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Isolation and Screening of Multifunctional Plant Growth Promoting Rhizobacteria from Rhizopshere of Different Crop Fields

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Abstract

Rhizospheric bacteria are naturally occurring soil microbes that are aggressively found in the plant rhizosphere, at root surface and in association with roots. They give satisfactory benefit plants by several mechanisms such as nitrogen fixation, phosphate solubilization, potassium decomposition, IAA production, antagonism against phytopathogenic microorganisms by production of siderophore, antibiotics and cell wall degrading enzymes. The total number of beneficial bacteria were isolated from different rhizospheric soil in agricultural lands. The isolated bacterial strains were screened for their plant growth promoting factors such as production of ammonia, siderophore, cellulase, chitinase, and pectinase enzyme. All of the isolates produced ammonia and 79% of the isolates produced siderophore on chrome azurole S agar plates. Furthermore, the bacterial isolates produced cell wall degrading enzyme; pectinase (69%), cellulase (94%), chitinase (51%), amylase (61%) and glucanase enzyme (59%) on agar plate method. The isolates also produced auxin type plant hormone (IAA), all the isolates produced IAA and the highest IAA producing strain is W1 and the produce amount was 21.91mg/L. Among the isolated bacteria, only two strains could produce HCN with the use of Feigl-Anger paper method. The recent study suggests that the use of these PGPR isolates as inoculants might be a promising source for sustainable agricultural use.

Keywords: Rhizobacteria, IAA, Siderophore, Phosphate solubilization, PGPR.

INTRODUCTION

The rhizosphere is a specialized region surrounding the plant root zone which possess a very intense chemical and biological activities due to root exudates. Rhizobacteria are found near the plant roots colonizing the root surfaces and closely adhering soil interface the rhizosphere [1]. Plants may be an important role in selecting and enriching the types of bacteria by using the constituents of their root exudates because rhizospheric bacterial communities could be efficient systems for the catabolism and uptake of organic compounds present in the root exudates. The rhizobacterial population may have various effects on plant growth and health. Plant growth promoting rhizobacteria (PGPR) can be used one or more direct or indirect mechanism of action to improve plant growth and nutrition. The direct activities of plant growth promotion are 1) fixation of atmospheric nitrogen 2) solubilization of phosphorus 3) decomposition of potassium 4) sequestering of iron with the production of siderophores 5) production of plant hormones such as auxins, cytokinins and gibbrellins and 6) lowering of ethylene concentration in the plants [2]. Indirect mechanism of plant growth promotion by PGPR are as follows 1) antibiotic production 2) depletion of iron from the rhizosphere 3) synthesis of antifungal compound 4) production of fungal cell wall lysing enzymes 5) competition for sites on roots and induced systemic resistance [3].

A large number of microorganisms such as algae, bacteria, fungi and protozoa can be found in a narrow sleeve around the axes of the root hairs in the rhizospheric zone. Plant growth promoting rhizobacteria (PGPR) are soil-borne bacteria which enhance the growth of plant directly by providing nutrients and growth promoting substances (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels to the plants or indirectly reducing the inhibitory effects of various plant pathogens and development in the forms of biocontrol agents [4]. Free living plant growth promoting rhizobacteria have been shown promise as biofertilizers. There have been a lot of studies reporting that inoculation with PGPR could increase plant growth promotion, increased crop yield, uptake of nitrogen, solubilization of phosphorus or decomposition of potassium. Some other studies have revealed that inoculation with PGPR could enhance

plant root growth leading to a root system with large surface area increasing number of root hairs [5]. Due to world's expanding population, plant nutrients become essential for the crop and healthy food production. Therefore, plant nutrients are a vital component of sustainable agriculture to meet rising demand of the food. Microbial inoculants could be constitute as an important component of integrated nutrient management and as an economic input to increase crop productivity leadings to a sustainable agriculture [6]. In this case, fertilizer doses can be lowered and more nutrients can be harvested from the soil. Biofertilizers are any inputs containing a formulation of living microorganisms which are able to fix atmospheric nitrogen in the available form of plants either by living freely in the soil or being symbiotically associated with plants or mobilizing nutritive elements from unusable form to usable form via biological processes [7]. Crop productions have been highly effected by pathogenic microbes threating plant health and affecting ecosystem stability in the world. Therefore, agricultural productions became highly dependent on agrochemicals as a reliable approach of crop protection helping economic stability of agricultural practices [8]. In this way, increasing chemical inputs may cause several negative effects such as developing resistant pathogen varieties, non-target environmental impacts and growing cost and accumulation of chemicals in edible plant parts [9]. Plant growth promoting rhizobacteria (PGPR) could be applied as crop inoculants for biofertilization, phytostimulation and biocontrol offering an attractive and eco-friendly alternative to decrease the application of chemical fertilizers which decrease soil fertility and cause adverse effects on the environment [10]. Crop plants have been protected from pathogenic microorganisms and crop productivity have been needed to be improved as a critical context of demand for food for the increasing human population.

The development of biofertilizer composed by beneficial rhizobacteria could minimize and even replace the use of chemical fertilizers while assuring a sustainable agriculture and maintaining environmental quality. [11] For this purpose, the current research focus on the isolation of bacteria from the rhizosphere of several crops cultured in the Hainan, China, and then screening of multifunctional PGPRs activities from soil samples of different crop cultivated lands to determine the possession of direct and indirect plant growth promoting rhizobacteria attributes.

MATERIALS AND METHODS

Soil Sample Collection

The rhizospheric soil samples were collected from the root zones of soil cultivated with different crops from Hainan and Anhui Province of China. The soil samples were carefully dug out at a depth of 5-10 inches and were taken in plastic bags with proper labelling. They were stored at 4°C in the refrigerator for future purpose.

Isolation, Identification and Purification of Bacterial Isolates

Microbial species have been isolated from each sample by serial dilution and spread plate method. The soil sample was weighed as 0.1g and put in a test tube containing 900µl of sterilized NaCl (0.9 %) and was shaken well using vortex mixer. This stock solution was then transferred to 900 µl of sterile NaCl to form 10^1 dilution. In this way, the stock solution was diluted serially up to 10^7 for each soil sample. The amount of 100 µl of each dilution was carefully spread on Burk's N free medium for about 48 hrs at 30±1°C. It was for the isolation of nitrogen fixing bacteria (NFB), similarly, on Pikovskaya's at the same incubation time and at the same temperature for the isolation of phosphate solubilizing bacteria (PSB) and on Aleksandrow's medium for seven days at the same temperature for the isolation of potassium solubilizing bacteria (KSB). Fine isolated and distinct bacterial colonies were picked and purified by subculture method on respective media and incubated at respective temperature. After the recovery isolates in pure form, the resulting PSB, NFB and KSB isolates were growing in LB broth. The amount of 500 µL of the overnight culture were added to 2 ml screw top tube containing 500 µL of 50% glycerol. The bacterial isolates were then stored in the glycerol stocks at -80°C in the freezer. The bacterial cultures were also lyophilized by mixing thoroughly with 15% skim milk and 15% rice powder using vortex mixture. Morphology and texture of each bacterial colony was recorded and then randomly selected and further purified on nutrient agar slant at 4°C for further studies [12].

Screening of Phosphate Solubilization Activity

Phosphate solubilization activity of the isolates was evaluated upon the solubilization of inorganic phosphate on Pikovskaya's (PVK) agar plates containing 0.5% tricalcium phosphate as the inorganic form of phosphate. The colonies on the isolation media were screened on PVK media for their P solubilisation activities. All the isolates were spot inoculated at the centre of PVK plates and they were then incubated at 37°C for 5 days. Each isolate was measured successively after culture incubation by phosphate solubilization efficiency approach measuring the ratio of total diameter (clear zone including bacterial growth and the colony diameter). The halo zone diameter of P solubilization was calculated by subtracting the colony diameter from the total diameter.

Formula for P solubilization index is PSI= colony diameter + halozone diameter/colony diameter. The appearance of clear halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria and these bacteria were easily identified as phosphate solubilizing bacteria (PSB) [13].

Nitrogen Fixing Activity

For the identification of nitrogen fixing activity of the isolates, the bacterial isolates were spot inoculated on nitrogen free minimal medium (NFMM) containing K_2HPO_4, $MgSO_4$, NaCl, $CaCl_2$, $FeSO_4$, $NaMoO_4$, $MnSO_4$, glucose and the amount 0.5% bromothymol blue as a pH indicator. After about 3-10 days of culture incubation, the color changing of the media from green to blue was identified as NFB isolates [14].

Potassium Solubilization Activity

For potassium solubilization activity of the isolates, the bacterial isolates were spot inoculated at the center of Aleksandrow's media containing $MgSO_4$, $CaCO_3$, $KAlSi_3O_8$, $FeCl_3$, $Ca_3(PO_4)_2$, glucose and agar [15]. The culture plates were incubated at 37°C and the clear zone diameter of the culture isolates were successively measured after 4 weeks of incubation.

Indole-3-Acetic Acid (IAA) Production Activity

For IAA production activity test, bacterial isolates were firstly cultured in sterilized nutrient broth supplemented with 1% tryptophan as a precursor for IAA production for seven days at the temperature of 28-30°C at 100-150 rpm of shaking incubation. Then, the different tubes containing bacterial cultures were centrifuged at 10000 rpm for 10 min. The supernatants were then separated in new tubes, 1 ml of each supernatant of particular isolates were mixed with 2ml of Salkowski reagent (1ml of 0.5M FeCl3 in 50mL of 35% HClO4) and the mixtures were kept for 30 minutes at room temperature [16]. The development of pink color was the indicator for production of IAA and the quantity of IAA was read at 530 nm in a UV-Vis spectrophotometer. IAA standard solution of 5 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml and 100 µg/ml were used to estimate the bacterial IAA production activity. A standard curve was plotted for IAA quantification and a reagent alone without bacterial inoculation was used as a control. The solutions were incubated at room temperature for 30 minutes and then read in UV-Vis spectrophotometer at OD_{530}. The different color across the gradient of different concentrations was observed in a few minutes. The different absorbance was recorded in an excel spreadsheet and the amount of IAA production by bacterial isolates were calculated using standard curve prepared with known concentrations of IAA.

Production of Ammonia (NH_3)

For the production of ammonia (in term of nitrogen fixing activity), bacterial isolates were tested in peptone water. Freshly grown bacterial cultures inoculated in each tube containing 10ml of peptone water and then incubated on a rotary shaker for 96hrs at 28 ± 2°C. After incubation period, 0.5 ml of Nesseler's reagent was added into each bacterial tube. The development of yellow to brown color was a positive test for

ammonia production and faint yellow color indicated as small amount of ammonia and deep yellow to brownish color indicates maximum amount of ammonia production [17].

Hydrogen Cyanide (HCN) Production Activity

HCN production was detected according to the method developed by Lorck [18]. The bacterial isolates were streaked on Nutrient agar media amended with glycine (NB 18g/l+ Agar 15g/l+ glycine 4.4g/l). Feigl-Anger paper were prepared by dipping the filter paper strips in a 1:1 mixture of two freshly prepared solutions: (1) 1% (w/v) methylenebis in chloroform and (2) 1% (w/v) copper ethyl acetoacetate in chloroform for 2 minutes. After drying, the paper strips were wrapped with aluminium foil and then put inside the plastic bag until used. These Feigl-Anger papers turned from a faint blue green to a bright blue in the presence of HCN and they can detect as little as 1µg HCN [19]. Feigl-Anger papers were placed in the lid of each Petridish and sealed air-tight with para film. After 48 hrs incubation at 30°C, development of faint blue to bright blue color was recorded for HCN production.

Siderophore Production Activity

Siderophores are special low molecular weight iron chelating compounds produced by bacteria under iron restricted condition. They have been implicated for both direct and indirect enhancement of plant growth. Siderophore production was detected by the universal CAS-agar plate assay using blue agar plates containing the dye chrom azurol S (CAS) [20]. Sterilized blue agar was prepared by mixing CAS (60.5 mg/50ml distilled water) with 5ml iron solution (1mM FeCl3.6H2O) and 5ml 10mM HCl). This solution was slowly added to hexadecyltrimethyl ammonium bromide (HDTMA) (72.9 mg/40ml distilled water). Thus, 50 ml CAS dye was prepared and poured into 500 ml nutrient agar and the plates were prepared. The bacterial cultures were spot inoculated on CAS agar medium and were incubated at 28°C for four days. The siderophore producing bacterial colonies could be found by showing orange color around the colony. The CAS assay is based on siderophore ability to bind to ferric iron with high affinity. The agar contains Chrome Azurol S (CAS) dye which, when complexed with Fe3+, is blue in color. If the inoculated organisms secrete siderophores, ferric iron is stripped from the dye, causing the media to change colors from blue to orange or yellow. Therefore, the presence of siderophores is indicated by coloration surrounding the inoculation site [21]. Orange halos around the bacterial colonies on blue media were positive indicator for the production of siderophore.

Amylase Enzyme Production

For amylase production activity, starch hydrolysis test was used. The isolated bacteria were spot inoculated on the starch agar medium and incubated at 28°C for seven days [22]. After culture incubation, Iodine solution was flooded in the plates using dropper. The plates were then kept undisturbed for 5-10 minutes and then the iodine solution was discarded from the plates. The clear halo around the colony was the positive indicator for the production of amylase.

Pectinase Enzyme Production

For pectinase enzyme production (casein degradation) activity, the bacterial isolates were spot inoculated in pectin amended nutrient medium. The plates were then incubated at 28°C for seven days. After culture incubation, the plates were flooded with 1% Congo red solution. They were washed with 1M NaCl solution in order to remove superficially adhered congo red dye. The isolates producing pectinase indicated the appearance of clear halo zone around the colonies according to Anand *et al*. [23]

Cellulase Enzyme Production

The ability to produce cellulase was determined by using the method of Wood *et al*. The isolates were spot inoculated into the agar plates containing $NaNO_3$, K_2HPO_4, KCl, $MgSO_4$, $FeSO_4$, yeast extract, agar, carboxy methyl cellulose (CMC) as a carbon source [24]. Bacterial strains were grown on this media and incubated for 2 days at 28°C. After cell growth, the presence of extracellular cellulase was detected qualitatively by formation of a clear zone by flooding the plates with 1% Congo red solution for 15minutes. The plates were then destained with 1M NaCl for 15 minutes. They were then visualized for halo zone indicating cellulase production.

β -Glucanase Enzyme Production Activity

β-1,3-glucanases are enzymes which are able to destroy cell walls of some fungi as well as split off the fragments possessing immunoregulating properties (endogenous elicitors and suppressors). β-1,3-glucanase detection was tested in agar containing nutrient media with 0.01% laminarin [25]. Microorganisms were spot inoculated in these agar media and incubated at 28°C up to 5 days. Laminarin depolymerized zones were seen on the culture plates after 5 days.

Chitinase Enzyme Production

Chitinases are chitin-degrading enzymes that hydrolyze the β -1, 4-glycosidic bonds between the N-acetyl glucosamine residues of chitin and are widely found in nature. These enzymes have the ability to degrade the fungal cell wall and this is an important mechanism of fungal inhibition. Activity of chitinase was detected on colloidal chitin agar plates 1% (w/v) [26]. Colloidal chitin was made by slowly adding chitin powder to 10M HCl with vigorous stirring and kept overnight at 4°C in the refrigerator. This suspension was added to chilled ethanol by stirring vigorously and kept overnight at 25°C. The precipitate was then collected by centrifugation at 10000 rpm for 20 mins. After that they were washed with sterile distilled water until colloidal chitin became neutral (pH 7). Finally it was stored at 4°C until further use. Basal chitinase detection medium was directly supplemented with colloidal chitin (4.5g/l) and bromocresol purple (0.15g/l). Resulting substrate had a bright yellow-color, and retained enough bromocresol purple even after pH was adjusted to 4.7 and sterilization at 121°C for 15 mins. Colloidal chitin media containing bromocresol purple (pH 4.7) were inoculated with chitinolytic bacterial isolates, resulted in breakdown of chitin into N-acetyl glucosamine causing a corresponding shift in pH towards alkalinity and change of color of pH indicator dye (BCP) from yellow to purple zone surrounding the inoculated fresh culture colonies in the region of chitin utilization [27, 28]. A single colony of each bacterial isolate was spot inoculated on this agar medium and incubated at 30°C for 4 days.

RESULTS AND DISCUSSION

Isolation and Purification of Bacteria

The total of 102 bacterial isolates were further screened on the respective media for their NPK activities. Among them, 39 bacterial strains with dual effects such as phosphate solubilization, nitrogen fixing and/or potassium solubilization were selected for further investigation of plant growth promoting activities such as IAA production, hydrolytic enzyme production and plant defence substances production. Not wanting to focus only on the potential to fix nitrogen, whole 39 bacteria isolates were assessed for the others potential PGPR, such as Tricalcium phosphate solubilization, IAA production, siderophore production, hydrolytic enzyme production and cell wall degrading enzyme production.

Identification of bacterial isolates

All the bacterial isolates were selected on the basis their particular activity was identified on the basis of gram's staining. The name of the isolates were given in this work. (Table 1)

Table 1: Colony Morphology and Gram's Reaction of the Selected Bacterial Isolates

No.	Isolates	Colony Morphology	Gram's Reaction	Cell Shape
1	BP2	Irregular, raised, lobate, creamish	+	Rod
2	BP3	Irregular, raised, lobate, creamish	+	Rod
3	BP4	Irregular, flat, lobate, creamish	-	Rod
4	RP4	Irregular, flat, lobate, creamish	-	Rod
5	SP2	Irregular, flat, lobate, creamish	-	Rod
6	SAP1	Irregular, flat, undulate, yellowish	-	Cocci
7	MAP3	Irregular, flat, undulate, yellowish	-	Cocci
8	MAP4	Irregular, flat, lobate, creamish	+	Rod
9	MAP5	Circular, convex, entire, creamish	-	Rod
10	WAP5	Circular, convex, entire, creamish	+	Rod
11	WP1	Irregular, flat, lobate, creamish	-	Rod
12	WP2	Circular, convex, entire, creamish	-	Cocci
13	WP5	Irregular, flat, undulate, yellowish	-	Cocci
14	TAP1	Irregular, flat, undulate, yellowish	-	Rod
15	B2	Irregular, flat, lobate, creamish	-	Rod
16	B3	Irregular, flat, lobate, creamish	-	Rod
17	B4	Irregular, flat, lobate, creamish	-	Rod
18	B5	Circular, convex, entire, creamish	-	Rod
19	R3	Circular, convex, entire, creamish	-	Rod
20	S2	Circular, convex, entire, creamish	-	Rod
21	S3	Irregular, flat, lobate, creamish	-	Rod
22	S4	Irregular, flat, lobate, creamish	-	Rod
23	T1	Irregular, flat, lobate, creamish	-	Rod
24	T4	Irregular, flat, lobate, creamish	+	Rod
25	T5	Irregular, flat, lobate, creamish	-	Rod
26	T6	Irregular, flat, lobate, creamish	-	Rod
27	MA4	Circular, convex, entire, creamish	+	Rod
28	WA4	Circular, convex, entire, creamish	-	Rod
29	WA6	Circular, convex, entire, creamish	-	Rod
30	TK1	Irregular, raised, lobate, creamish	-	Rod
31	TK6	Irregular, raised, lobate, creamish	-	Rod
32	TK7	Irregular, raised, lobate, creamish	-	Rod
33	SAK2	Irregular, raised, lobate, creamish	-	Rod
34	SAK5	Irregular, raised, lobate, creamish	-	Rod
35	SAK6	Irregular, flat, lobate, creamish	-	Rod
36	SuK2	Irregular, flat, lobate, creamish	-	Rod
37	SuK7	Irregular, flat, lobate, creamish	-	Rod
38	WK3	Irregular, flat, lobate, creamish	+	Rod
39	WK5	Irregular, flat, lobate, creamish	-	Rod

Phosphate solubilizing activity of the isolates

Phosphate solubilizing bacteria (PSB) play an important role to solubilize insoluble phosphates into soluble forms for the plants are able to use. Phosphorus is one of the macro elements for the plant mineral nutrition due to its importance to plant growth and development [29]. It is often a limiting mineral nutrient for many agricultural crop fields although it is abundant in many soils but poor assimilable forms [30]. The exploitation of phosphorus solubilizing rhizobacteria is a very promising approach to enhance the availability of soluble P in soils. In recent studies, the phosphate solubilizing bacteria are more abundant in the rhizospheric soils than non-rhizospheric soil [31, 32]. PSB has been considered as one of the most alternative way for the production of inorganic phosphate biofertilizers to improve plant growth and yield. The isolated bacterial strains were identified as potential phosphate solubilizers based on the screening of their solubilizing activities by producing clear zone around the colonies after seven days of culture incubation on Pikovskaya's agar plates. Among all 39 PSB isolates, the best strains were SP2, WP2, TK6, WA6 and SAK6 base on their PSI value and their P solubilizing index are 7.75, 4.17, 3.88, 3.71 and 3.6 respectively. Results for the phosphate solubilisation assay are shown in Table (2). (Figure 1.b)

Table 2: P Solubilization Activity of the Isolates

No.	PSB	P-Solubilization Index	NFB	P-Solubilization Index	KSB	P-Solubilization Index
1	BP2	2.56	B2	2.1	TK1	2.1
2	BP3	2.2	B3	3.07	TK6	3.88
3	BP4	2.3	B4	3	TK7	3.17
4	RP4	2.17	B5	2.1	SAK2	3.6
5	SP2	7.75*	R3	2.3	SAK5	3.22
6	SAP1	2.21	S2	2.4	SAK6	3.1
7	MAP3	2.32	S3	2.86	SuK2	2.03
8	MAP4	2.11	S4	2.3	SuK7	2.69
9	MAP5	2.77	T1	2.25	WK3	2.7
10	WAP5	2.15	T4	2	WK5	2.8
11	WP1	2.7	T5	2.5		
12	WP2	4.17	T6	2.9		
13	WP5	2.21	MA4	2.4		
14	TAP1	2.12	WA4	2		
15			WA6	3.71		

Nitrogen fixing activity of the isolates

The nitrogen fixation from atmospheric nitrogen is an important criterion for the selection of potential plant growth promoting bacteria. Plants are seen to be able to achieve their optimal growth only when they get enough amount nutrients such as nitrogen in the presence of N-fixing plant growth promoting bacteria [33]. Nitrogen fixing activity of the bacterial isolates is determined by the strain screening on nitrogen free minimum medium (NFMM). Among 39 bacterial strains, only 10 have showed satisfactory results on nitrogen fixing activity by changing color of the culture media from green to blue. The strains with color changed were B2, B3, B5, S2, S3, S4, T1, T4, WA4 and WA6. (Figure 1.a) Among these 10 strains, B3, S2, S3 and S4 strains indicated the best nitrogen fixing activities and the best phosphate solubilizing activities.

Potassium solubilizing activity of the isolates

The K solubilizing activities of the isolates were detected by spot inoculated them on Aleksandrow's agar media. Among 39 isolates, 10 isolates were able to possess potassium solubilizing activity on Aleksandrow's media plates containing $KAlSi_3O_8$ as insoluble K source. K solubilization index was obtained by formula: SI= Colony diameter + Diameter of zone/colony Diameter. KSB isolates namely TK1, TK6, TK7, SuK2, SAK5 and SuK2 strains have high potassium solubilizing activities and their K solubilizing index are 4.2, 3.75, 3.11, 3 and 2.9 respectively as shown in Table 2. Most of the KSB isolates were able to produce phosphate solubilizing activity. This findings are in agreement

with those studies, i.e. Silva *et al*. [34] found that 90% of the isolates were positive for phosphate solubilisation while Oteino *et al*. [35] reported that 75% of the isolates were phosphate solubilizers. (Table 3) (Figure 1. c)

Table 3: K. Solubilization Activity of the Isolates

No.	Isolates	Colony diameter (mm)	Zone diameter (mm)	Solubilization Index
1	TK1	7.5	24	4.2
2	TK6	8	22	3.75
3	TK7	8.5	18	3.11
4	SAK2	11	17	2.55
5	SAK5	7.5	15	3
6	SAK6	7.2	12.5	2.81
7	SuK2	12	17	2.42
8	SuK7	10.5	20	2.9
9	WK3	7	11	2.57
10	WK5	7.8	14	2.79

Estimation of IAA production from the isolates

Auxin is the most investigated hormone among other plant growth regulating substances. The most physiologically active auxin for the plant is indole-3-acetic acid (IAA) and it is known to stimulate both a short-term response (e.g., increased cell elongation) and a long-term response (e.g., cell division and differentiation) in plants. Production of IAA is an important trait of plant growth promoting rhizobacteria (PGPR) because it is most important phytohormones and function as signal molecule in the regulation of plant development. IAA producing by PGPR can vary among different bacterial species and also influenced by culture condition, growth stage and substrate availability [36]. About 80% of the rhizospheric bacteria are able to produce IAA when L- tryptophan is used as a precursor in bacterial culture media for IAA production [37]. Plant root exudates are natural source of L-tryptophan to enhance auxin biosynthesis in the plant root zone where rhizosphere microflorea are inhibited [38]. Several PGPRs for the genera *Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Pantoea and Pseudomonas* have been reported for the production of IAA in the presence of tryptophan as a precursor [39]. All the 39 bacterial isolates are found to produce plant defence hormone IAA. The best IAA producer strain was WP1 while it produced 21.91 µg/ml. (Figure 4) The second higher IAA producer strain was WA4 while producing 11.32 µg/ml. The remaining higher IAA producer strains were S4, T6, TK6, SP2 and B2 and their IAA production activities were as shown in Table (4).

Table 4: IAA Production of the Isolates

No.	PSB	IAA Concentration (µg/ml)	NFB	IAA Concentration (µg/ml)	KSB	IAA Concentration (µg/ml)
1	BP2	0.81	B2	8.44	TK1	5.94
2	BP3	0.97	B3	5.46	TK6	9.72
3	BP4	0.22	B4	0.97	TK7	3.19
4	RP4	5.12	B5	3.15	SAK2	1.59
5	SP2	8.53	R3	2.29	SAK5	2.00
6	SAP1	4.71	S2	0.69	SAK6	5.08
7	MAP3	2.82	S3	0.89	SuK2	2.82
8	MAP4	3.73	S4	10.5	SuK7	2.41
9	MAP5	5.74	T1	5.98	WK3	2.17
10	WAP5	2.41	T4	5.45	WK5	1.96
11	WP1	21.91*	T5	3.15		
12	WP2	4.67	T6	9.76		
13	WP5	1.34	MA4	3.47		
14	TAP1	0.32	WA4	11.32		
15			WA6	2.08		

Estimation of Siderophore production

Besides IAA production, most of the microorganisms also enhance plant growth by scavenging available iron (Fe3+), which involves secretion of high affinity, low molecular weight iron chelating substances called siderophores [40, 41]. Siderophore production of PGPR is one of the most prominent biocontrol properties against pathogenic microbes in iron limiting condition [42]. Plant growth promoting rhizobacteria can produce a certain amount of siderophore [43]. In the present study under iron limiting environment, totally 31 isolates were found to show the siderophore activity in the CAS agar medium. (Figure 1.d) Among siderophore producer strains in this sturdy, the maximum discoloration was with B2, S3, TK7 and SAK6 while the least was recorded in the isolates BP4, WP2, TAP2, R3, WA6, TK6 and SAK5. (Table 5)

Table 5: Siderophore Production of the Isolates

No.	PSB	Siderophore Activity	NFB	Siderophore activity	KSB	Siderophore Activity
1	BP2	++	B2	+++	TK1	++
2	BP3	++	B3	++	TK6	+
3	BP4	+	B4	++	TK7	+++
4	RP4	-	B5	++	SAK2	-
5	SP2	++	R3	+	SAK5	+
6	SAP1	-	S2	++	SAK6	+++
7	MAP3	++	S3	+++	SuK2	++
8	MAP4	++	S4	++	SuK7	-

9	MAP5	++	T1	++	WK3	-
10	WAP5	++	T4	-	WK5	-
11	WP1	++	T5	++		
12	WP2	+	T6	++		
13	WP5	++	MA4	-		
14	TAP1	+	WA4	++		
15			WA6	+		

- = no += low ++= medium +++= high production

Ammonia and HCN production

Production of ammonia is another important trait of the PGPR (plants can use ammonia as a nitrogen source) which impacts directly the growth of the plants [44]. The ammonia producing activity is an indicator for the process of ammonification which takes place in the rhizosphere rather than non-rhizosphere [45]. Ammonia and HCN are considered as indirect mechanisms of the plant growth and development. The excess production of HCN may play a critical role in the control of fungal diseases [46] and HCN synthesized by some rhizobacteria inhibits diseases in plant and thereby increasing the biocontrol mechanisms [47]. In the present study, ammonia production was observed in all 39 bacterial isolates and highest NH3 producer strains were BP2, SP2, MAP5, WAP5, WP1, WP2, B2, B3, R3, S2, S4, MA4, WA4, SAK5, SuK2 and WK5 as shown in Table (6). (Figure 3.c) HCN production of rhizobacteria is also essential to play an important role in the biological control of pathogenic microbes [48]. When the isolated bacteria were subjected to HCN production assay, only the isolates SAK6 and TK1 were found to be efficient hydrogen cyanide producers. (Figure 3.b) SAK6 produced higher HCN than TK1 strains according to color intensity from screening of Feigl-Anger paper method.

Table 6: Ammonia Production of the Isolates

No.	**PSB**	**NH_3 Production**	**NFB**	**NH_3 Production**	**KSB**	**NH_3 Production**
1	BP2	+++	B2	+++	TK1	++
2	BP3	+	B3	+++	TK6	++
3	BP4	+	B4	++	TK7	++
4	RP4	+	B5	++	SAK2	+
5	SP2	+++	R3	+++	SAK5	+++
6	SAP1	++	S2	+++	SAK6	++
7	MAP3	++	S3	++	SuK2	+++
8	MAP4	+	S4	+++	SuK7	++
9	MAP5	+++	T1	++	WK3	++
10	WAP5	+++	T4	++	WK5	+++
11	WP1	+++	T5	+		
12	WP2	+++	T6	+		
13	WP5	+	MA4	+++		
14	TAP1	+	WA4	+++		
15			WA6	++		

+= low ++= medium +++= high production

Production of Hydrolytic Enzymes (Chitinase, Glucanase, Amylase, Pectinase, and Cellulase)

Hydrolytic enzymes are the crucial agents for the prevention of plant diseases by pathogenic microorganisms in the close vicinity of the plants. They have the ability to secrete elevated level of lytic enzymes such as chitinase, glucanase, etc from the cell wall of pathogenic fungi [49]. Almong all the selected bacterial isolates, 20 isolates were seen to produce chitinase and the highest discoloration was found in the isolate SAK6 with the zone diameter of 75mm. (Table 7) Other high chitinase producers' strains were B3, B5, WA4 and WK5 while their zone diameter were 47mm, 33mm, 30mm and 27mm respectively. (Figure 2. a) 23 isolated bacteria could produce β-glucanase enzyme and the highest producer strains were BP2, MAP5, B3, B5, S3, S4 and WK5 according to their screening results. (Table 8) (Figure 2. b) Furthermore, amylase enzyme production has been detected by inoculating the isolates on starch agar media. Among 39 isolates, 24 bacterial isolates showed the positive results for amylase enzyme production and the other 15 isolates could not produce this enzyme. (Table 9) (Figure 2. d) The screening of pectin degrading enzymes was done by inoculating of the isolates in pectin amended nutrient medium. (Figure 2.c) It is observed that 12 isolates among all the tested strains, could not produce pectinase while the other 27 strains produce a minor amount of this enzyme. (Table 10) In another comparison, 37 isolates out of 39 isolates produced a certain amount of cellulase while screening in CMC media *in vitro*, (Figure 3. a) only the isolates SP2 and WP2 were not able to produce cellulase. (Table 11)

Table 7: Chitinase Enzyme Production of the Isolates

No.	**PSB**	**zone diameter (mm)**	**Activity**	**NFB**	**zone diameter (mm)**	**Activity**	**KSB**	**zone diameter (mm)**	**Activity**
1	BP2	14	++	B2	20	+++	TK1	0	-
2	BP3	0	-	B3	47	++++	TK6	0	-
3	BP4	0	-	B4	20	+++	TK7	0	-
4	RP4	9	+	B5	33	+++	SAK2	0	-
5	SP2	15	++	R3	0	-	SAK5	0	-
6	SAP1	13	++	S2	0	-	SAK6	75	++++
7	MAP3	0	-	S3	24	+++	SuK2	0	-
8	MAP4	12	+	S4	25	+++	SuK7	0	-
9	MAP5	0	-	T1	0	-	WK3	0	-
10	WAP5	15	++	T4	0	-	WK5	27	+++
11	WP1	15	++	T5	9	+			
12	WP2	13	++	T6	0	-			
13	WP5	0	-	MA4	13	++			
14	TAP1	0	-	WA4	30	+++			
15				WA6	0	-			

- = no += low ++= medium +++= high ++++= highest production

Table 8: Glucanase Enzyme Production of the Isolates

No.	PSB	β 1,3glucanase activity	NFB	β 1,3 glucanase activity	KSB	β 1,3 glucanase activity
1	BP2	+++	B2	+	TK1	-
2	BP3	-	B3	+++	TK6	-
3	BP4	-	B4	+	TK7	-
4	RP4	+	B5	+++	SAK2	-
5	SP2	-	R3	-	SAK5	+
6	SAP1	+	S2	++	SAK6	+
7	MAP3	+	S3	+++	SuK2	+
8	MAP4	-	S4	+++	SuK7	+
9	MAP5	+++	T1	-	WK3	-
10	WAP5	-	T4	++	WK5	+++
11	WP1	-	T5	++		
12	WP2	-	T6	++		
13	WP5	++	MA4	-		
14	TAP1	-	WA4	+		
15			WA6	++		

- = no += low ++= medium +++= high production

Table 9: Amylase Enzyme Production of the Isolates

No.	PSB	Amylase Production	NFB	Amylase Production	KSB	Amylase Production
1	BP2	+	B2	+	TK1	+
2	BP3	-	B3	+	TK6	+
3	BP4	+	B4	+	TK7	+
4	RP4	+	B5	+	SAK2	-
5	SP2	+	R3	-	SAK5	-
6	SAP1	-	S2	-	SAK6	+
7	MAP3	-	S3	+	SuK2	+
8	MAP4	-	S4	-	SuK7	+
9	MAP5	-	T1	-	WK3	-
10	WAP5	+	T4	+	WK5	-
11	WP1	+	T5	+		
12	WP2	-	T6	+		
13	WP5	+	MA4	-		
14	TAP1	+	WA4	+		
15			WA6	+		

- = Not detected + = Detected

Table 10: Pectinase Enzyme Production of the Isolates

No.	PSB	Pectinase Production	NFB	Pectinase Production	KSB	Pectinase Production
1	BP2	+	B2	+	TK1	-
2	BP3	+	B3	+	TK6	+
3	BP4	+	B4	+	TK7	+
4	RP4	+	B5	+	SAK2	-
5	SP2	-	R3	-	SAK5	+
6	SAP1	+	S2	-	SAK6	-
7	MAP3	-	S3	+	SuK2	+
8	MAP4	+	S4	-	SuK7	+
9	MAP5	-	T1	-	WK3	+
10	WAP5	+	T4	+	WK5	+
11	WP1	+	T5	+		
12	WP2	-	T6	+		
13	WP5	+	MA4	-		
14	TAP1	+	WA4	+		
15			WA6	+		

- = Not detected + = Detected

Table 11: Cellulase Enzyme Production of the Isolates

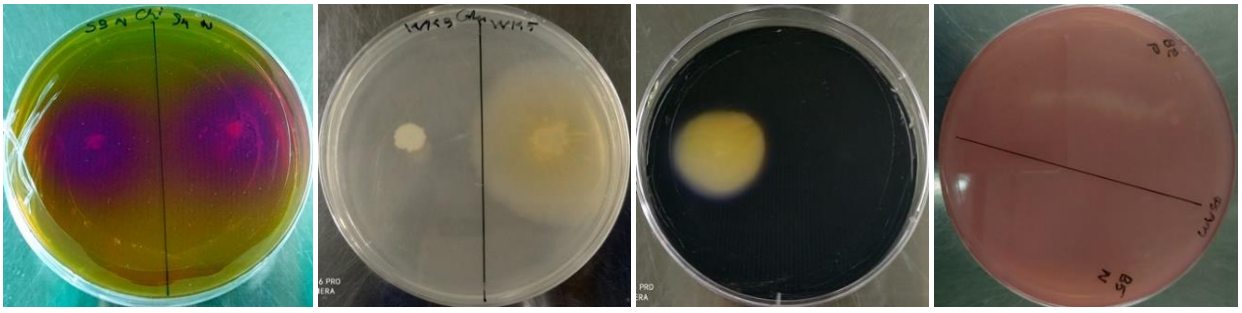
No.	PSB	Cellulase Production	NFB	Cellulase Production	KSB	Cellulase Production
1	BP2	+	B2	+	TK1	+
2	BP3	+	B3	+	TK6	+
3	BP4	+	B4	+	TK7	+
4	RP4	+	B5	+	SAK2	+
5	SP2	-	R3	+	SAK5	+
6	SAP1	+	S2	+	SAK6	+
7	MAP3	+	S3	+	SuK2	+
8	MAP4	+	S4	+	SuK7	+
9	MAP5	+	T1	+	WK3	+
10	WAP5	+	T4	+	WK5	+

11	WP1	+	T5	+		
12	WP2	-	T6	+		
13	WP5	+	MA4	+		
14	TAP1	+	WA4	+		
15			WA6	+		

- = Not detected + = Detected

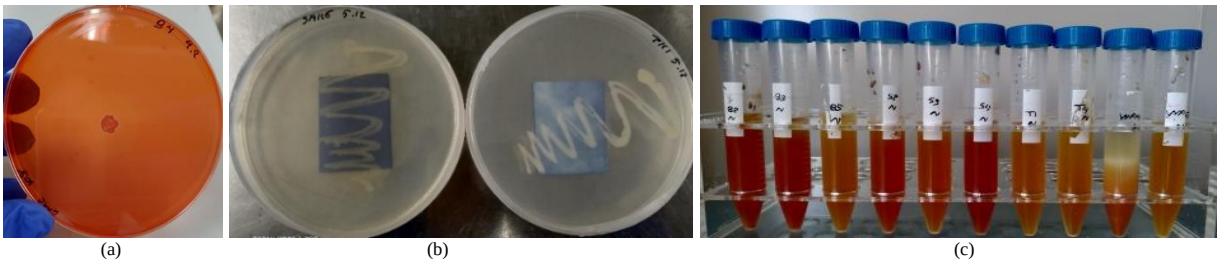
(a) (b) (c) (d)

Figure 1: (a) Screening of nitrogenase enzyme activity, **(b)** Phosphate solubilisation activity, **(c)** potassium solubilisation activity, **(d)** Siderophore production



(a) (b) (c) (d)

Figure 2: (a) Screening of Chitinase enzyme production, **(b)** Glucanase enzyme production, **(c)** Amylase enzyme production, **(d)** Pectinase enzyme production



(a) (b) (c)

Figure 3: (a) Screening of Cellulase enzyme activity, **(b)** HCN production, **(c)** Ammonia production

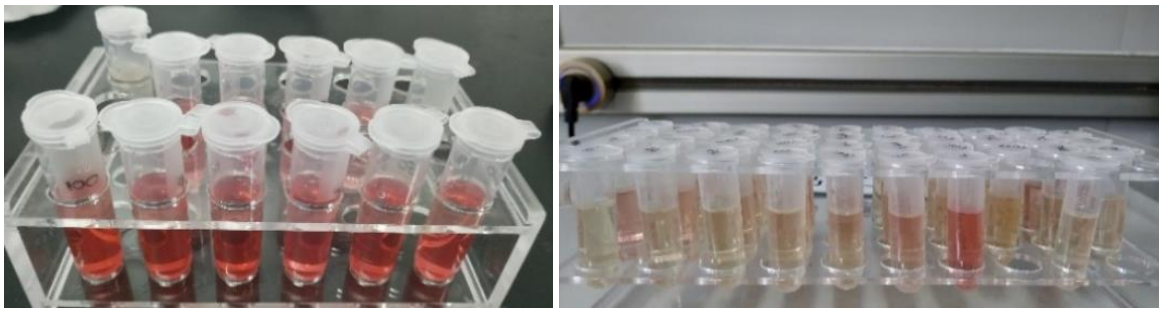


Figure 4: Quantitative estimation of IAA production

CONCLUSION

In the present study, 39 isolates from different crop fields (kidney bean, rice, sugarcane, tomato, maize, soybean and watermelon) were screened for their plant growth promoting activities. The capacity to produce phytoharmones such as IAA, is a desirable characteristic of PGPR [50]. Almost all the PGPR isolates have multifunctional activities for plant growth promoting acitvities such as IAA production, phosphate solubilization, ammonia production and hydrolytic enzyme production [51]. Based on the results, the currently isolated bacterial strains especially PSB isolate showing the highest IAA producer strain WP1, NFB isolates namely B2, B3, S3, S4 and KSB isolates such as TK1 and SAK6 could be useful for bio-fertilizer individually or as a consortium in order to be used for an alternative approach to chemical fertilizers. These bacterial isolates had dual activities such as nitrogen fixation and phosphate solubilisation, potassium solubilisation and phosphate solubilisation

rather than plant hormone IAA production and defence substances such as ammonia and siderophore production. PGPR ability can be exploited further by using it as bio-fertilizers in the field of medicinal plant, agriculture and crop plantation after further field studies to support these findings.

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Conflict of Interest

The author would hereby like to declare that there is no conflict of interests that could possibly arise in the future.

REFERENCES

1. Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, *et al*. Core microbiomes for sustainable agroecosystems. Nat. Plants. 2018; 4:247–257. doi: 10.1038/s41477-018-0139-4
2. Kloepper JW, Lifshitz R, Zablotowicz RM. Free living bacterial inocula for enhancing crop productivity. Trends in Biotechnol. 1989; 7:39-44.
3. Dunne C, Crowley JJ, Loccoz YM, Dowling DN, Bruijn FD, O Gara F. Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* 110 W81 is mediated by an extracellular proteolytic activity. Microbiol. 1993; 143:3921-3931.
4. JW, Leong Kloepper J, Teintze M, Scrhorth MN. 'Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting Rhizobacteria', *Nature*, 1980; 286:885-886.
5. Burk D. The influence of nitrogen gas upon the organic catalysis of nitrogen fixing by *Azotobacter*. Journal Physical Chemistry. 1930; 34:1174-1194.
6. Barriuso J, Pereyra M, García JL, Megías M, Manero FG, *et al*. Screening for putative PGPR to improve establishment of the symbiosis Lactarius deliciosus-*Pinus* sp. Adv Microb Ecol. 2005; 50:82-89.
7. Ahmad F, Ahmad I, Khan MS. Screening of Free- Living Rhizospheric Bacteria for Their Multiple Plant Growth Promoting Activities," *Microbiological Research*, 2006; 36(2):1-9.
8. Spaepen S. Plant hormones produced by microbes. Principles of plant-microbe interactions. Springer. 2015; 247-256.
9. Company S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 2005; 71(9):4951-4959.
10. Amir H Ai'shah ON, Keng CL, Othman A. Influence of Various Combinations of Diazotrophs and Chemical N Fertilizer on Plant Growth and N_2 Fixation Capacity of Oil Palm Seedlings (*Elaeis guineensis* Jacq.). *Thai J Agric Sci* 2009; 42:139-149.11
11. *Gram HC*.: Ueber die isolirte Färbung der Schizomyceten in Schnitt- und Trocken-präparaten. Fortschr Med. 1884; 2:185–189.
12. Pikovskaya RE, 'Mobilization of Phosphorus in Soil in Concentration with Vital Activity of Some Microbial Species', Microbiology, 1948; 17:362-37.
13. Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World, J. Microbiol. Biotechnol. 2012; 28:1327–1350.
14. Qureshi MA, Shakir MA, Iqbal A, Akhtar N, Khan A. Co-inoculation of phosphate solubilizing bacteria and rhizobia for improving growth and yield of mungbean (*Vigna radiata* L.). *Journal of Animal and Plant Sciences* 2011; 21(3):491-497.
15. Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN. Diversity of plant growth and soil health-supporting bacteria. *Current Science* 2005; 89(1):136-150.
16. Cappuccino JC, Sherman N. In: Microbiology. A Laboratory Manual, 3rd edn. Benjamin/Cummings Publ. Co., New York; 1992.
17. Cattelan AJ, Hartel PG, Fuhrmann JJ. Screening for Plant Growth- Promoting Rhizobacteria to Promote Early Soybean Growth. Soil Sci Soc Am J 1999; 63:1670-1680.
18. Lorck H. Production of hydrocyanic acid by bacteria. Physiologia Plantarum 1948; 1:142-146.
19. Mishra P, Singh PP, Singh SK, Verma H. Sustainable agriculture and benefits of organic farming to special emphasis on PGPR. In Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology; Elsevier Inc.: Amsterdam, The Netherlands, 2019.
20. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 1987; 160:47-56.
21. Egamberdiyeva D. The Effect of Plant Growth Promot- ing Bacteria on Growth and Nutrient Uptake of Maize in Two Different Soils," *Applied Soil Ecology*, 2007; 36(2-3):184-189. doi:10.1016/j.apsoil.2007.02.005
22. Chauhan H, Bagyaraj D, Selvakumar G, Sundaram S. Novel plant growth promoting rhizobacteria—prospects and potential. Appl. Soil Ecol. 2015; 95:38–53. doi: 10.1016/j.apsoil.2015.05.011
23. Anand A, Bass SH, Cho HJ, Klein TM, Lassner M, McBride KE. Methods and compositions of improved plant transformation. Patent publication WO2017078836 A1, 2017.
24. Chaparro JM, Badri DV, Vivanco JM. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 2014; 8:790. doi: 10.1038/ismej.2013.196
25. Cardinale M, Ratering S, Suarez C, Zapata Montoya AM, Geissler-Plaum R, Schnell S. Paradox of plant growth promotion potential of rhizobacteria and their actual promotion effect on growth of barley (Hordeum vulgare L.) under salt stress. Microbiol. Res. 2015; 181:22–32. doi: 10.1016/j.micres.2015.08.002
26. Robert WK, Selitrennikoff CP. Plant and bacterial chitinase differ in antifungal activity. J. Gen. Microbiol. 1988; 134:169-176.
27. Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R. Potential of Rhizobium and Bradyrhizobium Species as Plant Growth Promoting Rhizobacteria on Non-Legumes: Effect on Radishes (*Raphanus sativus L.*)," *Plant and Soil*, 1998; 204(1):57-67. doi:10.1023/A:1004326910584
28. Yadav J, Verma JP, Tiwari KN. Effect of Plant Growth Promoting Rhizobacteria on Seed Germination and Plant Growth Chickpea (*Cicer arietinum L.*) under in *vitro* conditions. Biological Forum-Annual of International Journal. 2010;2(2):15-18.
29. White PJ, Brown PH. Plant nutrition for sustainable development and global health. *Ann. Bot.*, 2010; 105:1073-1080.
30. Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M. Functional diversity among plant growth-promoting rhizobacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin Heidelberg, 2009; pp 105–132
31. Chen JH. The combined use of chemical and organic fertilizers and/ or biofertilizer for crop growth and soil fertility. In: International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use 2006; 16:20.
32. Muleta D, Assefa F, Börjesson E, Granhall U. Phosphate solubilisingrhizobacteria associated with *Coffeaarabica* L. in natural coffee forests of Southwestern Ethiopia. *J. Saudi Soc. Agric. Sci.*, 2013; 12:73-84.
33. Bray RH, Kurtz LT. Determination of Total, Organic and Available Forms of Phosphorus in Soils. *Soil Science*, 1945; 59(2):39-45.
34. Silva PRA, Sim~oes-Ara'ujo JL, Vidal MS, Cruz LM, Souza EM, Baldani JI. Draft genome sequence of Paraburkholderia tropica PPE8 strain, a sugarcane endo-phytic diazotrophic bacterium. Brazilian Journal of Microbiology. 2018; 49:210–211.https://doi.org/10.1016/j.bjm.2017.07.005
35. Oteino N. Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. *Frontiers in Microbiology*, 2015; 6(1):1-9.
36. Garcia de Salamone IE, Hynes RK, Nelson LM. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. 2001; 47:404–411. doi: 10.1139/w01-029
37. Khare A, Arora NK. Effect of indole-3-acetic acid (IAA) produced by Pseudomonas aeruginosa in suppression of charcoal rot disease of chickpea. Current microbiology. 2010; 61:64-68.
38. Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y. Rock phosphate-solubilizing *Actinomycetes*: screening for plant growth-promoting activities. World Journal of Microbiology and Biotechnology 2008; 24(11):2565- 2575.
39. Kumar A, Bahadur I, Maurya B, Raghuwanshi R, Meena V, Singh D, *et al*. Does a plant growth promoting rhizobacteria enhance agricultural sustainability. J. Pure Appl. Microbiol. 2015; 9:715–724.
40. Anitha G, Kumudini BS. Isolation and characterization of fluorescent pseudomonads and their effect on plant growth promotion. J Environ Biol 2014; 35:627-634.
41. Singh AL, Hariprassanal K, Solanki RM. Screening and selection of groundnut genotypes for tolerance of soil salinity. *Australian Journal of Crop Science* 2008; 1(3):69-77.
42. Ahmad F, Ahmad I, Khan MS. Screening of Free- Living Rhizospheric Bacteria for Their Multiple Plant Growth Promoting Activities," *Microbiological Research*, 2006; 36(2):1-9.
43. Hafeez FY, Abaid-Ullah M, Hassan MN. Plant growthpromoting rhizobacteria as zinc mobilizers: a promising approach for cereals biofortification," in Bacteria in Agrobiology: Crop Productivity, eds D. K. Maheshwari, M. Saraf, and A. Aeron (Berlin: Springer), 2013; 217–235.
44. Jha CK, Saraf M. Plant growth promoting rhizobacteria (PGPR): a review. E3 J. Agric. Res. Dev. 2015; 5:108–119.
45. Tomaz Rijavec L. "Hydrogen cyanide in the rhizosphere: not supressing plant pathogens, but rather regulating the availability of phosphate." Frontiers in microbiology. 2016; 7:1785.
46. Flaishman MA, Eyal ZA, Zilberstein A, Voisard C, Hass D. Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide

producing strains of *Pseudomonas putida*. Mol Plant Microbe Inter 1996; 9:642-645.
47. Schippers B, Bakker AW, Van Peer R. Beneficial and deleterious pseudomonads on rhizosphere interactions. Plant Soil 1990; 129:75-83.
48. Ramyasmruthi S, Pallavi O, Pallavi S, Tilak K, Srividya S. Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from *Solanaceae* rhizosphere effective against broad spectrum fungal phytopathogens. *Asian J. Plant Sci. Res.*, 2012; 2(1):16-24.
49. Gull M, Hafeez FY. Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. *Afr. J. Microbiol. Res.*, 2012; 6(33):6308-6318.
50. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 2003; 255(2):571-586.
51. Phale S. Isolation and Screening of Multifunctional Plant Growth Promoting Rhizobacteria (PGPR) from Onion Rhizosphere (*Allium cepa*). J Bioprocess Biotech, 2018; 8:335. doi:10.4172/2155-9821.1000335