

Original Research Article

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

Commented [PC1]: Traits

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 development of chilli plant. The main objective of this study was to isolate characterize and evaluation for its PGPR characters of PSB from weed rhizosphere of chilli ecosystem. Totally 40 PSB isolates were isolated from different weeds in chilli ecosystem and were subjected to biochemical characterization where 27 isolates were found positive for starch hydrolysis,35 isolates were positive for acid production,34 isolates were positive for gas production, 40 isolates were positive for catalase production,12 isolates were positive for H₂S 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.

Commented [PC2]: weed

Commented [PC3]: plants

Commented [PC4]: of the PGR characteristics

Commented [PC5]: the chilli

Commented [PC6]: the chilli

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the major commercial crop which belongs to the Solanaceae family. It was grown for vegetables, spices, condiments, sauces and pickle purpose. 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 enzymes amylase which helps in 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

Commented [PC7]: crops

Commented [PC8]: purposes

Commented [PC9]: enzyme

Commented [PC10]: the digestion

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 exist 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 (Vyas and Gulati, 2009).

Commented [PC11]: exists

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 of water levels, compaction and pH.

Commented [PC12]: chemical-based

Commented [PC13]: example water

In recent years, weeds are gaining an importance in one 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.

Commented [PC14]: weeds have gained importance in another way

MATERIALS AND METHODS

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 chilli ecosystem from Raichur district of Karnataka. The details about location and sample codes are given in table number 1.

Commented [PC15]: For the work to have complete credibility, I suggest introducing a map of the locations from which the soil samples were taken, marking the territory on the map of India or the administrative subdivision. Possibly, the locations can be labelled on the map. The suggestion is a GIS map, which will be able to form the foundation of a useful database for tracking soil dynamics.

Commented [PC16]: the chilli

Commented [PC17]: 1.

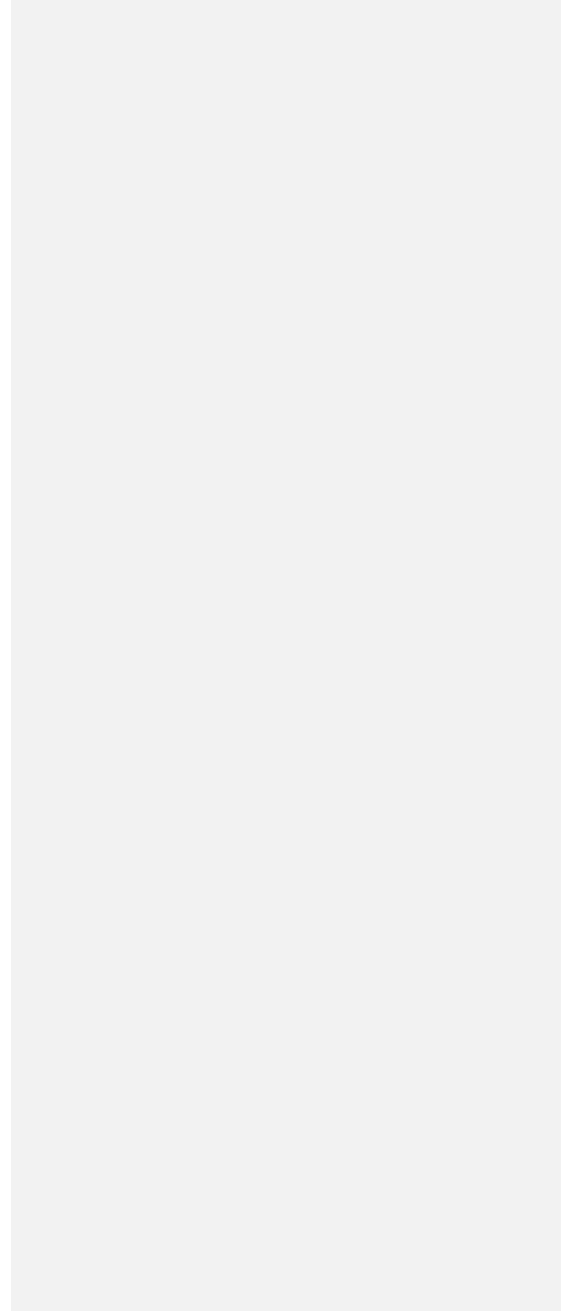
Table :1 Rhizosphere soil samples of weeds collected from Raichur distric.

SL. NO	Location	Soil type	Weed	Sample code
1	UAS Raichur Experimental of plot. Department of Genetics and Plant breeding	Black	<i>Cynodon dactylon</i>	PRCD 1
2		Black	<i>Cyperus rotundus</i>	PRCR 2
3		Black	<i>Digitaria marginata</i>	PRDM 3
4		Black	<i>Commelina benghalensis</i>	PRCB 4
5		Black	<i>Parthenium hysterophorus</i>	PRPH 5
6	UAS Raichur Administration block	Black	<i>Cynodon dactylon</i>	PRCD 6
7		Black	<i>Cyperus rotundus</i>	PRCR 7
8		Black	<i>Digitaria marginata</i>	PRDM 8
9		Black	<i>Commelina benghalensis</i>	PRCB 9
10		Black	<i>Parthenium hysterophorus</i>	PRPH 10
11	Rampur	Red laterite soil	<i>Cynodon dactylon</i>	PrCD 1
12		Red laterite soil	<i>Cyperus rotundus</i>	PrCR 2
13		Red laterite soil	<i>Digitaria marginata</i>	PrDM 3
14		Red laterite soil	<i>Commelina benghalensis</i>	PrCB 4
15		Red laterite soil	<i>Parthenium hysterophorus</i>	PrPH 5
16	Askial	Red laterite soil	<i>Cynodon dactylon</i>	PACD 1
17		Red laterite soil	<i>Cyperus rotundus</i>	PACR 2
18		Red laterite soil	<i>Digitaria marginata</i>	PADM 3
19		Red laterite soil	<i>Commelina benghalensis</i>	PACB 4
20		Red laterite soil	<i>Parthenium hysterophorus</i>	PAPH 5
21	Kalluru	Black	<i>Cynodon dactylon</i>	PKCD 1
22		Black	<i>Cyperus rotundus</i>	PKCR 2
23		Black	<i>Digitaria marginata</i>	PKDM 3
24		Black	<i>Commelina benghalensis</i>	PKCB 4
25		Black	<i>Parthenium hysterophorus</i>	PKPH 5
26	Alkur	Red laterite soil	<i>Cynodon dactylon</i>	PaCD 1
27		Red laterite soil	<i>Cyperus rotundus</i>	PaCR 2
28		Red laterite soil	<i>Digitaria marginata</i>	PaDM 3

29		Red laterite soil	<i>Commelina benghalensis</i>	PaCB 4
30		Red laterite soil	<i>Parthenium hysterophorus</i>	PaPH 5
31	Gillesugur	Red laterite soil	<i>Cynodon dactylon</i>	PGCD 1
32		Red laterite soil	<i>Cyperus rotundus</i>	PGCR 2
33		Red laterite soil	<i>Digitaria marginata</i>	PGDM 3
34		Red laterite soil	<i>Commelina benghalensis</i>	PGCB 4
35		Red laterite soil	<i>Parthenium hysterophorus</i>	PGPH 5
36	Janaki ram camp	Black	<i>Cynodon dactylon</i>	PJCD 1
37		Black	<i>Cyperus rotundus</i>	PJCR 2
38		Black	<i>Digitaria marginata</i>	PJDM 3
39		Black	<i>Commelina benghalensis</i>	PJCB 4
40		Black	<i>Parthenium hysterophorus</i>	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*.

UNDER PEER REVIEW



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 (Pikovskaya, 1948).

Commented [PC18]: In this subchapter, we suggest the introduction of experimental evidence in the form of sketches and/or photos, as in [1,2,3] from the literature proposed by the reviewer in the review form, after chapter 6. of the editor's questionnaire.

Commented [PC19]: contained

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 Bartholomew and Mitterer (1950).

Commented [PC20]: In this subchapter, we suggest the introduction of experimental evidence in the form of sketches and/or photos, as in [3,4] from the literature proposed by the reviewer in the review form, after chapter 6. of the editor's questionnaire.

Commented [PC21]: Bartholomew and Mitterer (1950). (see [5] from the literature proposed by the reviewer in the review form, after chapter 6. of the editor's questionnaire)

Biochemical characterization and tentative identification of the PSB isolates

Starch hydrolysis

All 40 PSB isolates were streaked on the media containing starch agar then incubated at $30 \pm 2^\circ\text{C}$ 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 (Eckford, 1927).

Commented [PC22]: In this subchapter, we suggest the introduction of experimental evidence in the form of sketches and/or photos, as in [5,6] from the literature proposed by the reviewer in the review form, after chapter 6. of the editor's questionnaire.

Commented [PC23]: and then

Commented [PC24]: incubation,

Acid and gas production

All PSB isolates were inoculated 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 (Cappuccino and Sherman, 1992).

Commented [PC25]: with five

Commented [PC26]: Durham

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

Commented [PC27]: Incubation,

peroxide and observed for production of gas bubbles. The occurrence of gas bubbles were scored positive for catalase activity (Blazevic & Ederer, 1975).

Commented [PC28]: was

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 black ring in the medium was taken as positive for H₂S production (Phillips and Steel, 1970).

Commented [PC29]: the black, or a black

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 (James and Sherman, 1992) (James & Sherman, 1992).

Commented [PC30]: double quote?

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, a pinpoint inoculation of PSB strains were made onto the plates under aseptic conditions. They were incubated at 28±2°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.

Commented [PC31]: tri-calcium

Commented [PC32]: and a

Commented [PC33]: was

$$\text{SEC} \% = \frac{Z-C}{C} \times 100$$

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

Commented [PC34]: 1)SEC or SE ?
2) Suggestion: write the equation and the mathematical variables using the mathematical editor (I indicated a form at the end of the review form).
In the form in which you wrote the equation, only C is divided by C not (Z-C). You must write 100(Z-C)/C in brackets. The proposed suggestion solves the problem more easily.

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

Commented [PC35]: nitrogen-free

culture broth after three days of incubation will be centrifuged at 5000 rpm for 5 min and determined by spectrophotometric method using Salkowski's reagent as given below:

Commented [PC36]: a spectrophotometric or the spectrophotometric

One ml of the supernatant was mixed with 1 ml of Salkowski's reagent (2 ml of 0.5 M $\text{FeCl}_3 + 98 \text{ ml } 35 \% \text{ HClO}_4$) 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 standard solution of indole acetic acid (Mali & Bodhankar, 2009).

Commented [PC37]: the red

Commented [PC38]: a spectrophotometer

Commented [PC39]: a standard

Siderophore production

Siderophore Production by phosphate solubilizing bacteria was assayed by plate assay method as described by Schwyn and Neilands (1987). The tertiary complex Chromoazurole 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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. Formation of bright zone with yellowish fluorescent colour in the dark blue coloured medium was the indication for production of siderophore.

Commented [PC40]: phosphate -olubilizing

Commented [PC41]: The formation

Commented [PC42]: a bright, or the bright

Commented [PC43]: blue coloured

Commented [PC44]: of the production

Giberellin production

Commented [PC45]: Gibberellin

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 methodology (Marmur1961) (Marmur, 1961). 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 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).

Commented [PC46]: The gibberellic

Commented [PC47]: double quote?

Commented [PC48]: a test, or the test

RESULTS AND DISCUSSION

Isolation of PSB

Phosphate solubilizing bacterial (PSB) isolates were isolated from the 40 rhizospheric soil samples collected from different weeds in 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 $27 \pm 2^\circ\text{C}$. The colonies showing clear zone 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.

Similarly, Tenzing *et al.* (2016) conducted 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.* (2013) 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 zone and 12 isolates were selected for further studies.

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 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 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).

Commented [PC49]: the chilli

Commented [PC50]: temperature

Commented [PC51]: zones

Commented [PC52]: a laboratory

Commented [PC53]: phosphate-solubilizing

Commented [PC54]: eggplant

Commented [PC55]: zones

Commented [PC56]: procedure,

Commented [PC57]: a compound, or the compound

Commented [PC58]: rod-shaped

Commented [PC59]: a microscope, or the microscope

Commented [PC60]: gram-positive

Commented [PC61]: gram-negative

Similarly, Kundu *et al.* (2009) (Kundu, Nehra, Yadav, & Tomar, 2009) 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.

Commented [PC62]: double quote?

Biochemical characterization of PSB isolates

All the 40 PSB isolates were subjected to the biochemical characterization and were tentatively identified up to generic level. Results of biochemical characterization are presented in the Table 2. Among 40 PSB isolates 27 isolates were found positive and 13 isolates were found negative 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 34 isolates were positive for gas production and 6 isolates were negative for gas production. All 40 isolates were positive for catalase test. Among 40 isolates 12 isolates were positive and remaining 28 were negative for H₂S production. Out of 40 isolates 13 isolates were positive for urease test and remaining were negative.

Commented [PC63]: biochemical

Commented [PC64]: the generic

Commented [PC65]: The results

Commented [PC66]: Table 2

Commented [PC67]: isolates,

Commented [PC68]: Column 1 of Table 2? Please check! I found 13 positive and 27 negative.

Commented [PC69]: isolates,

Commented [PC70]: isolates,

Commented [PC71]: Column 3 of Table 2? Please check! I found 12 positive and 28 negative.

Commented [PC72]: the catalase

Commented [PC73]: the remaining

Commented [PC74]: isolates,

Commented [PC75]: the urease

Commented [PC76]: the remaining

Commented [PC77]: Please verify!

Commented [PC78]: the same

Commented [PC79]: the dilution

Commented [PC80]: , and catalase test

Commented [PC81]: Double quote?

Similarly same results were obtained by (Ujah, 2022) in this they isolated 12 PSB isolates from soil samples of three different locations on the Pikovaskya's agar medium using 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. Same results also obtained by Kundu *et al.* (2009) (Kundu et al., 2009) 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.

Commented [PC82]: the same

Commented [PC83]: when

Commented [PC84]: isolated

Commented [PC85]: in the Lahore

Similarly, Hfiza *et al.* (2013) obtained same results that, they isolate PSB isolates from the rhizosphere of different plants of 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.* (2020) 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 that 72.72 per cent positive and 27.27 per cent negative catalase.

Commented [PC86]: 72.72

In conclusion, totally 40 PSB isolates were isolated from collected weed rhizosphere soil samples of chilli ecosystem from Raichur district. More than 80 per cent of PSB isolates showed positive for all biochemical tests, based on the biochemical characters of all 40 isolates they were tentatively identified as genera of *Bacillus* and *Pseudomonas*.

Commented [PC87]: Total of 40

Commented [PC88]: The chilli

Commented [PC89]: The Raichur

Commented [PC90]: Characteristics ?

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 the isolate PACD 1 was recorded highest PSI (10.00) followed by the isolate PrCB 4 (9.66), the isolate PRCD 6 (2.33) recorded lowest PSI.

Commented [PC91]: PACD 1 recorded

Commented [PC92]: The highest

Commented [PC93]: and the

Commented [PC94]: the lowest

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

Commented [PC95]: ranging

UNDER PUBLICATION

UNDER PEER REVIEW

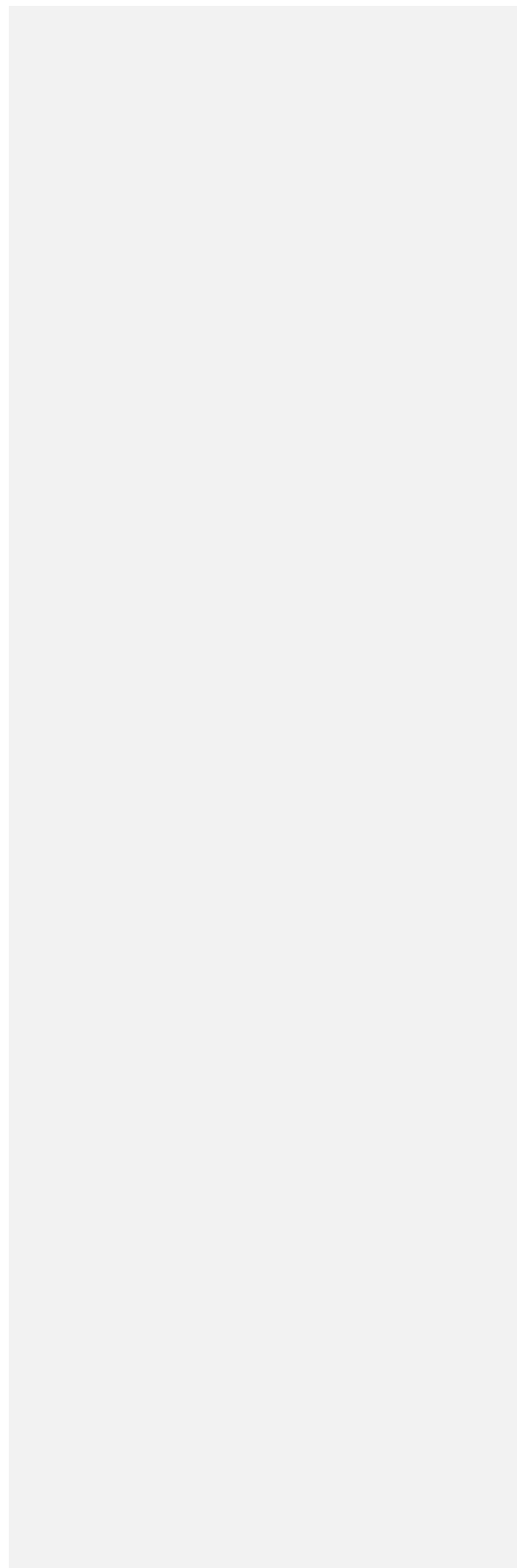


Table 2 : Morphological and biochemical characterises of PSB isolates of weeds rhizosphere.

SI No.	Isolates	Morphological characters	Biochemical characters								Probable species
			Motility	Gram's Reaction	1	2	3	4	5	6	
1.	PRCD 1	Round, white.	+	-	+	+	-	-	-	-	<i>Pseudomonas</i>
2.	PRCR 2	Round, pale yellow.	+	-	-	-	-	-	-	-	<i>Pseudomonas</i>
3.	PRDM 3	Transparent, raised white.	+	-	-	-	-	-	-	-	<i>Pseudomonas</i>
4.	PRCB 4	Light yellowish.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
5.	PRPH 5	Flat, irregular, cream.	+	+	+	-	-	+	-	-	<i>Bacillus</i>
6.	PRCD 6	Flat, irregular, light yellow.	+	-	-	+	+	-	-	-	<i>Pseudomonas</i>
7.	PRCR 7	Ovel, white	+	-	-	+	+	-	-	-	<i>Pseudomonas</i>
8.	PRDM 8	Round, white.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
9.	PRCB 9	Transparent, irregular.	+	-	-	+	-	-	+	-	<i>Pseudomonas</i>
10.	PRPH10	Bright yellow, ovel.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
11.	PrCD 1	Cream, round.	+	-	-	+	+	+	-	+	<i>Pseudomonas</i>
12.	PrCR 2	White, raised, pin head.	+	+	-	+	+	-	-	+	<i>Bacillus</i>
13.	PrDM 3	Orange, flat.	+	-	+	+	-	+	-	+	<i>Pseudomonas</i>

14.	PrCB 4	White, raised, large.	+	-	+	+	+	+	+	+	<i>Pseudomonas</i>
15.	PrPH 5	White, smooth, flat.	+	+	-	+	-	-	-	-	<i>Bacillus</i>
16.	PACD 1	Raised, transparent.	+	-	+	+	+	+	-	+	<i>Pseudomonas</i>
17.	PACR 2	Small, pin head.	+	+	-	+	-	+	-	+	<i>Bacillus</i>
18.	PADM3	Yellow, round.	+	-	+	+	+	+	+	+	<i>Pseudomonas</i>
19.	PACB 4	White, raised.	+	-	-	+	-	+	-	-	<i>Pseudomonas</i>
20.	PAPH 5	Orange, raised	+	-	-	+	+	-	-	-	<i>Pseudomonas</i>
21.	PKCD	Round, flat, yellow.	+	-	-	-	-	-	-	+	<i>Pseudomonas</i>
22.	PKCR 2	Yellow, circular.	+	-	-	+	-	+	-	-	<i>Pseudomonas</i>
23.	PKDM3	Flat, yellow, round.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
24.	PKCB 4	Light yellow, raised.	+	-	-	+	-	+	-	+	<i>Pseudomonas</i>
25.	PKPH 5	White, flat, round.	+	+	+	-	-	-	-	-	<i>Bacillus</i>
26.	PaCD 1	Light yellow.	+	-	+	+	-	-	-	-	<i>Pseudomonas</i>
27.	PaCR 2	Yellow, irregular.	+	-	+	+	-	-	-	-	<i>Pseudomonas</i>
28.	PaDM 3	Orange, raised.	+	-	-	+	-	-	-	+	<i>Pseudomonas</i>
29.	PaCB 4	Round, yellow.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>

30.	PaPH 5	Yellow, round.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
31.	PGCD 1	Yellow, round.	+	-	-	+	+	+	-	-	<i>Pseudomonas</i>
32.	PGCR 2	Round, yellowish.	+	-	+	+	+	+	-	+	<i>Pseudomonas</i>
33.	PGDM	Light yellowish, round.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
34.	PGCB 4	Irregular, round, cream.	+	+	-	+	-	-	-	-	<i>Bacillus</i>
35.	PGPH 5	Yellow, round.	+	-	-	+	+	+	-	-	<i>Pseudomonas</i>
36.	PJCD 1	Creamy, round.	+	-	-	+	-	-	-	+	<i>Pseudomonas</i>
37.	PJCR 2	Yellow, irregular.	+	-	+	+	-	-	-	-	<i>Pseudomonas</i>
38.	PJDM 3	Flat, white.	+	+	+	+	-	-	+	-	<i>Bacillus</i>
39.	PJCB 4	White, raised.	+	-	-	+	-	-	-	-	<i>Pseudomonas</i>
40.	PJPH 5	Light yellow, round.	+	+	+	+	+	+	+	+	<i>Bacillus</i>

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*.

UNDER PEER REVIEW

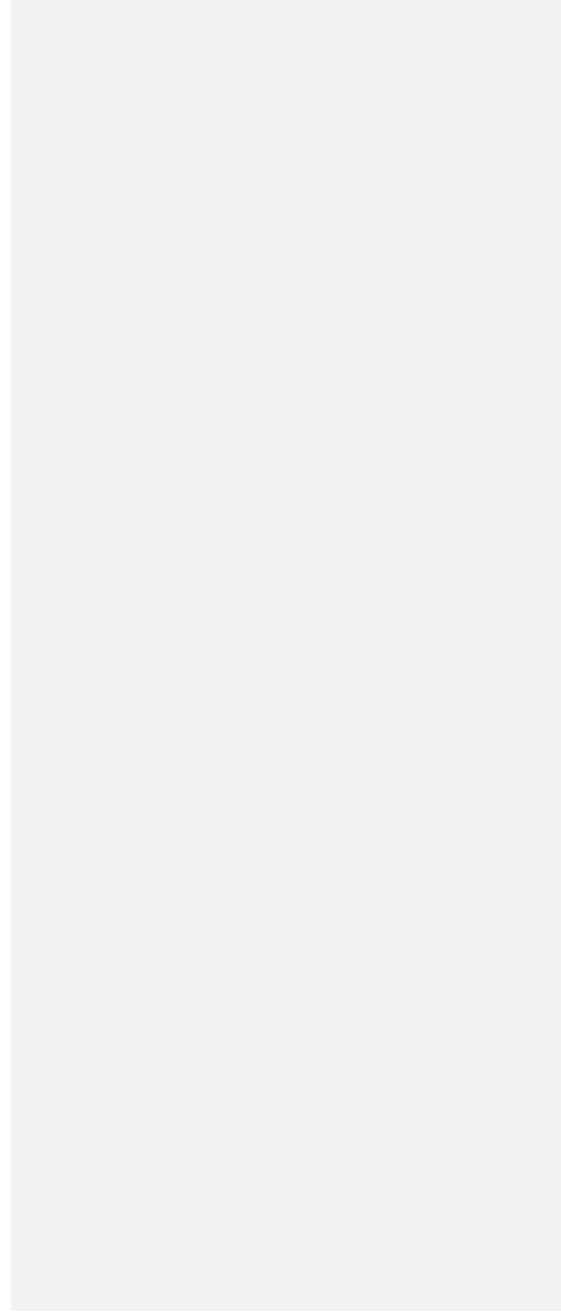


Table 3: The Phosphate Solubilizing Index, *in - vitro* P - solubilization by PSB isolates of weeds rhizosphere

SL. NO	Isolate	PSI	Broth assay 5 th day (µg/100ml)	Broth assay 10 th 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

Commented [PC96]: explain the abbreviation

39	PJCB 4	5.50	34.300	68.200
40	PJPH 5	6.00	41.453	86.932

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*.

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 there by having the ability of iron chelation and remaining 10 were negative. Out of 30 the isolate PACD 1 had 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.

Similar finding were recorded by Chaiharn *et al.* (2008) (Chaiharn, Chunnaleuchanon, Kozo, and Lumyong, 2008) they isolated PSB isolates from different rhizosphere soil and screened for their plant growth promoting and it was found that 23 per cent of isolates produced siderophore on Chrome Azurole S agar plates. Similarly, Aarab *et al.* (2015) (Aarab *et al.*, 2015) isolated 305 PSB isolates from the rhizosphere of rice fields in Northwestern Morocco, in which 136 were tri calcium phosphate solubilizers and 66 bacteria are producers of siderophores. The same results also obtained by Jai and Naveen (2019) isolated a phosphate solubilizing bacteria from the rhizosphere of *Stevia rebaudiana* and the bacteria was identified as *bacillus sp.* and it was able to produce siderophore (16.06 µg/ml).

4.4.10 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 produced 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

Commented [PC97]: thereby

Commented [PC98]: the remaining

Commented [PC99]: the most

Commented [PC100]: Table 4.

Commented [PC101]: findings

Commented [PC102]: promotion

Commented [PC103]: were

Commented [PC104]: phosphate-solubilizing

Commented [PC105]: *bacillus sp.*

Commented [PC106]: Table

Commented [PC107]: produce

(33.561 µg/ml) followed by the isolate PGCR 2 (31.243 µg/ml) and lowest IAA was produced by the isolate PRCD 1 (4.567 µg/ml).

Commented [PC108]: the lowest

The results showed that all the PSB isolates were isolated 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.

Commented [PC109]: isolates from

Similar research was carried out by Yasin *et al.* (2022) isolated and characterized PSB isolates 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 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 maximum amount of IAA (1260 ppm/L). Similar results were found by Ramakrishnan *et al.* (2012) and Sasmita *et al.* (2022).

Commented [PC110]: in soil

Commented [PC111]: and were

Commented [PC112]: the maximum

4.4.11 Gibberellin production by PSB isolates.

All 40 PSB isolates produced to GA₃ 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 GA₃ 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 GA₃ which improves cell division, cell elongation and growth of plants.

The above results were in accordance with the findings of Sasmita *et al.* (2022) (Sasmita, Anas, Anwar, Yahya and Djajakirana, 2022) 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 GA₃.

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 $\mu\text{g } 25 \text{ ml}^{-1}$ and GA₃ ranging from 2.08 to 12.55 $\mu\text{g } 25 \text{ ml}^{-1}$. 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 GA₁ (0.23 $\mu\text{g}/100 \text{ ml}$) GA₃ (5.11 $\mu\text{g}/100 \text{ ml}$) and GA₄ (2.65 $\mu\text{g}/100 \text{ ml}$) along with physiologically inactive GA₉, GA₁₂, GA₁₅, GA₂₀ and GA₂₄.

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

SL. NO	Isolate	IAA($\mu\text{g}/\text{ml}$)	GA ₃ ($\mu\text{g}/100\text{ml}$)	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, GA₃ – Gibberellic Acid, +ve - Positive, - ve - Negative. .CD - *Cynodon dactylon*. CR - *Cyperus rotundus*. DM - *Digitaria marginata*. CB - *Commelina benghalensis*. PH - *Parthenium hysterophorus*.

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* sps and *pseudomonas* sps with highest PSI, IAA and GA₃ production. Additionally research is need to be carried out to test the effect of isolate on plants under pot culture and field studies.

Commented [PC113]: revealed

Commented [PC114]: growth-promoting

Commented [PC115]: *bacillus* sp. ?

Commented [PC116]: the highest

Commented [PC117]: needs

Commented [PC118]: isolating

REFERENCES

- Aarab, S., Ollero, F. J., Megías, M., Laglaoui, A., Bakkali, M. and Arakrak, A. (2015). Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An in vitro study. *Int J Curr Microbiol App Sci*, 4(1), 260-269.
- Alok, R., Mahalakshmi, M. R. and Sridevi, M. (2013), Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. *Int. J. Nutr. Pharmacol. Neuro. Dis.*, 3:29-33.

- Bartholomew, J.W. and Mitterer, J. (1950). A simplified bacterial Strain. *Stain Tech.*, 25: 153.
- Blazevic, D. J., and Ederer, G. M. (1975). *Principles of biochemical tests in diagnostic microbiology*.
- Cappuccino, J. C. and Sherman, N. (1992), *Microbiology: A Laboratory Manual* (3rd ed.), Benjamin/Cummings Pub. Co., New York, 1:125-179.
- Chaiharn, M., Chunhaleuchanon, S., Kozo, A. and Lumyong, S. (2008). Screening of rhizobacteria for their plant growth promoting activities. *Current Applied Science and Technology*, 8(1):18-23.
- Eckford, M. O. (1927). Thermophilic Bacteria in Milk. *American Journal of Hygiene*, 7, 201-221.
- Hfiza, H. M., Jahangir, G. Z., Nasir, I. A., Iqtidar, M. and Iqbal, M. (2013), Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere soil. *Biotechnol. Biotechnol. Equip.*, 27(6):4248-4255.
- James, G. and Sherman, N. (1992). *Microbiology and laboratory manual, rockland community college, suffern, New York: The Benjamin/Cummings publishing Co. Inc., Redwood, City, California.*
- Kundu, B., Nehra, K., Yadav, R. and Tomar, M. (2009). Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian journal of microbiology*, 49:120-127.
- Mali, G., and Bodhankar, M. (2009). Antifungal and phytohormone production potential of *Azotobacter chroococcum* isolates from groundnut (*Arachis hypogea* L.) rhizosphere. *Asian J Exp Sci*, 23(1):293-297.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *Journal of molecular biology*, 3(2):208-IN201.
- Pikovskaya, R. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species [Internet].
- Sasmita, K. D., Anas, I., Anwar, S., Yahya, S., and Djajakirana, G. (2022). *Application of phosphate solubilizing microbes to promote the effectiveness of rock phosphate on cacao seedling growth in acid soil*. Paper presented at the AIP Conference Proceedings.

Sudewi, S., Ala, A., Patandjengi, B. and Basavaraj, D. R. (2020). *Application of phosphate solubilizing microbes to promote the effectiveness of rock phosphate on cacao seedling growth in acid soil*. Paper presented at the AIP Conference Proceedings.

Tenzing, B. N., Pandiarajan, G. and Kumar, B. M. (2016), Isolation, identification and characterization of phosphate solubilizing bacteria from different crop soils of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu. *Trop. Ecol.*, 573:465-474.

Ujah, I. (2022). Isolation and Biochemical Characterization of Phosphate Solubilizing Bacteria used in Biofertilizer. *EC Pharmacology and Toxicology*, 10:15-25.

Vyas, P. and Gulati, A. (2009). Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC microbiology*, 9:1-15.

UNDER PEER REVIEW