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Exploring the Hidden Treasures: Isolation, Characterization and Screening of Phosphate Solubilizing Bacteria (PSB) for Plant Growth-Promoting Traits from Chilli Field Weeds Rhizosphere

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plant-associated microbes affect growth, nutrient acquisition, tolerance to different stress conditions and *etc.* by the plant. However, beneficial microbes influencing weeds biosynthesis remain largely unexplored and unexploited. Phosphorus is one of essential macro-minerals for the growth and

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development of chilli plants. The main objective of this study was to isolate characterize and evaluation of the PGPR characters of PSB from weed rhizosphere of the chilli ecosystem. Totally 40 PSB isolates were isolated from different weeds in the chilli ecosystem and were subjected to biochemical characterization where 13 isolates were found positive for starch hydrolysis, 35 isolates were positive for acid production, 12isolates were positive for gas production, 40 isolates were positive for catalase production, 5 isolates were positive for H_2S production, 13 isolates were positive for urease production and remaining all isolated were negative for the respective tests. Based on morphological and biochemical characterization, the isolates were tentatively identified as *Bacillus* sp and *Pseudomonas* sp.

Keywords: Bacteria isolation; bacterial screening; phosphate solubilizing bacteria; rhizosphere; plant microbe; weeds biosynthesis.

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the major commercial crops which belongs to the Solanaceae family. It was grown for vegetables, spices, condiments, sauces and pickle purposes. Both green and dry chillies are used in our diet which will give the required pungency, colour, taste and flavor to our dishes. Chilli stimulates taste buds and there by increases the flow of saliva which contains enzyme amylase which helps in the digestion of starchy foods. The presence of capsaicin in chilli is responsible for its pungency and it is also used in pain balms, cosmetics and medicines related to heart diseases. Capsanthin, a pigment in chilli is used for natural colouration in jams and jellies *etc*, as it is a natural pigment and it has no side effects on human health. It also contains vitamins such as A, B and C, which gives it a high export value.

Phosphorus is one of the most important macronutrients that are essential for plant growth and development. Phosphorus in soils can exist in both organic (PO_4^{3-}) and inorganic (Pi) forms. The inorganic forms of phosphorus have been calculated to account for 35 – 70 per cent of total P in soil that exists in calcium, aluminium or iron-complexed forms that are unavailable for plant use. The Phosphate solubilizing bacteria (PSB) in the plant rhizosphere play a significant role in releasing P from its insoluble complexes to a form that is more readily usable by plants. The inorganic forms of P can be solubilized by microorganisms that secrete low molecular weight organic acids (citric, lactic, glycolic, 2-ketogluconic, oxalic, glyconic, acetic, malic, fumaric, succinic, tartaric, malonic, glutaric, propionic and butyric) to dissolve phosphate complex minerals and chelate cations that partner with P ions (PO_4^{3-}) to release P directly into the surrounding soil solution system [1].

Weeds are plants that are unwanted in a given situation and may be harmful, dangerous or economically detrimental. Weeds are a serious threat to primary production and biodiversity. They reduce agricultural productivity, displace native species and contribute significantly to land and water degradation. The costs of weeds to the natural environment are also high, with weed invasion being ranked second to habitat loss in causing biodiversity decline.

In spite of all the difficulties caused by weeds, they can offer some beneficial properties, particularly when occurring at low densities. These aspects should be utilized in the farming system, although this may make organic management more complicated than chemical-based systems. Weeds offer several potential benefits in conserving soil moisture and preventing erosion. A ground cover of weeds will reduce the amount of bare soil exposed helping to conserve nutrients, particularly nitrogen which could otherwise be leached away, especially on light soils. Weeds can also be valuable indicators of growing conditions in a field, for example water levels, compaction and pH.

In recent years, weeds have gained importance in another way; it may be due to drought tolerance and pest resistance. Keeping all the above information, the present study was conducted to isolate, characterize and screen the PSB from weeds rhizosphere from chilli fields.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Totally 40 weeds rhizosphere soil samples were collected from Bermuda grass (*Cyanodon dactylon*), Java grass(*Cyperus rotundus*),Crab

grass (*Digitaria marginata*), Bengal day flower *Commelina benghalensis*) and congress (*Parthenium hysterophorus*) weeds in the chilli ecosystem from Raichur district of Karnataka. The details about location and sample codes are given in Table 1.

Table 1. Rhizosphere soil samples of weeds collected from Raichur distric

Sl. No	Location	Soil Type	Weed	Sample Code
1	UAS Raichur Experimental plot. Department of Genetics and Plant breeding	Black	*Cynodon dactylon*	PRCD 1
2		Black	*Cyperus rotundus*	PRCR 2
3		Black	*Digitaria marginata*	PRDM 3
4		Black	*Commelina benghalensis*	PRCB 4
5		Black	*Parthenium hysterophorus*	PRPH 5
6	UAS Raichur Administration block	Black	*Cynodon dactylon*	PRCD 6
7		Black	*Cyperus rotundus*	PRCR 7
8		Black	*Digitaria marginata*	PRDM 8
9		Black	*Commelina benghalensis*	PRCB 9
10		Black	*Parthenium hysterophorus*	PRPH 10
11	Rampur	Red laterite soil	*Cynodon dactylon*	PrCD 1
12		Red laterite soil	*Cyperus rotundus*	PrCR 2
13		Red laterite soil	*Digitaria marginata*	PrDM 3
14		Red laterite soil	*Commelina benghalensis*	PrCB 4
15		Red laterite soil	*Parthenium hysterophorus*	PrPH 5
16	Askial	Red laterite soil	*Cynodon dactylon*	PACD 1
17		Red laterite soil	*Cyperus rotundus*	PACR 2
18		Red laterite soil	*Digitaria marginata*	PADM 3
19		Red laterite soil	*Commelina benghalensis*	PACB 4
20		Red laterite soil	*Parthenium hysterophorus*	PAPH 5
21	Kalluru	Black	*Cynodon dactylon*	PKCD 1
22		Black	*Cyperus rotundus*	PKCR 2
23		Black	*Digitaria marginata*	PKDM 3
24		Black	*Commelina benghalensis*	PKCB 4
25		Black	*Parthenium hysterophorus*	PKPH 5
26	Alkur	Red laterite soil	*Cynodon dactylon*	PaCD 1
27		Red laterite soil	*Cyperus rotundus*	PaCR 2
28		Red laterite soil	*Digitaria marginata*	PaDM 3
29		Red laterite soil	*Commelina benghalensis*	PaCB 4
30		Red laterite soil	*Parthenium hysterophorus*	PaPH 5
31	Gillesugur	Red laterite soil	*Cynodon dactylon*	PGCD 1
32		Red laterite soil	*Cyperus rotundus*	PGCR 2
33		Red laterite soil	*Digitaria marginata*	PGDM 3
34		Red laterite soil	*Commelina benghalensis*	PGCB 4
35		Red laterite soil	*Parthenium hysterophorus*	PGPH 5

Sl. No	Location	Soil Type	Weed	Sample Code
36		Black	*Cynodon dactylon*	PJCD 1
37	Janaki ram camp	Black	*Cyperus rotundus*	PJCR 2
38		Black	*Digitaria marginata*	PJDM 3
39		Black	*Commelina benghalensis*	PJCB 4
40		Black	*Parthenium hysterophorus*	PJPH 5

Note : P – Phosphate Solubilizing Bacteria, R – Raichur, r– Rampur, A – Askial, a – Alkur, G - Gillesugur, J – Janaki ram camp, K – Kallur.CD - Cynodon dactylon. CR - Cyperus rotundus. DM - Digitaria marginata. CB - Commelina benghalensis. PH - Parthenium hysterophorus

2.2 Isolation of Phosphate Solubilizing Bacteria

Phosphorus solubilizing bacterial isolates were isolated from each soil sample by serial dilution and spread plate method. The Pikovskaya's agar medium (PVK) was used for isolation which containing insoluble tricalcium phosphate and incubated at 27 to 30˚C for 3 to 4 days. Colonies showing halo zones were picked and purified to get pure culture for further studies [2].

2.3 Morphological Characterization of Phosphate Solubilizing Bacterial Isolates

All PSB isolates were examined for their colony morphology, cell shape, Motility test and gram reaction as per the standard procedures given by and Barthalomew and Mittewer [3].

2.4 Biochemical Characterization and Tentative Identification of the PSB Isolates

2.4.1 Starch hydrolysis

All 40 PSB isolates were streaked on the media containing starch agar and then incubated at 30 ± 2°C for three days. After incubation, the plates were flooded with Lugol's iodine solution and allowed to stand for 15 to 20 min. The clear zone around the colony will be considered as positive for the test [4].

2.4.2 Acid and gas production

All PSB isolates were inoculated with five ml of presterilized glucose broth medium in test tubes containing Durham's tube and bromocresol purple as pH indicator. The test tubes were incubated for seven days at 30 °C. The accumulation of gas in the Durham's tube was taken as positive for gas production and the change in colour of medium from purple to yellow was taken positive for acid production [5].

2.4.3 Catalase test

The nutrient agar slants were inoculated with all 40 PSB isolates and incubated at 30 °C for 24 h. After incubation, the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for production of gas bubbles. The occurrence of gas bubbles were scored positive for catalase activity [6].

2.4.4 Hydrogen sulphide production

All 40 PSB isolates were inoculated to test tubes containing 5 ml of SIM medium and were incubated at 28 °C. The formation of the black ring in the medium was taken as positive for H_2S production (Phillips and Steel, 1970).

2.5 Urease Test

Overnight grown culture were inoculated with sterilized urea broth and inoculated for 48 h at 30 °C. Change in color of the broth from orange to pink was taken as a positive for the urease activity [7].

2.6 Phosphate Solubilization Index (PSI)

Preliminary screening for phosphate solubilization was done by a plate assay method using Pikovskaya's (PVK) agar medium supplemented with tri-calcium phosphate (TCP). The pH of the media was adjusted to 7.00 before autoclaving. Sterilized PVK media was poured into sterilized Petri plates after solidification of the media, and a pinpoint inoculation of PSB strains was made onto the plates under aseptic conditions. They were incubated at 28±2^{0}C for seven days with continuous observation for colony diameter. The halo zone formation around the growing colony showing phosphate

solubilization efficiency was evaluated according to the following formula.

SEC % = (Z-C)/C×100

Where, Z: Solubilization zone, C: Colony diameter, SE: solubilization efficiency

2.7 Indole Acetic Acid (IAA) Production

The IAA production potential of isolates was tested on selective nitrogen-free broth supplemented with 0.005 M concentration tryptophan at 28°C. The concentration of IAA in the culture broth after three days of incubation will be centrifuged at 5000 rpm for 5 min and determined by a spectrophotometric method using Salkowski's reagent as given below:

One ml of the supernatant was mixed with 1 ml of Salkowski's reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35 % $HClO_4$) and the intensity of red color developed within 30 min will be checked at 530 nm using spectrophotometer. The concentration was determined by using a standard curve prepared from a standard solution of indole acetic acid [8].

2.8 Siderophore Production

Siderophore Production by phosphate-solubilizing bacteria was assayed by plate assay method as described by Schwyn and Neilands (1987). The tertiary complex Chromoazurol S (CAS) served as an indicator. To prepare one liter of the blue agar 60.5 mg CAS was dissolved in 50 ml water and mixed with 10 mM Fe^{3+} solution (1 mM $FeCl_3$ $6H_2O$ in 10 mm HCl) and 72.9 mg HDTMA dissolved in 40 ml water was added by constantly stirring. 48 h old cultures of phosphate solubilizing bacterial isolates were streaked on the nutrient agar medium amended with the indicator and incubated for two days. The formation of a bright zone with yellowish fluorescent colour in the dark blue coloured medium was the indication for production of thesiderophore.

2.9 Gibberellin Production

Gibberellic acid (GA_3) production potential of the isolates was determined in selective nitrogen media containing 0.005 M tryptophan at 28°C by following standard [9]. The isolates were grown in media containing 0.005 M tryptophan at 28°C for 3 days and were centrifuged at 5000 rpm for 5 min. The three ml of filtrate obtained was taken in a test tube and 2 ml of zinc acetate was added. After 2 min, 2 ml of potassium ferrocyanide was added and the solution was centrifuged at 1000 rpm for 5 min. Five ml of 30 per cent HCl was added to the supernatant (5 ml) and incubated at 20 °C for 2 h. The absorbance of the sample was measured at 254 nm using uv–Visspectrophotometer including the control (treated with 5 % HCl).

3. RESULTS AND DISCUSSION

3.1 Isolation of PSB

Phosphate solubilizing bacterial (PSB) isolates were isolated from the 40 rhizospheric soil samples collected from different weeds in the chilli ecosystem of Raichur district. Totally 40 PSB isolates were isolated by serial dilution and spread plate method on Pikovskaya's agar media and plates were incubated for 3 to 5 days at the temperature of 27 ± 2°C. The colonies showing clear zones around them were streaked repeatedly on Pikovskaya's agar media to get the pure culture. The obtained pure cultures of PSB isolates were streaked on Pikovskaya's agar slants for further use. Pathak et al., [10].

Similarly, Tenzing et al. [11] conducted a laboratory study to isolate, identify and characterize the phosphate-solubilizing bacteria from different crop soils such as okra, chilli, tomato, cotton and egg-plant. Among ten PSB strains, six strains were identified as *Bacillus megaterium*, two strains as *Pseudomonas putida* and two as *P. fluorescences.* The same results were also recorded by Alok et al. [12]isolated PSB isolates from maize, onion, jasmine and tomato rhizosphere soils from four different localities of Salem (Tamil Nadu), based on their ability to form clear zones and 12 isolates were selected for further studies.(Sharma et al., 2019)

3.2 Morphological Characterization of PSB Isolates

As per the standard procedure, the PSB isolates were examined for colony morphology, cell shape, motility and gram reaction by use of a compound microscope and the results of these tests are presented in Table 2. All the 40 PSB isolates were rod-shaped which was observed by simple staining. All the 40 PSB isolates were found to be motile when observed under a microscope. Out of 40 isolates, eight isolates were gram-positive and 32 isolates were gram-negative. The colony morphology of the PSB

isolates varied from irregular whitish creamy to yellow circular colonies (Table 2) [13].

Similarly, Kundu et al. [14] Kundu, Nehra, Yadav, & Tomar, [14] isolated and characterized 193 PSB isolates from the rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. In this majority of the isolates had white, round and gummy colonies, irrespective of the crop or the region.

3.3 Biochemical Characterization of PSB Isolates

All the 40 PSB isolates were subjected to biochemical characterization and were tentatively identified up to the generic level. The results of biochemical characterization are presented in the Table 2. Among 40 PSB isolates, 27 isolates were found negative and 13 isolates were found positive for starch hydrolysis. Out of 40 PSB isolates, 35 isolates were positive and 5 isolates were found negative for acid production. Out of 40 PSB isolates 12 isolates were positive for gas production and 28 isolates were negative for gas production. All 40 isolates were positive for catalase test. Among 40 isolates 5 isolates were positive and remaining 35 were negative for H_2S production. Out of 40 isolates 13 isolates were positive for urease test and remaining were negative.

Similarly the same results were obtained by Ujah, [15] in this they isolated 12 PSB isolates from soil samples of three different locations on the Pikovaskya's agar medium using the dilution plate technique. Characterization was done by identification of external colony morphology, colour, halo zone formation and appearance on the PVK agar plates. All isolates were positive for gram staining, starch hydrolysis, urease test, and catalase test. The same results also obtained by Kundu et al. [14] isolated total of 193 PSB isolates from 245 rhizosphere samples collected from south-west and north-east zones and using biochemical analysis isolates were tentatively identified as belonging to *Pseudomonas* sp. Mohamed et al., [16].

Similarly, Hfiza et al. [17] obtained the same results when they isolate PSB isolated from the rhizosphere of different plants in the Lahore District, Pakistan. Based on morphological and biochemical characterization they were identified as *Citrobacter freundii*, *Acinetobacter lwoffii* and *Pseudomonas fluorescens*.

In accordance with the above results Sudewi et al. [18] isolated 11 PSB isolates from the local aromatic rice rhizosphere. In this they recorded morphological characteristics such as shape and colour of the colony. The bacteria found were gram-positive and gram-negative, which are 81.81 and 18.18 per cent, respectively. The result of the catalase reaction test showed 72.72 per cent positive and 27.27 per cent negative catalase.

In conclusion, totally of 40 PSB isolates were isolated from collected weed rhizosphere soil samples of the chilli ecosystem from the Raichur district. More than 80 per cent of PSB isolates showed positive for all biochemical tests, based on the biochemical characters (Starch hydrolysis ,Acid production , Gas production, Catalase test, Hydrogen sulphide production, Urease test,) of all 40 isolates they were tentatively identified as genera of *Bacillus* and *Pseudomonas*.

All 40 isolates were screened for PSI and results are represented in Table 3. All 40 isolates formed the clear zone, the zone of solubilization was measure and PSI was calculate, which was ranged from 2.33 to 10.00. In this PACD 1 was recorded the highest PSI (10.00) followed by the isolate PrCB 4 (9.66), and the isolate PRCD 6 (2.33) recorded lowest PSI.

Similar findings were obtained by Thi and Trung (2022) isolated and screened seven bacterial strains that solubilized phosphate at different phosphate solubilization indexes, ranging from 4.2 to 6.1. In this, the most efficient isolate was PSB31.

3.4 Siderophore Production by PSB Isolates

Out of 40 isolates 30 isolates were able to change the colour of the media from greenish blue to bright yellow colour indicating production of Siderophore thereby having the ability of iron chelation and the remaining 10 were negative. Out of 30 the isolate PACD 1 had the most prominent yellow colour zone which indicated that the isolate was having highest siderophore production ability which is given in Table 4**.**

The generation of siderophores may be due to the activity of iron chelators. This iron-chelating property is an important factor for isolates because it deprives pathogens of environmental iron available to them.

Table 2. Morphological and biochemical characterises of PSB isolates of weeds rhizosphere

Sl No.	Isolates	Morphological Characters	Biochemical Characters							
			Motility	Gram's Reaction	1	2	3	4	5	6	Probable Species
1.	PRCD 1	Round, white.	+	_	+	+	_	+	_	_	*Pseudomonas*
2.	PRCR 2	Round, pale yellow.	+	_	_	_	_	+	_	_	*Pseudomonas*
3.	PRDM 3	Transparent, raised white.	+	_	_	_	-	+	_	_	*Pseudomonas*
4.	PRCB 4	Light yellowish.	+	_	_	+	_	+	_	-	*Pseudomonas*
5.	PRPH 5	Flat, irregular, cream.	+	+	+	_	_	+	_	_	*Bacillus*
6.	PRCD 6	Flat, irregular, light yellow.	+	_	_	+	+	+	_	_	*Pseudomonas*
7.	PRCR 7	Ovel, white	+	_	_	+	+	+	_	_	*Pseudomonas*
8.	PRDM 8	Round, white.	+	_	_	+	_	+	_	_	*Pseudomonas*
9.	PRCB 9	Transparent, irregular.	+	_	_	+	_	+	+	_	*Pseudomonas*
10.	PRPH10	Bright yellow, ovel.	+	_	_	+	_	+	_	_	*Pseudomonas*
11.	PrCD 1	Cream, round.	+	_	_	+	+	+	_	+	*Pseudomonas*
12.	PrCR 2	White, raised, pin head.	+	+	_	+	+	+	_	+	*Bacillus*
13.	PrDM 3	Orange, flat.	+	_	+	+	_	+	_	+	*Pseudomonas*
14.	PrCB 4	White, raised, large.	+	_	+	+	+	+	+	+	*Pseudomonas*
15.	PrPH 5	White, smooth, flat.	+	+	_	+	_	+	_	_	*Bacillus*
16.	PACD 1	Raised, transparent.	+	_	+	+	+	+	-	+	*Pseudomonas*
17.	PACR 2	Small, pin head.	+	+	_	+	_	+	_	+	*Bacillus*
18.	PADM3	Yellow, round.	+	_	+	+	+	+	+	+	*Pseudomonas*
19.	PACB 4	White, raised.	+	_	_	+	_	+	_	_	*Pseudomonas*
20.	PAPH 5	Orange, raised	+	_	_	+	+	+	_	_	*Pseudomonas*
21.	PKCD	Round, flat, yellow.	+	_	_	_	_	+	_	+	*Pseudomonas*
22.	PKCR 2	Yellow, circular.	+	_	_	+	_	+	_	_	*Pseudomonas*
23.	PKDM3	Flat, yellow, round.	+	_	_	+	_	+	_	_	*Pseudomonas*
24.	PKCB 4	Light yellow, raised.	+	_	_	+	_	+	_	+	*Pseudomonas*
25.	PKPH 5	White, flat, round.	+	+	+	_	_	+	_	_	*Bacillus*
26.	PaCD 1	Light yellow.	+	_	+	+	_	+	_	_	*Pseudomonas*
27.	PaCR 2	Yellow, irregular.	+	_	+	+	_	+	_	_	*Pseudomonas*
28.	PaDM 3	Orange, raised.	+	_	_	+	_	+	_	+	*Pseudomonas*
29	PaCB 4	Round, yellow.	+	_	_	+	_	+	_	_	*Pseudomonas*
30.	PaPH 5	Yellow, round.	+	_	_	+	_	+	_	_	*Pseudomonas*

Sl No.	Isolates	Morphological Characters	Biochemical Characters								
			Motility	Gram's Reaction	1	2	3	4	5	6	Probable Species
31.	PGCD 1	Yellow, round.	+	_	_	+	+	+	_	_	*Pseudomonas*
32.	PGCR 2	Round, yellowish.	+	_	+	+	+	+	_	+	*Pseudomonas*
33.	PGDM	Light yellowish, round.	+	_	_	+	_	+	_	_	*Pseudomonas*
34.	PGCB 4	Irregular, round, cream.	+	+	_	+	_	+	_	_	*Bacillus*
35	PGPH 5	Yellow, round.	+	_	_	+	+	+	_	_	*Pseudomonas*
36.	PJCD 1	Creamy, round.	+	_	_	+	_	+	_	+	*Pseudomonas*
37.	PJCR 2	Yellow, irregular.	+	_	+	+	_	+	_	_	*Pseudomonas*
38.	PJDM 3	Flat, white.	+	+	+	+	_	+	+	_	*Bacillus*
39.	PJCB 4	White, raised.	+	_	_	+	_	+	_	_	*Pseudomonas*
40.	PJPH 5	Light yellow, round.	+	+	+	+	+	+	+	+	*Bacillus*

Note : + - positive, - = negative, 1 – Starch hydrolysis, 2 – Acid production , 3 – Gas production, 4 – Catalase test, 5 – Hydrogen sulphide production, 6 – Urease test, P – Phosphate Solubilizing Bacteria, R – Raichur, r– Rampur, A – Askial, a – Alkur, G - Gillesugur, J – Janaki ram camp, K – Kallur. .CD - Cynodon dactylon. CR - Cyperus rotundus. DM - Digitaria marginata. CB - Commelina benghalensis. PH - Parthenium hysterophorus

Table 3. The phosphate solubilizing index, *In - vitro* P - solubilization by PSB isolates of weeds rhizosphere

Sl. No	Isolate	PSI	Broth Assay 5th Day (µg/100ml)	Broth Assay 10th Day(µg/100ml)
1	PRCD 1	3.50	34.345	35.547
2	PRCR 2	3.3	38.567	37.387
3	PRDM3	4.00	40.532	56.000
4	PRCB 4	5.00	40.843	67.580
5	PRPH 5	2.25	41.546	66.984
6	PRCD 6	2.33	36.945	51.680
7	PRCR 7	7.00	38.758	55.900
8	PRDM8	3.00	39.923	57.570
9	PRCB 9	2.75	28.546	47.960
10	PRPH10	3.00	34.124	45.550
11	PrCD 1	7.00	29.354	39.627
12	PrCR 2	5.00	25.067	33.780
13	PrDM 3	8.00	32.876	61.612
14	PrCB 4	9.66	51.450	97.239
15	PrPH 5	7.66	42.564	67.350
16	PACD 1	10.00	67.536	126.649
17	PACR 2	7.40	34.982	56.547
18	PADM3	6.33	43.600	91.487
19	PACB 4	7.00	39.010	44.670
20	PAPH 5	4.00	29.987	59.460
21	PKCD 1	2.75	40.423	74.900
22	PKCR 2	4.00	39.657	51.567
23	PKDM3	2.66	41.001	67.735
24	PKCB 4	6.00	29.398	49.765
25	PKPH 5	2.33	35.543	58.000
26	PaCD 1	5.00	38.964	67.457
27	PaCR 2	5.40	36.800	67.540
28	PaDM 3	7.00	29.675	47.000
29	PaCB 4	2.50	37.568	81.860
30	PaPH 5	5.50	29.875	59.347
31	PGCD 1	4.55	39.647	79.340
32	PGCR 2	9.50	52.298	103.376
33	PGDM3	3.75	26.678	52.500
34	PGCB 4	5.60	38.500	76.300
35	PGPH 5	7.00	40.165	80.328
36	PJCD 1	3.80	37.760	72.820
37	PJCR 2	5.50	27.430	55.070
38	PJDM 3	2.50	20.670	43.405
39	PJCB 4	5.50	34.300	68.200
40	PJPH 5	6.00	41.453	86.932

Note: P – Phosphate Solubilizing Bacteria, PSI - Phosphate Solubilizing Index R – Raichur, r– Rampur, A – Askial, a – Alkur, G - Gillesugur, J – Janaki ram camp, K – Kallur. CD - Cynodon dactylon. CR - Cyperus rotundus. DM - Digitaria marginata. CB - Commelina benghalensis. PH - Parthenium hysterophorus

Similar findings were recorded by Chaiharn et al. [19] (Chaiharn, Chunhaleuchanon, Kozo, and Lumyong, 2008) they isolated PSB isolates from different rhizosphere soil and screened for their plant growth promotion and it was found that 23 per cent of isolates produced siderophore on Chrome Azurole S agar plates. Similarly, Aarab et al [20] isolated 305 PSB isolates from the rhizosphere of rice fields in Northwestern Morocco, in which 136 were tri calcium phosphate solubilizers and 66 bacteria were producers of siderophores. The same results also obtained by Jai and Naveen (2019) isolated a phosphate-solubilizing bacteria from the

Table 4. Indole Acetic Acid (IAA), Gibberllic Acid (GA3) and siderophore production by weeds rhizosphere PSB isolates

Sl. No	Isolate	IAA(µg/ml)	GA_3(µg/100ml)	Siderophore
1	PRCD 1	4.567	91.400	+
2	PRCR 2	6.457	45.320	+
3	PRDM3	12.974	56.569	+
4	PRCB 4	17.777	99.480	-
5	PRPH 5	6.932	27.452	-
6	PRCD 6	25.713	80.472	+
7	PRCR 7	21.332	44.730	+
8	PRDM8	7.654	74.675	+
9	PRCB 9	22.445	36.000	+
10	PRPH10	18.874	77.900	-
11	PrCD 1	21.971	37.680	+
12	PrCR 2	6.582	46.761	+
13	PrDM 3	11.218	89.450	-
14	PrCB 4	31.162	121.396	+
15	PrPH 5	18.108	29.548	+
16	PACD 1	33.561	144.138	+
17	PACR 2	11.218	58.000	+
18	PADM3	30.989	97.914	+
19	PACB 4	22.357	88.106	+
20	PAPH 5	21.649	36.469	-
21	PKCD 1	23.774	55.000	-
22	PKCR 2	21.134	52.496	-
23	PKDM3	21.971	77.084	+
24	PKCB 4	21.520	65.105	+
25	PKPH 5	13.601	81.904	+
26	PaCD 1	20.812	29.055	-
27	PaCR 2	18.816	71.084	+
28	PaDM 3	15.017	67.505	+
29	PaCB 4	13.665	85.370	+
30	PaPH 5	18.623	69.526	-
31	PGCD 1	19.073	77.024	+
32	PGCR 2	31.243	127.316	+
33	PGDM3	14.244	57.300	+
34	PGCB 4	28.410	61.084	+
35	PGPH 5	19.460	47.000	+
36	PJCD 1	22.164	79.550	+
37	PJCR 2	24.546	46.309	-
38	PJDM 3	11.218	55.175	+
39	PJCB 4	6.582	48.000	+
40	PJPH 5	30.193	93.016	+

Note : P – Phosphate Solubilizing Bacteria, R – Raichur, r– Rampur, A – Askial, a – Alkur, G - Gillesugur, J – Janaki ram camp, K – Kallur, IAA – Indole Acetic Acid, GA_3 – Gibberellic Acid, +ve - Positive, - ve - Negative. .CD - Cynodon dactylon. CR - Cyperus rotundus. DM - Digitaria marginata. CB - Commelina benghalensis. PH - Parthenium hysterophorus

rhizosphere of *Stevia rebaudiana* and the bacteria was identified as *bacillus sp*. and it was able to produce siderophore (16.06 µg/ml).

3.4.1 Indole Acetic Acid (IAA) production by PSB isolates

Results of IAA produced by isolates are presented in Table 4. All 40 PSB isolates were able to producer IAA and the concentration of IAA produce by isolates varied from 33.561 to 4.567 µg/100 ml. Among 40 PSB isolates the highest IAA was produced by the isolate PACD 1 (33.561µg/ml) followed by the isolate PGCR 2 (31.243 µg/ml) and the lowest IAA was produced by the isolate PRCD 1 (4.567 µg/ml).

The results showed that all the PSB isolates from weed rhizosphere could produce IAA. The production of IAA ultimately improves the metabolism of the plant. PSBs released the greatest amount of IAA in the presence of physiological precursors in the medium. It is affected by culture conditions, growth stage and substrate availability [21].

Similar research was carried out by Yasin et al. (2022) isolated and characterized PSB isolates in soil samples collected from Jimma, Ethiopia. Out of 10 isolates, three of them (JEC3, JEC4 and JEC7) produced IAA, in this JEC4 produced maximum IAA (10.21 µg/ml). Similarly, Dipanwita et al. (2015) screened and identified three IAA producing phosphate solubilising rhizobacteria from the rhizosphere soil of Dhaincha (*Sesbania bispinosa*) and screened for their Phosphate solubilization ability. In this seven isolates showed positive results and were further monitored for their IAA production. All seven PSBs were found to be producing IAA which was confirmed by colorimetric method and the isolate DD4 produced the maximum amount of IAA (1260 ppm/L) Similar results were found by Ramakrishnan et al. (2012) and Sasmita et al. [22].

3.4.2 Gibberellin production by PSB isolates

All 40 PSB isolates produced to GA_3 in the broth and the ranged from 144.138 to 29.055 µg /100 ml of broth. Among all isolates the isolate PACD 1 was able to produce highest GA_3 of 144.138 µg /100 ml followed by the isolate PGCR 2 which was of 127.316 µg /100 ml and the lowest was produced by the isolate PaCD 1 of 29.055 µg /100 ml. The results are presented in the Table 4**.**

As per the above results it indicates that all the weeds rhizosphere PSB isolates were able to produce GA_3 which improves cell division, cell elongation and growth of plants.

The above results were in accordance with the findings of Sasmita et al. (2022) [22] they evaluated five PSB isolates for their ability to solubilize insoluble phosphate in liquid medium produced phytohormones. The results showed that all the isolates produced GA_3 [23].

Same results were also obtained by Ramakrishnan et al. (2012) isolated 23 PSB isolates from 19 samples collected from different locations in Karnataka and Madhya Pradesh of India. The results revealed that all the PSB isolates were able to produce both IAA ranged from 0.74 to 9.53 µg 25 ml^{-1} and GA_3 ranging from 2.08 to 12.55 µg 25 ml^{-1}. Similarly, Gil et al. (2009) isolated 864 bacterial isolates from 553 soil samples and screened them for plant growth promotion. The culture filtrate of the bacterium was analyzed for the presence of gibberellins and they found physiologically active gibberellins GA_1 (0.23 µg/100 ml) GA_3 (5.11 µg/100 ml) and GA_4 (2.65 µg/100 ml) along with physiologically inactive GA_9, GA_{12}, GA_{15}, GA_{20} and GA_{24} [24].

4. CONCLUSION

Findings from the current study revealed the potential of weeds rhizosphere as a source of plant growth-promoting bacteria which were tentatively identified as *bacillus sp* and *pseudomonas sp* with the highest PSI, IAA and GA_3 production. Additionally research need to be carried out to test the effect of isolating plants under pot culture and field studies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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