop varieties and microbial strains. Presence of variation in salt tolerance, in different crops and microbial inoculants was reported by different scientists in wheat (Akhter *et al.* 2003) [2], rice (Lee *et al*, 2003) [23] and AM Fungi (Zou and Wu, 2011) [46]. Commercial strains of rhizobia usually cannot tolerate or function under high levels of osmotic stress caused by salinity (Green, 1991) [15]. Thus strategies to improve legume production in saline environments include selection of host genotypes that are tolerant to high salt conditions and inoculation with salt tolerant rhizobacteria could constitute another approach (Sharma *et al.* 2013) [8, 41].
Soil salinity in arid regions is frequently an important limiting factor for cultivating agricultural crops. Many technologies have been implicated in improving salt tolerance of plants and PGPR have also been reported to alleviate salt stress in plants. Salt tolerant plant growth promoting rhizobacteria have been found to improve growth of tomato, pepper, canola, bean, soybean and lettuce under saline conditions (Barassi *et al.* 2006; Glick *et al.* 1998) [3, 13] Use of these microorganisms can alleviate stresses in agriculture thus opening a new and emerging application of microorganisms.
The isolation of indigenous microorganisms from the stress affected soils and screening on the basis of their stress tolerance and PGP traits may be useful in the rapid selection of efficient strains that could be used as bio inoculants for stressed crops (Pooja and Kumar, 2015) [34]. Bacteria isolated from different stressed habitats possess stress tolerance capacity along with the plant growth promoting traits and therefore are potential candidates for seed bacterization. When inoculated with these isolates, plants show enhanced root and shoot length, biomass and biochemical levels such as chlorophyll, carotenoids and protein (Tiwari *et al.* 2011) [43]. Therefore the present study focused to isolate phosphorous solubilizing rhizobacteria from saline environment (*i.e.*, which will confer resistance to salt and soulubilize phosphate) and to characterize the isolates for cultural, morphological characteristics and screen the isolates for plant growth promoting attributes, ACC deaminase activity and EPS production.

Material and Methods

Soil sampling

Saline soils have been identified and samples were collected from different saline rhizospheric soils of Telangana state. The soil samples were selected based on salinity and sampling was done to a depth of 10 to 15 cm. The plant was uprooted and the soil intimately adhering to the roots was collected and mixed to provide a composite soil sample. All the samples were separately bagged, labelled, air dried and stored in a refrigerator at 4 ºC for further studies.

Isolation of Rhizobacteria

For isolation of rhizobacteria the method proposed by Vlassak *et al.* (1992) [45] was followed. In this procedure 10 g of soil from each soil sample was taken in a conical flask to which 90 ml of normal saline was added. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 0.1 ml of sample was spread on sterilized petri plates containing Trypticase Soy Agar (TSA) media with 2% NaCl and the petri plates were incubated at room temperatures for 24 - 48 h. Two replicates were maintained for each dilution. The plates were examined for bacterial colonies and the colonies were purified and evaluated for salinity tolerance.

Screening for salinity tolerance

Trypticase soy agar medium plates were prepared with different salt concentrations *i.e.,* 2%, 5%, 10% and 15%. All the isolates were spot inoculated on these prepared plates. For each treatment three replications were maintained. The petri plates were incubated at 28 ± 2 ºC for 24 - 48 h. Based on observations, cultures showing growth on different concentrations of salts were chosen for further study (Buchan *et al.* 2000) [4].

Qualitative assay of phosphate solubilization

Pure cultures of phosphate solubilizing bacterial isolates were spot inoculated in the prepared plates of Pikovskaya's agar medium. The plates were incubated at 30 ± 1 ºC for 7-10 days and the zone of phosphate solubilization (mm) formed around colonies was recorded (Pikovskaya, 1948) [33].

Cultural and morphological characterization

The bacterial isolates were tentatively identified on the basis of morphological, cultural, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt *et al.* 1984) [16].

All the isolated bacterial cultures were grown and purified by the streaking as single colony in the TSA medium and allowed to grow for 24 - 48 h and the isolates were grown on different mediums like Nutrient agar (NA), King's B agar (KB), Waksman's 77 agar & Yeast Extract Mannitol Agar with Congo red (YEMA) medium for further confirmation. Pure cultures were preserved at 4 ºC for further studies.

Biochemical and physiological characterization

Different biochemical tests were conducted using standard procedures. The protocols followed are briefly outlined below.

Indole production

Sterilized hydrogen sulfide indole motility agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2 ºC, after incubation 10 drops of Kovac's Indole reagent was added to each tube. The isolates showed production of red colour was recorded as positive for indole production (Isenberg and Sundheim, 1958).

Catalase test

This test was conducted to study the presence of catalase enzyme in bacterial colonies. Pure isolates (24 h old) were taken on glass slides and one drop of H_2O_2 (30%) was added. Appearance of gas bubble indicated the presence of catalase enzyme (Rangaswami and Bagyaraj, 1993) [36].

Oxidase test

The overnight grown cultures of the test isolates were spotted on plates poured with sterile Trypicase Soy Agar and the plates were incubated for 24 h at 28 ± 2 ºC. After incubation, 2 - 3 drops of N, N, N', N'- tetramethyl- p-phenylene diamine dihydrochloride (Wurster's reagent) was added on to the surface of growth of each test organism. The isolates showed change of colour to maroon was noted as oxidase positive (Collins and Lyne, 1970) [7].

Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ± 2 ºC. Then the tubes were placed in the refrigerator for 30 minutes at 4 ºC. The isolates showed liquefied gelatin was taken as positive and those which resulted in solidification of gelatin on refrigeration was recorded as negative for the test (Macfaddin, 2000) [25].

Methyl red test

Sterilized glucose phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 ºC for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Development of red colour indicated positive for the test while yellow colour production indicated negative for the test (Macfaddin, 2000) [25].

Voges - prausker's test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 ºC for 48 h. After incubation ten drops of baritt's reagent A was added and gently shaken followed by addition of 10 drops of baritt's reagent B. Pink colour development in the broth was taken as positive for the test (Macfaddin, 2000) [25].

Citrate utilization

Isolates were streaked on simmon's citrate agar slants and incubated at 28 ± 2 ºC for 24 h. Change in colour from green to blue indicated positive reaction for citrate utilization (Macfaddin, 2000) [25].

Starch hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2 ºC for 24 - 48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive for the test (Macfaddin 2000) [25].

Hofer's alkaline agar test

The isolates were streaked on hofer's alkaline media plates and incubated at 28 ± 2 ºC for 48 - 72 h. Rhizobium does not grow on the media plates (Vincent, 1970).

Screening of PSB isolates for PGP attributes

Siderophore production

Production of siderophores was estimated qualitatively on aqueous ferric chloride solution for siderophores detection. 60.5 mg CAS (Chrome azurol sulfonate) was dissolved in 50 ml of distilled water and mixed with 10 ml of iron (III) solution (1 mM $FeCl_3$. $6H_20$ in 10 mM HCl). This was added to 72.9 mg of hexadecyl trimethyl ammonium bromide (HDTMA) in 40 ml of distilled water. The dark blue colored CAS reagent was then autoclaved for 15 minutes. This reagent was added to PIPES agar medium (30.24 g of PIPES buffer dissolved in 750 ml of Distilled water +15 g of agar, the pH was adjusted by using 0.1 N NaOH pellets). The medium is allowed to solidify in the plates and all the isolates were spot inoculated on prepared plates and incubated at optimum temperature for 3 - 4 days to allow the growth (Schwyn & Neilands, 1987) [40].

Hydrocyanic acid production (HCN)

The HCN production was tested by the method of Castric and Castric (1983). Medium plates *i.e*., modified nutrient agar was prepared by adding 4.4 g per litre of glycine separately. One ml of culture of each test isolate was inoculated on plates. 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 upperlid 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 ºC for 48 - 72 h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Indole Acetic Acid (IAA) production

Indole acetic acid production was quantitatively measured by the method given by Gordon and Weber (1951). Bacterial cultures were grown in a Luria-Bertani broth amended with tryptophan (5 mM) for 3 - 4 days. Cultures were centrifuged at 10,000 rpm for 20 min. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of salkowski reagent. Tubes were incubated at room temperature for 25 min. The intensity of pink color was recorded at 530 nm spectrophotometrically and the amount of IAA produced was extrapolated from the standard curve.

Screening for ACC deaminase activity

Screening for ACC deaminase activity of isolates was done based on their ability to use ACC as a sole nitrogen source. All the isolates were grown in 5 ml of trypticase soy broth medium incubated at 28 ºC at 120 rpm for 24 h. The cells were harvested by centrifugation at 3000 *g* for 5 min and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified Dworkin and Foster (DF) salts minimal medium (Dworkin and Foster, 1958) [11] supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC was taken as negative control and with $(NH_4)_2SO_4$ (0.2% w/v) as positive control. The plates were incubated at 28 ºC for 72 h. Growth of isolates on ACC supplemented plates were compared to negative and positive controls and concluded based on growth by utilizing ACC as nitrogen source.

Exo polysaccharide (EPS) production

Exo polysaccharide was extracted from 3 day old cultures grown in trypticase soy broth (15% PEG 6000 was added to trypticase soy broth for inducing stress). The culture was centrifuged at 20,000 *g* for 25 min and the supernatant was collected. Highly viscous cultures were diluted with 0.85% KCl before centrifugation. The pellet was washed twice with 0.85% KCl to completely extract EPS. The possible extraction of intracellular polysaccharides was ruled out by testing the presence of DNA in the supernatant by DPA reagent (Burton, 1956).

Results and Discussion

Collection of soil samples

Saline rhizospheric soil samples were collected from Mahabubnagar, Nalgonda and Rangareddy districts of Telangana.

Isolation of salinity tolerant rhizobacteria

Bacterial isolation was carried out using Trypticase Soy Agar (TSA) medium with 2% NaCl. Based on the differences in the colony morphology and gram staining, individual bacterial colonies were selected. Single colonies were further streaked on the agar media and single pure colonies were selected. All the plates were incubated at 37 ºC and the pure bacterial isolates were maintained at -20 ºC in 50% (w/v) glycerol stocks.

Inorder to overcome the problem of salinity we have isolated salinity tolerant PSB which will tolerate salt stress and also help in promotion of plant growth by solubilization of phosphorous and making it available. In addition, stress tolerant bacterial strains have an important role in improving the tolerance of plants and establish symbiosis. Similarly Leite *et al.* (2014) [24] isolated and selected bacterial isolates of sugarcane, tolerant to salinity. Five diazotrophic salt tolerant bacteria were isolated from the roots of a halophyte, Arthrocnemum indicum by Sharma *et al.* (2016) [42]. Ramadoss *et al*. (2013) [35] isolated eighty four halo tolerant bacterial strains from the saline habitats and screened for growth at different NaCl concentrations.

Screening of the isolates for salinity tolerance

All the isolates were screened for salinity tolerance at 2%, 5%, 10% and 15% NaCl concentration in Trypticase soy agar (TSA) medium. The presence of growth was recorded as positive (+). All the 40 isolates which showed tolerance upto 15% NaCl were further evaluated for phosphorous solubilization (Table 1).

Phosphate solubilization ability of isolates

Among 80 isolates 40 isolates which have shown tolerance upto 15% NaCl were screened for their ability to solubilize tri calcium phosphate (TCP) on Pikovskaya's agar medium. Bacterial strains showed different abilities to solubilize tri calcium phosphate according to the formation of visible dissolution halos on the medium (Table 1). Of the 40 best salinity tolerant strains, 16 were able to solubilize phosphate in the range of 3 to 15 mm and depicted in Table 1. Among 16 isolates PJ 66 recorded the highest solubilization zone (15 mm) followed by PJ 28 and PJ 37 (14 mm), PJ 70 (13 mm), 12 mm zone was shown by the isolates PJ 23, PJ 25, PJ 38, PJ 54 and PJ 56, PJ 8 (11 mm), PJ 21 (9 mm), PJ 19, PJ 27 and PJ 44 showed 8 mm zone, PJ 20 (5 mm) and least was shown by the isolate PJ 31 (3 mm) and 24 isolates showed no solubilization. Reference strain showed 9 mm zone of solubilization.

Organic acids such as lactic acid, oxalic acid, tartaric acid,

formic acid, malic acid, citric acid, succinic acid and propionic acid, have also been reported to be produced by bacteria in the rhizosphere of many plants which lead to the solubilization of minerals (P, K and Zn). As the isolates have been isolated from the saline rhizospheric soil, they also have the ability to solubilize minerals.

Similarly Patel *et al.* (2012) [31] isolated 176 strains of which 62 bacterial strains were able to tolerate 1 M NaCl. Twenty eight isolates of the 62 strains showed good tricalcium phosphate solubilization in solid medium in the range of 9 - 22 mm and 15 isolates showed good phosphate solubilization in liquid medium in the range of 9 - 45 g/ml. Ghevariya and Desai, 2014 reported that among 50 isolates, 46 isolates exhibited positively for Tri-calcium phosphate solubilization on pikovskaya agar, 25 isolates were able to solubilize zinc, 24 isolates were able to solubilize potassium and all isolates produced IAA in the presence of L-Tryptophan.

Table 1: *In vitro* screening of salinity tolerant rhizobacteria for "P" solubilization

Isolate	NaCl tolerance				P Solubilization (mm)
	2% (0.34 M)	5% (0.85 M)	10% (1.70 M)	15% (2.60 M)	
Ref 1	+	+	+	+	9
PJ 8	+	+	+	+	11
PJ 19	+	+	+	+	8
PJ 20	+	+	+	+	5
PJ 21	+	+	+	+	9
PJ 23	+	+	+	+	12
PJ 25	+	+	+	+	12
PJ 27	+	+	+	+	8
PJ 28	+	+	+	+	14
PJ 31	+	+	+	+	3
PJ 37	+	+	+	+	14
PJ 38	+	+	+	+	12
PJ 44	+	+	+	+	8
PJ 54	+	+	+	+	12
PJ 56	+	+	+	+	12
PJ 66	+	+	+	+	15
PJ 70	+	+	+	+	13

+ = Growth present and - = No growth

Cultural and morphological characterization

The isolates were studied for their cultural and morphological characteristics. The isolates were streaked onto different specific mediums like Jensen's agar (JA), King's B (KB) agar, yeast extract mannitol agar (YEMA) with 2% NaCl and other non-specific bacteria were checked for their growth on nutrient agar (NA) with 2% NaCl. Of the total 16 salinity tolerant phosphorous solubilizing isolates, 2 (PJ 56 & PJ 70) were gram negative, sporulation negative, white colored with convex elevation, smooth shiny surface and regular margin. The isolates were oval shaped and the isolates produced cyst on Waksman No.77 N free agar medium, PJ 70 isolate showed brown pigmentation and the 2 isolates were tentatively identified as *Azotobacter* spp. (Table 2).

Among 16 isolates, 5 isolates (PJ 27, PJ 28, PJ 31, PJ 44 & PJ 66) were gram positive, rod shaped (2 small, 2 medium and 1 large rods) and showed formation of endospores. All were convex elevated without pigmentation, of which 4 were white colored and one was yellow. Surface was smooth and margin was irregular in all 5 isolates. These 5 were tentatively identified as Bacillus spp., according to the Bergey's manual of systemic bacteriology (Table 2).

Among 16 isolates, 8 isolates (PJ 8, PJ 19, PJ 20, PJ 21, PJ 23, PJ 25, PJ 37 & PJ 54) were gram negative and didn't produce endospores, all were rod shaped (6 small and 2 medium rods). Five isolates were white and 3 are yellow colored. All showed convex elevation with smooth surface. All isolates have regular margin and 4 isolates showed light green pigmentation (PJ 8, PJ 20, PJ 21 and PJ 54) and these 8 isolates were tentatively identified as *Pseudomonas* spp. based on the cultural and morphological characteristics. One isolate (PJ 38) among 16, was tentatively identified as *Rhizobium* spp. with white translucent color, gram negative and sporulation negative. Rod shaped with raised colony, regular margin and mucoid surface without pigmentation (Table 2). The pure culture was checked for their purity and found pure and the culture couldn't show growth on different mediums like Hofer's alkaline, glucose peptone agar and lactose agar plates.

Similarly Patil, 2014 [32] isolated and identified salt tolerant phosphate solubilizing bacterium from the soil as *Bacillus* spp. based on its morphological, cultural and biochemical characteristics. Damodaran *et al.* (2013) [8] isolated 16 rhizobacteria through natural selection from saline soils and characterized them by using morphological and biochemical characters.

Biochemical characterization

After the study of cultural and morphological characteristics, the isolates were characterized with different biochemical tests *viz.*, IMVIC test, oxidase test, catalase test, carbohydrate utilization test, H_2S production, starch hydrolysis and gelatin liquefaction tests (Table 3).

Of the 16 salinity tolerant PSB isolates only one isolate produced Indole (PJ 38), 12 isolates were positive for MR test (PJ 8, PJ 19, PJ 21, PJ 25, PJ 27, PJ 28, PJ 31, PJ 37, PJ 38, PJ 44, PJ 56 & PJ 70) and 4 were negative. For Voges - prausker's test 9 isolates were positive (PJ 8, PJ 19, PJ 20. PJ 21, PJ 23, PJ 25, PJ 37, PJ 44 & PJ 54), citrate was utilized by 11 isolates (PJ 8, PJ 20, PJ 21, PJ 23, PJ 27, PJ 28, PJ 31, PJ 37, PJ 54, PJ 56 & PJ 66) and all the isolates were positive for catalase and oxidase tests. Starch was hydrolzed by 8 isolates *i.e.*, PJ 8, PJ 19, PJ 23, PJ 27, PJ 31, PJ 44, PJ 54 & PJ 66, gelatin was liquefied by 11 isolates (PJ 19, PJ 20, PJ 21, PJ 23, PJ 25, PJ 27, PJ 28, PJ 31, PJ 37, PJ 44 & PJ 66). H_2S was produced by only 10 isolates *i.e.*, PJ 8, PJ 19, PJ 20, PJ 21, PJ 23, PJ 25, PJ 31, PJ 44, PJ 54 & PJ 66.

Table 2: Cultural and morphological characteristics of PSB isolates from saline soils of Telangana

Isolate name	Size	Shape	Color	Elevation	Surface	Margin	Pigmentation	Gram reaction	Sporulation
PJ 8	Medium	Rod	White	Convex	Smooth shiny	Regular	Light green	Negative	Negative
PJ 19	Small	Rod	Yellow	Convex	Smooth shiny	Regular	-	Negative	Negative
PJ 20	Small	Rod	Dull white	Convex	Smooth shiny	Regular	Light green	Negative	Negative
PJ 21	Small	Rod	White	Convex	Smooth	Regular	Light green	Negative	Negative
PJ 23	Medium	Rod	White	Convex	Smooth	Regular	-	Negative	Negative
PJ 25	Small	Rod	White	Convex	Smooth, gummy	Regular	-	Negative	Negative
PJ 27	Medium	Rod	White	Convex	Smooth	Irregular	-	Positive	Positive
PJ 28	Small	Rod	Yellow	Convex	Smooth	Irregular	-	Positive	Positive
PJ 31	Large	Rod	White	Convex	Smooth, Gummy	Irregular	-	Positive	Positive
PJ 37	Small	Rod	Yellow	Convex	Smooth	Regular	-	Negative	Negative
PJ 38	Small	Rod	White	Convex	Mucoid	Regular	-	Negative	Negative
PJ 44	Small	Rod	White	Convex	Smooth shiny	Irregular	-	Positive	Positive
PJ 54	Small	Rod	Yellow	Convex	Smooth	Regular	Light green	Negative	Negative
PJ 56	Small	Oval	White	Convex	Smooth shiny	Regular	-	Negative	Negative
PJ 66	Medium	Rod	White	Convex	Smooth	Irregular	-	Positive	Positive
PJ 70	Small	Oval	White	Convex	Smooth shiny	Regular	Dark brown	Negative	Negative

-absent

Table 3: Biochemical characterization of the PSB isolates from saline soils of Telangana

Isolate	Indole test	MR test	VP test	Citrate utilization	Catalase	Oxidase	Starch hydrolysis	Gelatin liquefaction	H_2S	Carbohydrate utilization			
										Lactose	Dextrose	Sucrose	Mannitol
PJ 8	-	+	+	+	+	+	+	-	+	+	-	-	-
PJ 19	-	+	+	-	+	+	+	+	+	+	-	-	-
PJ 20	-	-	+	+	+	+	-	+	+	-	+	-	-
PJ 21	-	+	+	+	+	+	-	+	+	+	+	+	+
PJ 23	-	-	+	+	+	+	+	+	+	+	+	-	+
PJ 25	-	+	+	-	+	+	-	+	+	+	+	+	-
PJ 27	-	+	-	+	+	+	+	+	-	-	-	+	-
PJ 28	-	+	-	+	+	+	-	+	-	+	+	+	-
PJ 31	-	+	-	+	+	+	+	+	+	-	+	+	-
PJ 37	-	+	+	+	+	+	-	+	-	+	+	+	-
PJ 38	+	+	-	-	+	+	-	-	-	+	-	-	+
PJ 44	-	+	+	-	+	+	+	+	+	+	+	+	-
PJ 54	-	-	+	+	+	+	+	-	+	+	-	+	-
PJ 56	-	+	-	+	+	+	-	-	-	-	+	-	+
PJ 66	-	-	-	+	+	+	+	+	+	-	+	+	+
PJ 70	-	+	-	-	+	+	-	-	-	-	-	+	+

+ Positive – Negative **MR**-Methyl Red test **VP**-Voges Prausker's test **H_2S**-Hydrogen sulphide test

In the carbohydrate utilization test, lactose was utilized by 10 isolates (PJ 8, PJ 19, PJ 21, PJ 23, PJ 25, PJ 28, PJ 37, PJ 38, PJ 44 and PJ 54), dextrose was utilized by 10 isolates (PJ 20, PJ 21, PJ 23, PJ 25, PJ 28, PJ 31, PJ 37, PJ 44, PJ 56 & PJ 66), sucrose was also utilized by 10 isolates (PJ 21, PJ 25, PJ 27, PJ 28, PJ 31, PJ 37, PJ 44, PJ 54, PJ 66 & PJ 70) whereas mannitol was utilized by only 6 isolates *i.e.*, PJ 21, PJ 23, PJ 38, PJ 56, PJ 66 & PJ 70). Similarly Jaymin *et al.* (2013) [18] Out of six isolates, two showed salt tolerance upto 10% NaCl concentration. Biochemical and molecular (16S rDNA sequencing) characterization revealed that the strains to be *Exiguobacterium* spp. and *Serratia* spp. Patil *et al.* (2014) [32] isolated 450 salt tolerant strains and all the isolates could able to grow at 12 per cent salt. Among which, 20 isolates were selected and subjected to morphological, biochemical examinations which exhibited the presence of great diversity.

Screening of PSB isolates for PGP attributes

Siderophore Production

Reference strain showed weak siderophore production and out of sixteen salinity tolerant PSB isolates, only 6 isolates produced siderophores (Table 4). Strong production (+++) was shown by the three isolates *i.e.*, PJ 21, PJ 31 and PJ 54 and weak production (+) was shown by three isolates *i.e.*, PJ 23, PJ 27 and PJ 28 whereas ten isolates didn't produce siderophores *i.e.*, no production (-).

Similarly change in the colour of the chrome-azurol S agar from blue to orange red confirmed the siderophore producing ability of *P. aeruginosa* FP6. The maximum siderophore production was obtained in succinate medium (125 µM) followed by King's B medium (105 µM) (Sasirekha and Srividya, 2016) [39].

Hydrocyanic acid Production

Hydrogen cyanide is a secondary metabolite produced by many antagonistic bacterial species from glycine. Twelve among 16 isolates produced HCN, and all the 12 isolates produced weak HCN (+) *i.e.*, PJ 8, PJ 19, PJ 21, PJ 23, PJ 25, PJ 27, PJ 28, PJ 31, PJ 37, PJ 54, PJ 56 & PJ 66 and four isolates doesn't produce HCN (Table 4). Similarly Deshwal and Kumar (2013) reported that all *Pseudomonas* strains produced medium HCN in 0 to 1% NaCl but as the concentration increased from 1.25 to 2.25% in medium, HCN production time varied from 24 h to approximately 48 h and above 1% NaCl concentration. Ahmadzadeh and Sharirifi (2009) [1] identified the production of HCN by six isolates out of the 41 selected Pseudomonads.

IAA production

Reference strain produced moderate amount of IAA and out

of 16 salinity tolerant PSB isolates only 8 isolates produced IAA, among these strong (+++) production was shown by PJ 21 isolate, 2 isolates (PJ 31 and PJ 54) showed moderate production (++), 5 isolates (PJ 8, PJ 23, PJ 25, PJ 38 & PJ 70) were weak (+) producers of IAA and 8 isolates were negative for IAA production (Table 4).

Nghia *et al.* (2017) [29] isolated 213 IAA producing bacteria from fifteen soil samples within the salt affected areas of rice crop. One out of ten efficient producers, the isolate ST2-1 was identified as the most promising strain and produced 33.13 $mg.L^{-1}$ concentration of IAA after 8 days of incubation. Ozdal *et al.* (2017) [30] isolated 8 IAA producing bacteria from the rhizosphere of Verbascum vulcanicum. Among them, Arthrobacter agilis A17 gave maximum IAA production (75 mg/L).

Screening for ACC deaminase activity

The isolates were evaluated for ACC deaminase activity and found its presence in four isolates among the 16 selected salinity tolerant PSB. They include PJ 21, PJ 23, PJ 28, and PJ 70. Reference strain also was negative for ACC deaminase activity (Table 4).

Similarly Bacillus licheniformis B2r was selected for its ability to utilize ACC as a sole nitrogen source under salinity stress and it also showed a high ACC deaminase activity at 0.6 M NaCl salinity (Kannika and Kedsukon, 2012) [20]. Mishra *et al.* (2017) [26] isolated thirty eight ACC deaminase producing PGPR which belong to 12 distinct genera and the isolates exhibited ACC deaminase activitiy ranging from 0.106–0.980 μM α- ketobutyrate μg $protein^{-1}$ h^{-1}.

Exo Polysaccharide Production

Among the 16 strains 13 showed positive for EPS production and 3 isolates were unable to produce EPS. One isolate (PJ 25) showed weak production (+), seven isolates (PJ 8, PJ 19, PJ 20, PJ 28, PJ 38, PJ 54 and PJ 70) produced moderate (++) amount of EPS, strong production (+++) was shown by five isolates (PJ 21, PJ 23, PJ 27, PJ 37 & PJ 44) and reference strain was negative for EPS (Table 4). The results were similar with Muminah *et al.* (2015) [27] isolated 74 exopolysaccharide producing bacteria, of which 34 formed a thick slime when cultured on media Mac Conkey and 15 isolates produced best EPS dry weight in the range of 0.10 to 2.24 mg / ml and the best dry weight was produced by the isolate P3.69 (2.24 mg / ml).

Table 4: Screening of salinity tolerant PSB for PGP attributes, ACC deaminase and EPS production

Isolate name	Siderophore production	HCN production	IAA production	ACC deaminase*	EPS production
Ref 1	+	-	++	-	-
PJ 8	-	+	+	-	++
PJ 19	-	+	-	-	++
PJ 20	-	-	-	-	++
PJ 21	+++	+	+++	+	+++
PJ 23	+	+	+	+	+++
PJ 25	-	+	+	-	+
PJ 27	+	+	-	-	+++
PJ 28	+	+	-	+	++
PJ 31	+++	+	++	-	-
PJ 37	-	+	-	-	+++
PJ 38	-	-	+	-	++
PJ 44	-	-	-	-	+++
PJ 54	+++	+	++	-	++
PJ 56	-	+	-	-	-
PJ 66	-	+	-	-	-
PJ 70	-	-	+	+	++

– No production IAA – Indole Acetic Acid HCN - Hydrogen Cyanide

+ Weak production ++ Moderate production +++ Strong production

*ACC deaminase (+ presence and - absence) EPS - Exo polysaccharide

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