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Isolation, Characterization and Plant Growth Promotion abilities of PSB Isolates from Millet Grown Fields

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ABSTRACT: Despite the various forms of phosphorus present in soils, both organic and inorganic, they commonly serve as primary growth-limiting factors for plants. Although soil contains organic compounds with total phosphorus, the majority remains dormant and inaccessible to plants. Enhancing phosphorus solubilization through bacteria and inoculants can significantly boost crop production and the absorption of phosphorus by plants. The phosphate-solubilizing bacteria (PSB) released a substantial amount of phosphorous, with productive strains in rhizosphere soils showing particularly high levels of phosphorus release. To eliminate duplication among isolates from the same sample, three strains were carefully chosen based on distinct colony morphological traits and ability of phosphate solubilization. Morphological and biochemical tests, coupled with growth promotion studies, were conducted on these isolates for identification. Subsequently, these selected isolates were made available for further molecular research, revealing their classification as members of *Burkholderia* sp. through 16s rRNA sequence analysis. Roll towel assay was used for assessing the seedling vigour index of Bajra after seed priming with the PSB isolates. Hence, phosphate solubilising bacteria isolated from millet fields could be used as plant growth promoting bioagents.

Keywords: PSB, pikovyskayas medium, phosphate solubilization, millets, organic acids.

INTRODUCTION

Numerous bacteria possess the capacity to positively impact plant development, with a significant number demonstrating this capability within the rhizosphere—the soil region encircling plant roots. Within this cadre of beneficial bacteria, phosphate-solubilizing microorganisms (PSM) assume a pivotal role by enzymatically breaking down both organic and inorganic phosphorus molecules into soluble forms (Sumanth *et al*., 2021). Distinguished PSMs encompass diverse groups, encompassing Arbuscular mycorrhizal (AM) fungi, Actinomycetes, as well as bacterial strains such as *Bacillus*, *Pseudomonas*, and *Rhizobium*. Furthermore, fungal counterparts such as *Penicillium* and *Aspergillus* also showcase prowess in phosphorus solubilization (Al-Ali *et al*., 2018). Given the heightened metabolic activity of PSMs in the rhizosphere, these microorganisms are frequently sourced from this specific niche. This deliberate isolation facilitates researchers in exploring and harnessing the potential of these phosphate-solubilizing bacteria for augmenting plant growth and nutrient accessibility in a targeted fashion.

Comprehending the dynamics of phosphorus availability and the pivotal role of Plant Growth-Promoting Rhizobacteria (PGPR) in augmenting nutrient absorption can guide the development of sustainable agricultural methodologies, aiming to optimize crop yields while mitigating environmental repercussions linked to excessive fertilizer application. Employing PGPR as biofertilizers emerges as a promising avenue for bolstering nutrient accessibility to plants and fine-tuning agricultural efficiency (Eramma *et al*., 2021). Various bacterial species, including Azotobacter, Rhizobium, Azospirillum, and Burkholderia, are widely harnessed as PGPR owing to their proficiency in promoting plant growth and enhancing yield. Their contributions encompass nitrogen fixation, synthesis of plant growth regulators, phosphorus solubilization, and augmentation of nutrient assimilation, among other mechanisms (Suleimanova *et al*., 2023).

A crucial component of agricultural soil stewardship revolves around the utilization of phosphorus fertilizers. Inorganic phosphate stands as an indispensable nutrient for plant development, with its presence in soil exerting a significant impact on overall plant productivity. Nevertheless, a noteworthy fraction of the phosphorus fertilizers administered to the soil undergoes fixation into insoluble forms, attributed to interactions with soil cations. Findings from the study conducted by Walpola

and Yoon (2012) underscore that cations possess the capability to convert inorganic phosphate, resulting in the fixation of approximately 70 to 90 percent of the phosphorus fertilizers applied to the soil.

The rhizosphere hosts a diverse array of microorganisms, including the essential plant growth-promoting rhizobacteria (PGPR). These PGPRs wield direct influences on plant physiology, demonstrated through hormone synthesis, mediation of inorganic phosphate solubilization, augmentation of iron uptake via siderophore secretion, and modulation of plant signaling cascades through the emission of volatile compounds (Yadav *et al*., 2023). Harnessing phosphorus-solubilizing bacteria as inoculants represents a promising avenue for enhancing phosphorus assimilation in plants. These phosphate solubilizing bacteria (PSB) can be deployed individually or in combination to evaluate their impacts on crop growth and biomass accrual. By capitalizing on such microbial inoculants, there lies the potential to significantly reduce reliance on synthetic fertilizers (Suleimanova *et al*., 2023). A focal point of investigation revolves around the screening and identification of promising phosphate solubilizing strains for utilization as bio-inoculants, with the goal of bolstering plant vigor and amplifying crop yields.

Millets thrive especially in nutrient-deficient environments, including soils with low phosphorus levels. Employing phosphate fertilizers in conjunction with P-solubilizing microorganisms (PSM) has demonstrated efficacy in advancing ecological agricultural intensification principles. This approach diminishes reliance on synthetic inputs in such settings while simultaneously introducing beneficial microorganisms. PSM possess the capability to transform insoluble phosphate compounds into soluble forms readily accessible to plants. They achieve this conversion by generating organic acids, siderophores, protons, and hydroxyl ions. (Gomes *et al*., 2023). Phosphorus (P) stands as one of the indispensable nutrients crucial for plant growth and development. It plays vital roles in numerous physiological functions, including respiration, energy metabolism, photosynthesis, nitrogen transportation within the plant, and the transmission of DNA from one generation to another. Therefore, phosphorus is indispensable for tissue expansion and cell division. Efforts to enhance the energy efficiency of agricultural systems and mitigate fertilizer usage highlight the escalating significance of the rhizosphere in sustainable agricultural practices (Silva *et al*., 2021). Rhizosphere activity facilitates the conversion, mobilization, and solubilization of nutrients from the soil's limited reservoir, enabling plants to absorb vital nutrients and attain their genetic potential for yielding abundant crops. In this study, Phosphate solubilising bacteria were isolated from millets grown areas, phylogenetically identified, and tested *in vitro* plant growth-promoting potential and studied seedling vigour index of bajra seeds using biopriming of PSB isolates.

MATERIALS AND METHODS

A. Isolation of PSB from millets grown field

In Tamil Nadu, soil sampling was conducted in millet-growing regions, specifically in areas such as Villupuram, Srivilliputhur, and Edappady, targeting eight millet fields. The rhizosphere of millet plants served as the primary sampling site for soil collection. Sampling was performed at a depth of 15 cm below the soil surface to capture the microbial community associated with the root zone. The soil samples were serially diluted and appropriate dilutions were taken for the isolation of Phosphate solubilizing bacteria.

B. Primary screening of PSB isolates

Primary screening of phosphate-solubilizing bacteria (PSB) involves initial assessments to identify and select potential isolates for further study. Inoculate PSB isolates obtained from soil samples onto the Pikovskaya's agar medium. Incubate the plates at optimal conditions for bacterial growth, typically at a temperature of around 37°C for 3-7 days. After the incubation period, visually inspect the plates for the presence of clear zones surrounding bacterial colonies. These clear zones indicate phosphate solubilization by the bacteria, as they release organic acids or enzymes that dissolve insoluble phosphate compounds in the agar medium.

C. Plate Assay by PSB Isolates

The strains were assessed for their capacity to solubilize insoluble tricalcium phosphate, a common form of phosphate, within Pikovskaya's agar medium, as described by Pikovskaya (1948); Gupta *et al*. (1994). Each quadrant of Pikovskaya's agar plates (3 mm thickness) was spot-inoculated with a loopful of culture grown in Pikovskaya's broth, allowing for direct comparison and observation of phosphate solubilization by the PSB bacteria. The experiment was replicated three times, and the plates were then incubated at 37°C for a duration of 5 days. Phosphate solubilization was identified by the presence of clear zones surrounding bacterial colonies, and the diameter of these zones was measured in millimeters to quantify the extent of solubilization.

$$\text{Phosphate solubilizing index} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}} \times 100$$

D. Broth Assay by PSB Isolates

The quantitative assessment of phosphate solubilization potential by PSB isolates followed the methodology outlined by Clesceri *et al*. (1998). Twenty-four-hour-old bacterial cultures were inoculated into 5 mL of sterile Pikovskaya's broth and incubated for a period of 10 days at 37°C in a shaker operating at 100 rpm. Following centrifugation, 5 mL of supernatant was carefully collected into screw-capped vials. Subsequently, the supernatant underwent treatment with 5 mL of Vanadomolybdate solution. The total volume was adjusted to 25 mL and left to incubate overnight to facilitate the development of a yellow color indicative of phosphate presence. This process was conducted in duplicate for each treatment. Phosphate solubilization per 5 g of tricalcium phosphate amended in one liter of

broth was determined by referencing a standard phosphorus curve and expressed in mg/L.

E. Characterization of PSB isolates

The PSB isolates were streaked purified on pikovskayas medium to obtain individual well isolated colonies. Colony characters such as elevation, color, margin of colony, colony size (mm), grams reaction and cell arrangements were examined and the results were recorded.

(i) Bio chemical Characterization of PSB isolates. These biochemical tests are commonly employed in microbiology laboratories for the identification and differentiation of bacteria: Oxidase Test, Nitrate Reductase Test, Catalase Activity, Indole Production, Methyl Red and Voges Proskauer Test, Citrate Utilization Test, Starch Hydrolysis, and Cellulose Degradation.

F. Plant growth promotion traits of PSB isolates

The isolated Phosphate solubilizing bacteria were screened for their plant growth promotion traits using standard protocols. Qualitative and quantitative assessment of nitrogen fixation, phosphorous solubilization (Pikovskaya, 1948), potassium solubilization (Lu and Huang 2010), IAA production (Gordon and Weber 1951), gibberellic acid production (Holbrook *et al*., 1961), ammonia (Cappuccino and Sherman 1992), Siderophore production (Schwyn and Neilands 1987) and ACC deaminase activity (Penrose and Glick 2003) were done for all the isolates.

G. Seedling Vigour Index

The roll towel assay was employed for assessing seedling vigour index (Abdel Latef and Tahjib-Ul-Arif Rhaman 2021). Seeds of Bajra were surface sterilized using 4% sodium hypochlorite for 3 minutes, followed by three washes with sterile distilled water, and blot dried with sterile tissue paper. PSB isolates selected based on phosphate solubilization were separately cross-streaked heavily on Pikovskaya's agar plates and incubated at 37°C for 3-5 days. Bacterial suspension was obtained by drenching the culture plates with 10 ml sterile distilled water under aseptic conditions (Saoussen *et al.,* 2020). The surface sterilized seeds were soaked in the bacterial suspension for 3 h and kept overnight for drying (Athira *et al.,* 2022). These bacterized seeds were then arranged in rows of ten on moist towel paper. The rolled paper towels were kept at 28°C in a beaker with sterile water at the bottom, and adequate moisture was maintained by daily watering. Three replications were kept for each treatment. After 10 days, the rolled towel papers were retrieved, and observations were recorded. Unprimed seeds were kept as control.

RESULTS

A. Isolation of PSB isolates

Twenty five bacterial isolates capable of phosphorous solubilizing bacteria were obtained from the collected soil samples. The isolates were named as PSB1 to PSB 25. Only three superior isolates were selected based on the phosphorous solubilization. The selected isolates were PSB 2, PSB 9, and PSB13 (Table 1 and Fig. 1).

B. Primary screening of PSB isolates

Phosphatase activity was identified in the current study by selecting bacterial colonies that formed within a given incubation period and had an appropriate clear zone diameter. Only three isolates with a clearance zone diameter greater than 10 mm were deemed noteworthy based on phosphate solubilization (Table 2).

C. Plate assay for Phosphate solubilization by PSB isolates

In the current study, three (PSB1, PSB2, PSB3) isolates were selected based on the phosphate Solubilization. The diameter of the clear zone, colony diameter, and hydrolytic values of selected isolates are indicated in Fig. 2. The highest hydrolytic zone was seen in PSB13 and the lowest value was seen in PSB9 and PSB2.

D. Broth Assay by PSB isolates

The efficient three PSB (PSB1, PSB2, PSB 13) isolates were selected based on the phosphate Solubilization and inoculated into the pikovyskaya's broth. After incubation, the readings were taken at 430nm. The isolate PSB 13 showed highest value because of its solubilization ability (Table 3). The highest hydrolytic zone was seen in PSB13 and the lowest value was seen in PSB9 and PSB2.

E. Characterization of PSB isolates

(i) Morphological Characters of the Selected Isolates. Morphological and microscopic characteristics of the PSB isolates are presented in Table 4 and 5. The colony characters such as colony shape, colony size, colony color, elevation, margin, opacity, and texture were visually noted. The majority of the isolates had raised round-shaped mucoid colonies with yellow in color and entire margin. All the isolates were measured the colony size of 1mm and the cell size of 1µm. Gram staining of the cells revealed that three isolates were Gram-negative and rod in shape (Fig. 3).

(ii) Biochemical characters of PSB isolates. Various biochemical tests were carried out for all three isolates. Most of the isolates showed negative reactions for the Indole production test. Voges Proskauer test, Glucose, Citrate utilization test, and cellulose degradation were also negative for all isolates. Also, the isolate showed a negative reaction towards the test, for Catalase, Arabinose, lactose, Sorbitol, Mannitol, Rhamnose, Sucrose, and Fructose also had positive reactions except for PSB2. The reactions like the Nitrate reduction Urease test and oxidase test are also positive reactions for all the isolates. Starch hydrolysis and cellulose degradation were also negative for all the isolates (Table 6 and Fig. 4).

(iii) Molecular Characterization. The promising PSB isolates PSB 2, PSB9, and PSB13 underwent 16 S rDNA sequencing. To identify the isolates at the generic level, the gene sequence was compared to the available data in GEN Bank using BLAST homology search. PSB 2 was highly homologous to *Curtobactrium citrum*, PSB 9 was homologous to *Burkholderia seminalis*, and PSB 13 was homologous to *Burkholderia cepacia*.

F. Assessment of Plant Growth Promotion Properties

The plant growth promotion properties like nitrogen fixation, phosphorous solubilization, IAA production, gibberellic acid production, ammonia, Siderophore production and ACC deaminase activity are listed in Table 7 and Fig. 5. Isolates *viz.*, PSB 2 isolate exhibited all such PGP traits, but there was no ACC deaminase activity in isolates PSB 9 and PSB 13.

G. Seedling Vigour Index

The plant growth parameters such as germination percentage, number of days taken for germination, Root length, Shoot length seedling vigour index and Root shoot ratio are presented in Table 8. The isolates showed significant difference in germination percentage. The isolate PSB 9 showed maximum germination percentage (90%) followed by PSB 13 (83.33%). With regard to seedling vigour index (SVI), maximum value was recorded with the seedlings treated with the isolate PSB 13 (2608.83) followed by PSB 9 (2533). The minimum SVI was recorded in uninoculated control (1195.29) (Fig. 6).

DISSCUSION

The present study focused on potency of biopriming of Bajra seeds with phosphate solubilizing bacteria for growth promotion. The common PSB isolates from most of the millet crops are *Bacillus* sp., *Burkholderia* sp., and *Curtobacterium* sp., which are recognized for their safety profile, and with the potential to produce a diverse array of beneficial substances for agricultural applications (Harinathan *et al*., 2016). Additionally, The prevalence of *Burkholderia* and *Curtobacterium* species are attributed to several factors, notably the production of organic acids, which enables their phosphate solubilisation under the presence of insoluable phosphorous. Therefore, an effort has been undertaken for the isolation of phosphate solubilizing bacteria from different regions of millet grown areas of Tamil Nadu. The isolation of phosphate solubilizing bacteria was reported in cereals and millet crops using insoluble phosphate amended medium and many were isolated and selected based on the zone of clearance shown by the potential P solubilizing bacteria (Kour *et al*., 2020a; Kour *et al*., 2020b; Tahir *et al*., 2013; Suleman *et al*., 2018).

Totally twenty five phosphate solubilizing bacteria were isolated from rhizosphere regions of millets such as ragi, bajra and sorghum. The selection of bacteria is mainly by the production of clear zone around the colony. The PSB isolates showed clear zone of solubilization on Pikovskaya's agar medium were selected for further study. The zone of solubilization was recorded maximum by PSB13 (1.5 mm), PSB9 (1.3 mm) and PSB2 (1 mm). Similar results were obtained by Karpagam and Nagalakshmi (2014) with three strains of phosphorus solubilizing bacteria psm1, psm2, psm6 which showed maximum Phosphate Solubilization Index (PSI) of 2.23, 2.15 and 2.11 in agar plates.

The broth assay of phosphate solubilizing bacteria exhibited quantitative measure of phosphorus solubilization on 10 days incubated broth culture and highest amount was recorded by PSB13 (206 µg mL^{-1}), PSB9 (184.94 µg mL^{-1}) and PSB2 (81 µg mL^{-1}). Saddick (2020) identified *Burkholderia* sp. with maximum concentration of soluble P (84.8 mg of soluble P L^{-1}) isolated from rice while the lowest concentration was reported as 10.85 mg of soluble P L^{-1}. Similarly, Lin *et al*. (2006) studied the P solubilization mechanism of *Burkholderia cepacia* CC-Al74 with high ability for solubilizing tricalcium phosphate and also, demonstrated that the P-solubilization increased from l g mL^{-1} to 200 g mL^{-1} during exponential growth, when the pH decreased from 8 to 3.

In the present study the colony pattern of selected isolates were studied. The selected isolates were mostly raised with circular shape, entire margin, mucoidal appearance possessing white color and of size ranging from 1mm. The similar study was done by Shankar *et al.,* (2013) isolated *Pseudomonas* sp. in *chilli* and maize crops having circular to irregular shaped colonies with raised elevation, smooth margin, and mucoid texture by following keys present in Bergey's manual of determinative bacteriology.

Even though morphological and biochemical characterization of the isolates cannot precisely reveal the identity of the isolates it will give an insight into the nature of the isolates and the nutritional requirements which can in turn help in selecting an efficient isolate suitable for our purpose. So, the best three bacterial isolates of PSB2, PSB9 and PSB13 were selected and molecular characterizations of these isolates were done at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram. The isolates were identified as *Curtobactrium citrum* PSB 2, *Burkholderia seminalis* PSB9, *Burkholderia cepacia PSB13*, based on 16S rRNA sequence homology.

In vitro screening techniques are usually employed to select efficient bacterial strains with multiple plant growth-promotions (Ahmad *et al.,* 2008; Cakmakci *et al.,* 2007). Even though there are reports of inconsistency in correlation between *in vitro* PGP activity and *in vivo* growth promotion (Khalid *et al.,* 2004; Yobo *et al.,* 2004), these techniques are simple and efficient for screening large numbers of isolates (Campbell, 1989). Combination of in *vitro* and *in vivo* screening can help in identification of effective strains for sustainable agriculture.

Some common techniques include nutrient uptake assay, nitrogen fixation tests, indole 3 acetic acid (IAA) production, phosphate solubilization assays, siderophore production tests, ammonia production, antagonistic activity against pathogens, tolerance to abiotic stress and green house or field trials (Lally *et al.,* 2017; Abdel Latef *et al.,* 2021). Green house or field trials are more expensive and time consuming. Hence less expensive and quick *in vitro* screening techniques help to identify bacterial strains that possess desirable traits for promoting plant growth.

In the present study, the superior isolates were subjected to analysis of plant growth promotion activity such as IAA, GA and ammonia production under *in vitro* conditions. Secondary metabolites such as auxin and gibberellin, as natural phytohormones involve in

mitigating abiotic stress, dormancy and stunted growth (Restu *et al*., 2019). In this study the highest production of plant growth attributes such as IAA production, ACC deaminase activity, nitrogen fixation, phosphorus solubilization, and potassium solubilization were reported by PSB13 *Burkholderia cepacia*. Ammonia, gibberellic acid and siderophore production were highest in the isolate PSB 2 *Curtobactrium citrum.* Many researchers have reported the presence of plant growth promoting traits in bacteria isolated from the rhizosphere soils, which enhanced shoot length, root length and plant biomass. Some plant growth promoting bacteria have also been attributed to exhibit biocontrol activity against plant pathogens (Navarro-Noya *et al.,* 2012; Kumar *et al*., 2014; Abdallah *et al*., 2019).

Seed germination and seedling vigour play pivotal roles in productive capacity of a crop, exerting substantial influence on both crop yield and quality. Seed vigour encompasses the overall health and robustness of seeds, indicative of their potential for successful germination and subsequent seedling establishment. This characteristic serves as a measure of the seed's capacity to thrive diverse environmental conditions (Rudolph *et al.,* 2015). Inoculation of plant growth promoting bacteria on pre-sowing seeds improve seed germination and increase rate of germination, seedling vigour, growth and yield of many crops (Raj and Raj 2021; Kloepper 2004). In the current study biopriming with the isolates PSB 2, PSB9 and PSB13 showed maximum germination percentage compared to uninoculated control. Similarly, Gunjal and Kapadnis (2013) reported the benefit of millets such as wheat, maize, jowar and bajra inoculated by PSB isolates improved the germination percentage, shoot length and root length of above mentioned seedlings. It is proved that *Burkholderia* and *Curtobactrium* have the capability to produce gibberellins and cytokinins. This biochemical activity contributes to enhanced root growth, an increased number of root hairs, and the establishment of a close and intimate association between the seeds and the bacteria thereby resulting in resilience of the seedlings (Ahmad *et al*., 2017).

Table 1: Phosphorous solubilizing bacterial isolates obtained from different regions of millet fields in Tamil Nadu.

Sr. No.	Isolates	Location	Medium	Sr. No.	Isolates	Location	Medium
1.	PSB1	Palladam	PKM	19	PSB19	Srivilliputhur	PKM
2.	PSB2	Kuniyamuthur	PKM	20	PSB20	Arappukkottai	PKM
3.	PSB3	Kinathukada	PKM	21	PSB21	Kolli malai	PKM
4.	PSB4	Edappady	PKM	22	PSB22	Kolli malai	PKM
5.	PSB5	Arappukkottai	PKM	23	PSB23	Srivilliputhur	PKM
6.	PSB6	Kolli malai	PKM	24	PSB24	Kinathukada	PKM
7.	PSB7	Villupuram	PKM	25	PSB25	Kinathukada	PKM

Table 2: Preliminary Screening of PSB isolates based on phosphorous solubilization.

Sr. No.	Name of the isolates	Diameter of zone (mm)*
1.	PSB 1	0.5
2.	PSB 2	1
3.	PSB 3	0.2
4.	PSB 4	0.3
5.	PSB 5	0.1
6.	PSB 6	0.2
7.	PSB 7	0.1
8.	PSB 8	0.3
9.	PSB 9	1.3
10.	PSB 10	0.2
11.	PSB 11	0.4
12.	PSB 12	0.3
13.	PSB 13	1.5
14.	PSB 14	0.5
15.	PSB 15	0.4
16.	PSB 16	0.5
17.	PSB 17	0.4
18.	PSB 18	0.8
19.	PSB 19	0.3
20.	PSB 20	0.4
21.	PSB 21	0.3
22.	PSB 22	0.6
23.	PSB 23	0.8
24.	PSB 24	0.6
25.	PSB 25	0.7

Table 3: **Selection of efficient PSB isolates by broth assay.**

Sr. No.	Name of the isolates	Phosphorous mg/mL^{-1}
1.	PSB 2	119.13
2.	PSB 9	184.94
3.	PSB 13	206

Table 4: Morphological characteristics of the PSB isolates.

Sr. No.	Isolates	Colony Shape	Colony Size(mm)	Colony Margin	Elevation	Colony colour	Opacity	Texture
1	PSB 2	Circular	1	Entire	Convex	Yellow	Opaque	Smooth
2	PSB9	Circular	1.5	Entire	Convex	Yellow	Opaque	Smooth
3	PSB13	Circular	1.4	Entire	Convex	Yellow	Opaque	Smooth

Table 5: Colony characteristics of the PSB isolates.

Sr. No. Isolates	Isolates	Cell Shape	Cell Size(µm)		Arrangement
			Diameter	Length	
1	**PSB2**	**Rod**	**0.2**	**1**	**Single rod**
2	PSB9	Rod	0.3	1	Single rod
3	PSB13	Rod	0.3	1	Single rod

Table 6: Biochemical characteristics of the PSB isolates.

Sr. No.	Bio-chemical Test Hi MVIC KB 001	PSB2	PSB9	PSB13
1.	Indole	_	-	-
2.	Methyl red	+	+	+
3.	Voges Proskauors	-	-	-
4.	Citrate	-	-	-
5.	Glucose	+	-	-
6.	Adonitol	-	-	-
7.	Arabinose	+	-	-
8.	Lactose	+	-	-
9.	Sorbitol	+	-	-
10.	Mannitol	+	-	-
11.	Rhamnose	-	-	-
12.	Sucrose	+	-	-

Table 7: Plant growth promotion traits of PSB isolates.

Isolates	IAA (µg mL^{-1})	Ammonia (µmol mL^{-1})	GA (µgmL^{-1})	Total Nitrogen (µg mL^{-1})	P. solubilization (mg L^{-1})	Potassium solubilization (mg L^{-1})	Siderophore (%)	ACC deaminase (µmol mL^{-1})
PSB 2	18.74	10.81	81.00	310	119.13	-	79.00	21.50
PSB 9	29.88	3.52	62.68	340	184.94	11.00	64.20	19.46
PSB 13	75.31	3.30	75.05	340	206	12.66	63.21	25.58

Table 8: Seedling vigour index of Bajara.

Sr. No.	Sample code	Root length (cm)/plant	Shoot length (cm)/plant	Germination percentage	Seedling vigour index
1.	PSB 2	13.475±0.452^{c}	8.136±0.036^{b}	80±0^{b}	1728.905±39.019^{c}
2.	PSB 9	21.016±2.134^{a}	9.456±0.33^{a}	83.333±5.774^{b}	2533±74.646^{a}
3.	PSB 13	19.638±0.553^{a}	9.349±0.336^{a}	90±0^{a}	2608.833±78.429^{a}
4.	CONSORTIUM	17.592±0.972^{b}	9.469±0.427^{a}	80±0^{b}	2164.933±74.722^{b}
5.	CONTROL	10.721±0.137^{d}	6.355±0.329^{c}	70±0^{c}	1195.292±16.707^{d}
	CV (%)	6.658	3.748	3.201	3.021
	SE(m)	0.634	0.185	1.491	35.691

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to LSD ($p \leq 0.05$)

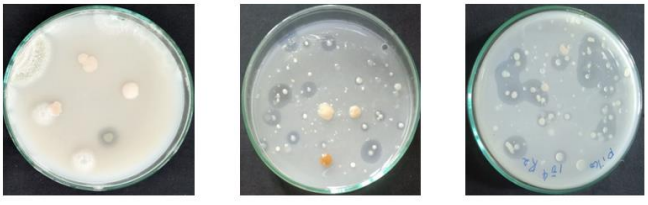

Fig. 1. Isolation of phosphate solubilizing bacteria from millets grown area of Tamil Nadu.

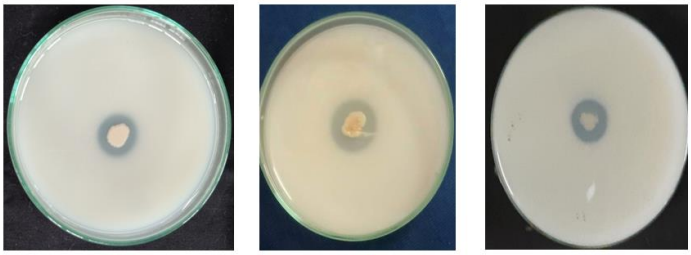

Fig. 2. Plate assay of selected PSB isolates.

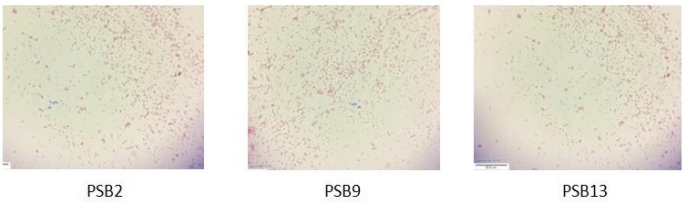

Fig. 3. Gram staining of selected PSB isolates.

Fig. 4. Biochemical characterization of PSB isolates.

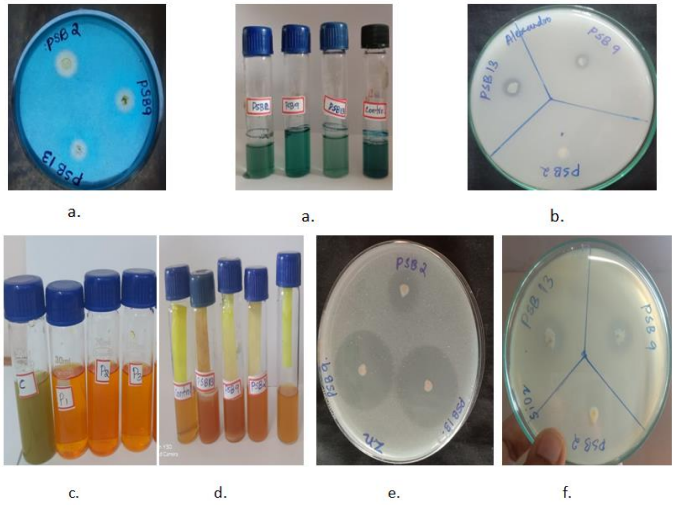

Fig. 5. Plant growth promotion properties of PSB isolates (a. siderophore production, b. potassium solubilization, c. ammonia production d. HCN production, e. zinc solubilization f. silicate solubilization).

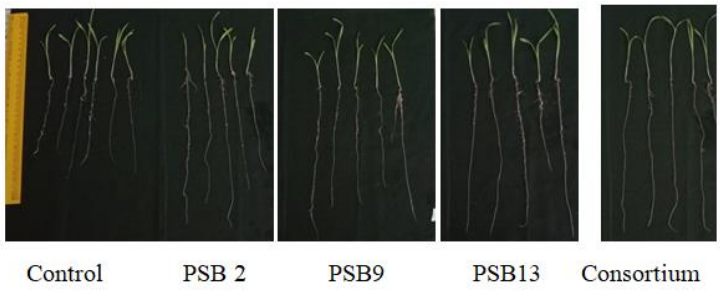

Fig. 6. Seedling Vigour Index of bioprimed PSB isolates on Bajra seedlings.

CONCLUSIONS

The current findings suggest that phosphate solubilising bacterial isolates had better efficiency in plant growth promotion by production of phytohormones and seedling vigour index assay. It is revealed that *Burkholderia cepacia* PSB 13 could be a promising phosphate solubilizing bioagent with plant growth promotion activity. The increase in plant growth promotion might be due to the higher production of IAA and GA. Phosphate solubilizing isolates with plant growth promoting potential to be applied as biofertilizer for sustainable agriculture has been identified in this study. They can be exploited for commercial production after further evaluations. It is advisable to have a consortium made with different isolates obtained from the same crop plant than the individual isolates for the use in plant health management as many with varying PGP traits act together and perform in a better way. The biopriming treatment enhanced the plant growth through various direct mechanisms such as production of IAA, GA, ammonia, HCN, siderophore, ACC deaminase activity, nitrogen fixation and phosphorus solubilization. Moreover, it is concluded that, PSB isolates offer promising opportunities for improving soil fertility, promoting plant growth, and enhancing environmental sustainability in agriculture.

FUTURE SCOPE

The findings of this study serve as a catalyst for further exploration into selected phosphate-solubilizing bacterial isolates, aiming to cultivate a bioagent conducive to fostering plant growth with broad applicability across various fields.

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