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Prospecting Phosphate Solubilizing Rhizobacteria for Enhanced Growth in Finger Millet

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Abstract

The experiment was conducted in *kharif,* 2020 (July to October) at SG College of Agriculture and Research Station, Jagdalpur, Chhattisgarh, India to explore the phosphate solubilizing bacteria from finger millet rhizosphere. Thirty rhizobacteria from finger millet rhizosphere were screened for their *in-vitro* phosphate solubilizing potential on solid and liquid National Botanical Research Institute's (NBRIP) media containing insoluble tricalcium phosphate by two methods: visual evaluation of solubilization zone around colonies (halo) and determination of solubilized phosphates in liquid medium by the colorimetric method of vanado-molybdate yellow. Nineteen isolates tested positive for P-solubilization on NBRIP-Bromophenol medium with P-solubilization index ranging from 1.53–2.55. Based on the results of halo method, 19 positive isolates were inoculated in liquid medium and periodically analyzed for P-solubilization by the colorimetric method and effect on pH of the media. In the liquid NBRIP media, the bacterial isolates showed phosphate solubilization ranging from 21.31–114.87 mg l^{-1}. The liquid media with insoluble tricalcium phosphate turned from turbid to clear after rhizobacterial cultivation and reduction in broth pH was recorded. Screening of PSB isolates for IAA production revealed that 17 out of 19 isolates produced a pink halo with varied intensity around the colony immobilized on nitrocellulose membrane suggesting IAA production with halo diameter range of 1.1–2.9 cm. Five most potent phosphate solubilizing rhizobacterial isolates were selected to assess their effect on finger millet growth. The isolates significantly improved the plant growth in terms of plant height, root length, plant biomass and chlorophyll content compared to the control.

Keywords: Bioinoculants, IAA, P-solubilization, plant growth promotion

1. Introduction

Phosphorus (P) is one of the major elements required for growth and development of plants. It is an essential nutrient for diverse metabolic and physiological processes encompassing energy metabolism, cell division, DNA synthesis and phospholipid biosynthesis, primarily as phosphate (Pi) or Pi esters (Stigter and Plaxton, 2015; Isidra-Arellano et al., 2021). Usually, the phosphorus content of soil is about 0.05% (w/w); however, only 0.1% of this phosphorus is available for plant use (Alori et al., 2017). Inadequate P in soil adversely affects fruit production, quality traits during vegetative growth and root development, eventually resulting in reduced crop yields (Khan et al., 2023). Usually, soil phosphorus deficiency is managed by the application of phosphorus fertilizers to ensure higher crop productivity (Amri et al., 2023). However, the major problem with the application of chemical fertilizers is that a large part of the soluble form of inorganic phosphate applied to the soil is rapidly immobilized and becomes unavailable to plants (Kumar and Shastri, 2017, Paz-Ares et al., 2022). Only 10–20% of the total P applied to soil is taken up by plants as inorganic phosphates (Helfenstein et al., 2018). Additionally, excessive application of inorganic fertilizers in excess of the amount that is commonly employed can lead to environmental problems such as, groundwater contamination and waterway eutrophication (Liu et al., 2021; Ibrahim et al., 2022). It is therefore of great interest to investigate management strategies that can improve phosphorus fertilization efficiency, increase crop yields and reduce environmental pollution. Phosphate solubilizing microbes (PSMs) are a group of beneficial microorganisms mainly bacteria but also fungi and archaea capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants (Raymond et al., 2020, Li et al., 2021).

Among PSMs, phosphate-solubilizing bacteria (PSB) are of significant interest owing to their ubiquitous nature and as an important part of Plant Growth Promoting Bacteria (Luo et al., 2024). PSB can solubilize soil-insoluble phosphates through mechanisms as the secretion of organic acids, the production of enzymes, and the excretion of siderophores (Puri et al., 2020; Pan and Cai, 2023). In addition, PSB can promote plant growth through the production of hormones like auxins, cytokinins and gibberellic acid, ACC-deaminase, hydrogen cyanide and siderophores (Rawat et al., 2020). PSM provide an ecofriendly and economically sound approach to overcome P scarcity and its subsequent uptake by plants (Kalayu., 2019; Bargaz et al., 2021).

Finger millet is an important climate-resilient nutri-cereal crop for the low socio-economic group. It is a highly significant crop due to its rich nutritional and bioactive profile, along with high climate resilience (Kaur et al., 2024; Mbinda and Mukami, 2021). However, it is mainly grown under low nutrient management condition including meager phosphatic fertilizers application which leads to poor yield potential. Moreover, cultivation of finger millet is mainly confined to rainfed conditions, wherein nutrient and moisture stresses are greatest constraints in its production (Prabhakar et al., 2023). Phosphorus is one of the essential nutrients that is mostly deficient in the rainfed conditions. Major part of the water-soluble P of added P fertilizers soon become unavailable due to chemical fixation in soils (Shen et al., 2023). P is often a limiting factor because most of P in soil exists as unavailable form for plants (Meng et al., 2021). Use of PSMs is a viable strategy for improving the P availability in soil. Considering this, the present study was intended towards exploration of Phosphate Solubilizing Bacteria from finger millet rhizosphere which may help in increasing the use of fixed P in soil and improving crop production under nutrient poor rainfed condition.

2. Materials and Methods

The experiment was conducted in *kharif*, 2020 (July to October) at SG College of Agriculture and Research Station, Jagdalpur, Raipur, Chhattisgarh, India. Thirty antagonistic rhizobacteria isolated from finger millet rhizosphere were selected from our previous experiment (Kumari et al., 2020). The bacterial isolates were screened for their *in-vitro* phosphate solubilizing potential on solid media and liquid medium.

2.1. Solubilization of phosphates

2.1.1. On solid agar medium

The bacterial isolates were screened for their *in-vitro* phosphate solubilizing potential on NBRIP solid medium (ingredients (g l^{-1}): glucose, 10.0; tricalcium phosphate (TCP), 10.0; $MgCl_2.6\ H_2O$, 5.0; $MgSO_4.7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1 with bromophenol blue) containing blue bromophenol, which produced yellow-colored halos around the colonies due to organic acid production (Gupta et al., 1994). The ability of bacteria to solubilize the TCP was determined by measuring the halo diameter around the colonies after the inoculation of fresh bacterial suspension on the NBRIP agar media containing bromophenol. The solubilization index (SI) was calculated after 3rd, 6th, 9th and 12th day of incubation at 28±2°C using the following formula:

SI=(CD+HD)/CD

where CD is the colony diameter, and HD is the halo zone diameter

2.1.2. Quantitative estimation of phosphate solubilization in liquid medium

The phosphate solubilizing capacity of the PSB isolates was also evaluated based on the colorimetric measurement of the concentration of solubilized phosphates in liquid medium. 100 ml NBRIP broth (containing (g l^{-1}): glucose, 10.0; tricalcium phosphate (TCP), 10.0; $MgCl_2.6\ H_2O$, 5.0; $MgSO_4.7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$) was inoculated aseptically with 1 ml of culture broth having OD of 1.5 at 600 nm. The aliquots were incubated with shaking at 28°C up to 12 days. Five ml of the growth medium from each flask was taken out on 3rd, 6th, 9th and 12th day, filtered through Whatman No. 1 filter paper, and centrifuged at 10,000 rpm for 20 minutes. Quantification of solubilized phosphorus in the cell free culture supernatant was carried out by the colorimetric method of vanado-molybdate yellow at 430 nm (Dipak and Sankar, 2017). For this, 0.5 ml or 1 ml of the supernatant was taken, 2.5 ml of Barton's reagent was added and volume was made up to 50 ml with double distilled water (ddw). After 10 minutes, the intensity of yellow color was read at 430 nm and the amount of P-solubilized was extrapolated from the standard curve prepared using potassium dihydrogen orthophosphate. The pH of the broth medium was also measured with a digital pH meter at 3rd, 6th, 9th and 12th day of incubation. All experiments were carried out in triplicate for each isolate.

2.2. IAA-producing ability determination of PSB

The qualitative assay for IAA production by PSB rhizobacterial isolates was conducted by the method of Bric's et al. (1991). Sterilized Petri dishes containing Luria agar supplemented with 3 Mm L-Trp was inoculated with bacterial inoculums, then, overlaid with an 82-mm-diameter disk of Whatman no. 1 filter paper, incubated at 27°C for 2 to 4 days. Salkowski's reagent (1 ml of 0.4 M $FeCl_3$ in 50 ml of 35% perchloric acid) was added on the filter paper after 48h of incubation. Pink colouration indicated production of IAA. The results were also analyzed visually on a three point scale (+: low; ++: medium and +++: high) and diameter of pink colour around immobilized bacterial colony was measured.

2.3. Study of effect of seed bacterization with phosphate solubilizing bacteria on finger millet growth under polyhouse condition

The ragi cultivar Uddru mallaige was used to study the effect of selected phosphate solubilizing rhizobacterial isolates on plant growth. Finger millet seeds were surface sterilized with 0.1% mercuric chloride for 30 s followed by consecutive 5–6

washings with sterile distilled water. The bacterial inoculum was prepared by inoculating in nutrient broth followed by incubation at 28°C for 24 h at 150 rpm. Bacterization of seeds was carried out by imbibing in selected bacterial suspension (1×10^8 cfu ml^{-1}) @ 50 g 10 ml^{-1} for 3-4 h with gentle shaking at 150 rpm while control seeds were incubated with uninoculated nutrient broth under the same conditions. Ten seeds were sown in each cup and were maintained under greenhouse conditions at 35±2˚C with a photoperiod of 12/ 12 h (light/dark) and watered periodically. Seeds soaked in sterile uninoculated nutrient broth served as control. The experiment was carried in a completely randomized block design with three replications per treatment. Growth parameters including seed germination, plant and root length, plant and root fresh weight, and chlorophyll content (Arnon, 1949) were recorded 20 days after germination.

2.4. Statistical analysis

Experimental data were analyzed using standard analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using CPCS1 software. Differences were considered significant at the $p<0.05$ level. Standard errors were calculated for all mean values.

A one-way ANOVA, followed by Tukey's test, was conducted to analyze the data sets obtained from the quantitative estimation of PGP traits. Student's t-test was used to analyze the data of seed germination/vigor index experiments. The significance level for all analyses was $p=0.05$.

3. Results and Discussion

Soil microorganisms play an important role in maintaining natural ecological balance through active participation in carbon, nitrogen, sulfur, and phosphorous cycles maintaining the stability of the plant-soil ecosystem (Huet et al., 2023) . Phosphate-solubilizing bacteria (PSB) are of high importance in the rhizosphere, enhancing the solubilization of inorganic phosphorus complexes into soluble forms available for plant nutrition. The investigation of this species of bacteria is of major interest in agriculture, as they can be used as biofertilizers for crops.

3.1. Screening of rhizobacteria for P-solubilization

All the rhizobacterial isolates in the present study were screened for phosphate solubilization on National Botanical Research Institute's phosphate-bromophenol blue (NBRIP-BPB) solid media supplemented with TCP as the only source of phosphorus. The phosphate solubilization ability was marked by the formation of yellow halos around the bacterial colony. The isolates showed a clear halo zone around their colonies, which could be the result of the production of organic acids or polysaccharides, or the activity of phosphatase enzymes (Paul and Sinha, 2013). The average Phosphate Solubilization Index (PSI) of the isolates is presented in Table 1 which ranged from 1.53–2.55 (Table 1). In a similar finding, Batool and Iqbal (2019) reported that phosphate solubilization index of 10 most promising PSB from wheat rhizosphere ranged between 4-6. Further, formation of yellow halo around the colonies on blue background (Figure 1) illustrated the role of organic acids in the solubilization of inorganic phosphates. Supplementing our finding, acidification *via* release of organic acids has been reported to be major contributing factor in solubilization of different insoluble compounds including phosphates and zinc (Costerousse et al., 2018).

Table 1: P-solubilization potential of rhizobacterial isolates on NBRIP medium

Isolates	P-solubilization index		
	Days after incubation		
	2	5	7
A-1	-	-	-
A-2	1.44±0.20	1.64±0.13	1.66±0.13
A-3	-	1.50±0.12	1.58±0.13
A-5	-	-	-
A-6	1.30±0.20	1.42±0.12	1.61±0.13
A-7	1.66±0.30	1.81±0.14	1.92±0.14
A-8	1.63±0.12	1.67±0.14	2.00±0.24
A-9	1.50±0.13	1.60±0.22	1.70±0.13
A-10	1.38±0.14	1.70±0.13	2.00±0.15
A-11	1.22±0.11	1.45±0.11	1.67±0.12
P-1a	1.22±0.11	1.89±0.15	1.80±0.14
P-4c	-	-	-
P-5a	1.88±0.14	2.11±0.26	2.00±0.24
P-5c	-	-	-
P-8b	-	1.90±0.14	1.91±0.24
P-8d	-	1.50±0.13	1.61±0.12
P-8e	-	-	-
B-11c	-	-	-
B-12a	-	-	-
B13a	-	1.47±0.12	1.58±0.12
B-13b	-	1.63±0.13	1.80±0.13
B-16a	1.22±0.11	2.00±0.14	2.30±0.15
P-17a	1.44±0.12	1.78±0.14	2.00±0.13
P-17b	1.33±0..11	1.46±0.11	1.66±0.12
B-19a	1.38±0.14	1.50±0.12	1.53±0.11
B-19d	-		-
P-20a	-	-	-
P-21a	1.5±0.13	2.22±0.26	2.55±0.25
P22a	-	-	-
B24a	-	-	-

Values represent mean±SE of three replicates

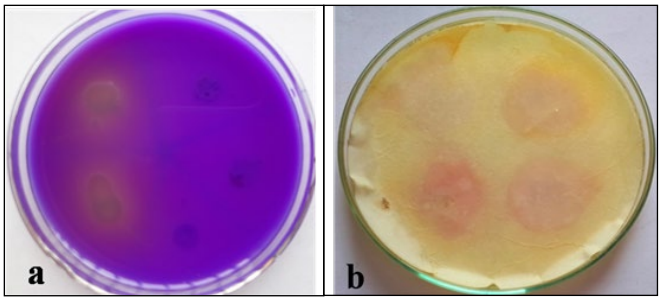

Figure 1: (a) Yellow halo around the bacterial isolates on NBRIP-Bromophenol agar medium indicative of P-solubilization; (b) Formation of pink halo around the bacterial colonies overlaid with Whatman no.1 filter paper ion treatment with Salkowski reagent indicating IAA production

The isolates tested positive for P-solubilization on solid medium were inoculated in liquid medium and periodically analyzed for P-solubilization and effect on pH of the media. It was observed that water soluble inorganic phosphate values in the supernatants harboring phosphate solubilizing strains were all higher than those of the control. All liquid media with insoluble tricalcium phosphate turned from turbid to clear after rhizobacterial cultivation. The phosphate value and the pH of the 19 phosphate solubilizing strains are shown in Table 2. The abilities of most phosphate solubilizing bacterial isolates to dissolve calcium phosphate varied significantly. After calibration with the control, the phosphorus content in the culture medium was in a range of 21.31–107.95 mg l^{-1}. In general, these results were consistent with the above-mentioned halo-zone experiments but with slight differences, which may be due to the non-diffusion or unclear diffusion of the acid produced by the strains on the solid plates. Similarly, Batool and Iqbal (2019) reported the P solubilization capacity of selected strains from agar plate in the range of 30–246 mg ml^{-1} in liquid NBRIP broth containing tricalcium phosphate as a source of insoluble P. However, they reported no direct correlation between 'P' solubilizing activity in solid and liquid assay.

In the liquid medium, compared with the control group, the pH of all the strains was found to decline, indicating that acidic substances were produced during the culture of phosphate solubilizing bacteria. Similar finding of decrease in pH in liquid

Table 2: P-solubilization by rhizobacterial isolates in liquid medium

Isolates	P-solubilization (ppm)								
	0 d	3d		6d		9d		12 d	
	pH	pH	ppm	pH	ppm	pH	ppm	pH	ppm
A-2	7.4±0.00	5.2±0.03	42.55±1.47	5.0±0.06	55.59±1.49	4.8±0.03	69.56±1.47	4.8±0.11	75.78±2.18
A-3	7.4±0.00	5.9±0.11	12.42±0.70	5.1±0.03	27.95±0.09	5.2±0.11	43.48±2.01	4.9±0.12	62.55±2.31
A-6	7.4±0.00	5.5±0.06	20.50±0.73	5.0±0.02	40.99±1.14	5.1±0.12	55.90±1.97	5.2±0.08	63.35±1.73
A-7	7.4±0.03	5.3±0.08	19.87±1.07	4.9±0.11	38.82±1.05	5.0±0.08	42.23±1.28	5.0±0.06	83.85±2.31
A-8	7.4±0.00	5.3±0.03	27.33±1.34	5.3±0.08	44.72±0.99	5.3±0.17	48.45±1.41	5.4±0.00	59.32±1.34
A-9	7.4±0.00	5.8±0.11	33.97±1.14	5.6±0.11	51.11±0.64	5.5±0.15	62.05±1.18	5.1±0.03	97.95±1.70
A-10	7.4±0.00	5.4±0.06	29.57±0.90	5.1±0.03	54.84±1.06	5.5±0.14	74.84±1.64	5.1±0.09	100.99±1.15
A-11	7.4±0.00	5.3±0.03	26.09±0.62	5.1±0.03	42.55±1.47	5.2±0.08	41.61±0.92	5.2±0.12	41.62±1.51
P-1a	7.4±0.00	5.5±0.08	23.29±0.74	5.3±0.06	22.67±0.96	5.3±0.11	25.46±1.42	5.1±0.15	29.81±1.62
P-5a	7.4±0.00	5.8±0.12	25.78±1.02	5.8±0.11	32.23±1.28	5.7±0.15	38.82±1.05	5.1±0.08	55.59±2.07
P-8b	7.4±0.00	5.6±0.11	15.53±0.88	5.4±0.12	22.36±1.33	5.4±0.11	78.88±1.66	5.3±0.11	31.99±1.72
P-8d	7.4±0.00	5.4±0.06	18.94±0.84	5.4±0.08	53.73±2.15	5.4±0.06	71.12±1.22	5.2±0.12	101.86±3.10
B13a	7.4±0.00	5.5±0.03	51.55±0.89	5.3±0.07	61.80±1.03	5.2±0.03	90.68±1.12	5.2±0.03	79.81±2.20
B-13b	7.4±0.00	5.6±0.11	36.33±0.76	5.1±0.06	54.04±1.17	5.2±0.06	92.24±2.44	4.8±0.06	82.61±2.08
B-16a	7.4±0.00	5.5±0.12	46.46±1.84	5.4±0.08	65.71±2.98	5.2±0.11	89.19 ±1.26	5.5±0.07	114.87±3.76
P-17a	7.4±0.00	4.8±0.06	52.48±0.85	4.8±0.11	72.36±1.93	4.7±0.12	77.02±1.18	4.8±0.11	111.49±3.46
P-17b	7.4±0.00	5.3±0.03	25.47±0.84	5.1±0.00	68.32±1.91	5.1±0.11	78.88±2.24	4.9±0.17	80.59±2.88
B-19a	7.4±0.00	5.5±0.11	24.84±0.48	5.0±0.09	51.24±0.71	5.1±0.03	62.42±1.39	4.9±0.12	86.02±2.32
P-21a	7.4±0.00	5.0±0.09	68.63±1.51	4.9±0.12	73.60±2.07	5.0±0.00	73.91±2.25	4.8±0.06	99.10±1.21
Control	7.4±0.00	7.3±0.06	4.21±0.12	7.0±0.08	5.83±0.47	7.0±0.03	6.33±0.31	7.0±0.06	6.64±0.67

Values represent mean±S.E. of three replicates

NBRIP medium accompanied by phosphate solubilizing activity of rhizobacterial isolates has been reported (Batool and Iqbal, 2019). Nevertheless, a lower pH did not mean a stronger ability to dissolve phosphorus. For example, the isolate A2 showed phosphorus content of 75.78 ppm, much lower than the highest P-solubilization exhibited by isolate P17d i.e. 114.59 ppm, in the culture even when the pH was as low as of 17d (4.8). In a similar experiment, Amri et al. (2023) reported that bacterial strains isolated from forest and agricultural soil of five Tunisian regions showed phosphate solubilization ranging from 535.70 to 618.57 µg ml^{-1} in the NBRIP medium, and 374.20 to 544.28 µg ml^{-1} in the PVK medium. The best phosphate solubilization ability and higher reduction in broth pH, which indicates higher organic acid production, were achieved in NBRIP broth for most of the PSB. The microbial ability to solubilize P has led to several hypotheses. Some revealed a significant correlation between the culture pH and the P-solubilizing ability, while others revealed the P-solubilizing effect of phosphate-solubilizing microorganisms was caused by various organic acids like gluconic acid, citric acid, oxalic acid, and tartaric acid into the surroundings s that chelate the phosphate-bound cations like $Ca^{2+,}$ Fe^{3+}, Fe^{2+} and Al^{3+} ions to dissolve the insoluble phosphate (Rawat et al., 2021). Another mechanism for inorganic phosphate solubilization is the production of inorganic acids like Sulfuric acid, carbonic acid, hydrochloric acid, and nitric acid by reducing the same soil pH level as organic acids but with less efficiency (Elhaissoufi et al., 2020) Role of proton extrusion has also been reported to be another important mechanism in P-solubilization (Kalayu, 2019; Javed et al., 2023). Therefore, the mechanism of phosphorus solubilization by microorganisms needs to be investigated further.

3.2. Assessment of IAA production of p-solubilizing bacteria

Endogenously produced or exogenously applied organic substances are known as regulators of plant growth and development (Kadmiri et al., 2018). They are active in a very low concentration and affect several morphological and physiological processes. Among the different types of plant growth regulators, IAA has important functions in cell proliferation, apical dominance, tropic responses, and reproduction (Puri et al., 2020). Various rhizobacterial strains have been reported to produce IAA in significant amounts and help in plant growth promotion. Many PSB are reported as plant growth promoters, with their beneficial effects on plants including alleviation of nutrient deficiency *via* phosphorus solubilization (Amri et al., 2023, Parnell et al., 2016).

The present study revealed that Indole acetic acid production was characteristic of almost all the PSB bacterial isolates. Seventeen out of 19 PSB isolates immobilized on nitrocellulose membrane produced a pink ring around the colony suggesting production of IAA (Figure 1). Qualitative determination carried out using Whatman no.1 filter paper provides an easy, time saving protocol for IAA production. Intensity of colour further signified the amount of IAA production. Bric et al. (1991) reported that the colorimetric reaction to IAA is limited to a region immediately surrounding each colony, is specific to isolates producing IAA, occurs within 1 h after the membrane is placed in the reagent, and is sensitive to as little as 50 pmol of IAA in a 2-mm spot. Development of pink colour with addition of salkowski reagent was observed with 17 isolates highest diameter being observed with isolate P1a (2.9 cm) followed by A9 (2.7), B8b (2.7) and B8d (2.7) (Table 3).

Table 3: IAA production by rhizobacterial isolates by filter paper assay

Isolates	Intensity of color	Dia (in cm)
A-2	++	1.1±0.11
A-3	+	1.9±0.05
A-6	+	1.8±0.08
A-7	++	2.3±0.11
A-8	+++	2.6±0.05
A-9	+++	2.7±0.17
A-10	++	2.5±0.08
A-11	+	1.6±0.02
P-1a	+++	2.9±0.17
P-5a	-	-
P-8b	++	2.7±0.08
P-8d	+++	2.7±0.02
B13a	+++	2.6±0.11
B-13b	++	2.5±0.11
B-16a	+	2.0±0.05
P-17a	++	2.4±0.11
P-17b	++	1.6±0.11
B-19a	-	-
P-21a	++	1.4±0.11

+: light; ++: moderate; +++: high intensity; Values represent mean ±S.E. of three replicates

3.3. Effect of seed bacterization with potent phosphate solubilizing bacteria on finger millet growth

Of 19 phosphate solubilizing rhizobacterial isolates, 5 most promising phosphate-solubilizing bacterial strains (viz. A9, A10, B16a, P17a, P21c) were selected for seed bacterization and to assess their effect on finger millet growth. Finger millet seeds inoculated with different strains exhibited significantly different increases in plant height, plant fresh weight, root length, root fresh weight and chlorophyll content, which was, in all cases, significantly higher than that of control seedlings ($p < 0.05$). Among inoculated seedlings, A10 treated seedlings exhibited the highest increase in plant height (20.19 cm), which was about 1.16 times that of the control followed by B16a and A9 seedlings with 1.12-fold and 1.119-fold increases plant height, respectively, as compared to that of control

seedlings. Of 5 rhizobacterial isolates used for seed priming, 10 isolates significantly improved the plant growth in terms of plant height, root length, plant biomass and chlorophyll content compared to the control (Table 4). Although, higher germination percent was recorded in treated plants, increase was not significant compared to control. Treatment recorded 13.6–34.8% increase in plant fresh wight over control, highest being with A-10 followed by B16a which was significantly higher than in control. Significant increase in root length was recorded with all the isolates ranging from 13.44–38.2% increase over the uninoculated control. Significant increase in root fresh weight was recorded with all the isolates in the range of 18.8–30.4% over control. In a similar report, Batool and Iqbal., (2019) reported that Phosphate solubilizing rhizobacteria (PSRB) based biofertilizer led the significant (p<0.05) effect on growth parameters of wheat such as decrease seed germination date while increase shoot, root length and biomass of wheat. Chlorophyll content also recorded an increase with seed bacterization which was in the range of 9.7–15.0%, 1.31–21.68% and 9.58–18.17% in case of chlorophyll a, chlorophyll b and total chlorophyll content. While all the isolates recorded significant increase in chlorophyll a, chlorophyll b and total chlorophyll content, P17a showed highest significant increase in chlorophyll a while B16a exhibited highest chlorophyll b and total chlorophyll content among all the treatments. Studying the influence of PSB on the morphological and physiological characteristics of finger millet seedlings and thereby unraveling the growth-promoting properties of these bacteria can prove useful for the application of PSB to plants. Plant height, root length, plant and root biomass and other morphological growth indicators are a direct manifestation of the efficiency of seedling growth. The results of this study showed that phosphorus-solubilizing bacteria promote the growth of finger millet seedlings in terms of plant height and root length, plant and root biomass to varying degrees. This might be due to the PSB strains dissolving the insoluble phosphate in the soil and enhancing the available P content by producing organic acid and extracellular phosphatases (Chen et al., 2021). Another possibility might be related to the metabolism of PSB, producing a variety of plant hormones, acids, and vitamins (Yu et al., 2011). This is consistent with the findings of Cui et al. (2020) who demonstrated that phosphorus-solubilizing bacteria can promote plant growth and increase rhizome and leaf biomass. Similarly, (Chen et al., 2021) found that the inoculation of PSB significantly promoted plant height, ground diameter, and biomass, which can be due to the organic acids production (such as gluconic, formic, and citric acids) by these strains. In this study, the plant height growth and biomass of seedlings treated with bacteria were significantly higher than those of control seedlings. In a similar experiment, Harinathan et al. (2016) reported that inoculation of phosphate solubilizing bacteria in finger millet and pearl millet increased the plant height, numbers of tillers, root length and shoot length. Multiple growth promoting factors have been reported to be responsible for significant responses. IAA plays significant role in plant signaling pathways to coordinate the physiological and morphological responses especially in induction of root development in young seedlings (Sekar et al., 2018).

Table 4: Effect of rhizobacterial isolates on growth parameters of finger millet

Treatments	% germination	Plant height (cm)	Plant fresh weight (g)	Root length (cm)	Root fresh weight (g)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
A9	93.33	19.42±0.60	0.379±0.01	17.2±0.554	0.429±0.011	18.57±0.81	9.32±0.42	37.23±1.5
A10	100.00	20.19±0.29	0.496±0.01	19.75±0.449	0.5±0.021	19.53±0.56	9.83±0.46	36.90±2.52
B-16a	100.00	19.55±0.736	0.461±0.017	18.6±0.557	0.513±0.015	19.54±0.41	10.33±0.40	40.89±0.79
P17a	93.33	18.17±0.327	0.447±0.012	18.2±0.646	0.499±0.019	19.87±0.15	9.93±0.47	37.61±0.84
P21a	90.00	18.4±0.64	0.374±0.016	14.3±0.484	0.481±0.011	18.86±0.50	9.86±0.48	36.98±0.51
Control	86.67	17.35±0.435	0.323±0.011	12.2±0.374	0.348±0.013	16.76±0.27	8.09±0.25	33.46±0.14
CD (p=0.05)	NS	1.51	0.037	1.473	0.045	1.552	1.359	3.96

In the same column, significant differences at p<0.05 levels are indicated by different letters. Data followed by same letter in the same column are not significantly different from each other according to analysis of variance (ANOVA)

4. Conclusion

The present investigation clearly depicted that rhizosphere of finger millet contained a diverse group of bacteria which could solubilize phosphate through an array of mechanisms making it available for plant growth. The selection and application of locally available PSB with high capabilities of phosphate solubilization could emerge as a green and sustainable way to make phosphates effectively available for plant growth.

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