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RESEARCH ARTICLE

Isolation, characterisation and *in vitro* evaluation of phosphorus solubilising and mineralising rhizobacterial consortia for rice (*Oryza sativa* L.)

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Abstract

Phosphorus (P) is a vital macronutrient for plant growth, however, its availability in soils is limited due to fixation and poor solubility. This limitation results in high reliance on chemical fertilisers, contributing environmental concerns such as nutrient runoff and soil degradation. This study aimed to isolate, characterise and evaluate phosphorus solubilising bacteria (PSB) and phosphorus mineralising bacteria (PMB) for plant growth promotion in rice (*Oryza sativa* L.) under *in vitro* conditions. A total of 45 distinct bacterial isolates were obtained from five different soil samples using Pikovskaya's (dicalcium phosphate (DCP) and tricalcium phosphate (TCP)), phytate, lecithin and Sperber's agar media. Further, the isolates were screened for plant growth-promoting (PGP) traits such as solubilisation of phosphorus, potassium and zinc, mineralisation of phosphorus and production of siderophore, indole-3-acetic acid, ammonia and hydrogen cyanide. Compatible isolates were grouped into three consortia, designated as C1, C2 and C3. Each consortium was formulated to ensure collective expression of all the screened PGP traits. Under *in vitro* conditions, consortium C2 showed the highest seed germination (100 %), root length (9.45 ± 0.31 cm), shoot length (6.70 ± 0.22 cm), seedling length (16.16 ± 0.52 cm) and vigour indices (I: 1616 ± 42.6; II: 5.47 ± 1.9). These values were significantly higher than those of other consortia and the control. The bacterial isolates constituting consortium C2 were identified as *Bacillus* sp., *Priestia megaterium* and *Bacillus subtilis*. These findings highlight the potential of C2 consortia as a sustainable bioinoculants for enhancing rice growth, thereby supporting eco-friendly and sustainable agricultural practices.

Keywords: microbial consortium; phosphorus mineralising bacteria; phosphorus solubilising bacteria; plant growth promoting traits

Introduction

Phosphorus (P) is an essential macronutrient for plant growth and development, playing a key role in energy metabolism, photosynthesis, nucleic acid synthesis and root development (1). Although phosphorous is one of the most important nutrients in agricultural systems after nitrogen, its availability in soils is often limited due to the formation of insoluble complexes with metal ions such as Fe^{3+}, Al^{3+} and Ca^{2+} (2). Large-scale application of phosphorous fertilisers has resulted in several environmental and economic concerns, including eutrophication of water bodies and depletion of finite phosphate rock reserves. These challenges have intensified efforts to identify sustainable alternatives that enhance phosphorus availability while reducing dependence on chemical fertilisers in agricultural systems. In this context, phosphorus solubilising bacteria (PSB) and phosphorous mineralising bacteria (PMB) have emerged as the potential biological agent, which can be utilised to release insoluble phosphorous compounds into plant available forms (3).

Phosphorus solubilising bacteria (PSB) and phosphorous mineralising bacteria (PMB) constitutes a heterogeneous group of microorganisms belonging to genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterobacter* and *Serratia*, with the capacity to solubilise organic and inorganic phosphorus compounds. These bacteria employ multiple phosphorus forms solubilisation mechanisms, including the production of organic acids (citric, gluconic, oxalic and lactic acids), phosphatases, phytases and other enzymes that facilitate the release of phosphorus from bound forms (4). The organic acids produced by these bacteria solubilise phosphates through chelation and acidification while also creating favourable rhizosphere conditions that enhance nutrient uptake (5).

In addition to the ability to solubilise and mineralise different phosphorus forms, most phosphorus solubilising bacteria (PSB) and phosphorous mineralising bacteria (PMB) have plant growth-promoting (PGP) activities that enhance the overall health and productivity of plants. These multifunctional bacteria contribute to nutrient solubilisation and synthesise phytohormones such as cytokinins, indole-3-acetic acid and gibberellins, which promote plant growth and development. Additionally, these bacteria have the ability to enhance plant resilience through the production of siderophores for iron

acquisition, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase for abiotic stress tolerance and antimicrobial compounds for biocontrol of plant pathogens (6).

The application of PSB as biofertilisers has shown promising results in various crops, demonstrating improved phosphorus uptake, enhanced root and shoot development, increased biomass production and better seed germination (7, 8). Recent studies have further validated the effectiveness of PSB in converting insoluble P into plant-available forms that can be readily absorbed by plants from the soil (9).

The application of plant growth-promoting rhizobacteria (PGPR) in sustainable agriculture has increased substantially over the past few decades. Several studies reported significant improvements in crop growth and yield following PGPR application. The present study aims to isolate phosphorus-solubilising and mineralising bacteria, characterise their PGP traits and evaluate their effects on seed germination and seedling vigour of rice under *in vitro* assays.

Materials and Methods

Site of experiment and soil sampling

The present experiment was conducted at ICAR-Indian Institute of Rice Research, Rajendranagar, Telangana, India. Rhizospheric soil samples were collected at 0–15 cm depth from rice-growing fields at Rajendranagar (Telangana), Nalgonda (Telangana), Nallamala (Andhra Pradesh) and Rajgir (Bihar), along with a forest soil sample from Nallamala (Andhra Pradesh). Samples were transported to the laboratory in sterile polyethylene bags and stored at 4 °C until further processing (10).

Isolation and purification of phosphorus-solubilising and mineralising bacteria

One gram of each soil sample was serially diluted up to 10^{-6} and aliquots (100 µL) from appropriate dilutions were spread onto selective media. These included Pikovskaya's medium supplemented with dicalcium phosphate (DCP) and tricalcium phosphate (TCP), phytate medium containing sodium phytate as an organic phosphorus source, lecithin medium containing lecithin and Sperber's medium for mineral phosphorus solubilisation (11–14). Distinct colonies exhibiting halo zones were selected, purified through repeated streaking and maintained on nutrient agar slants at 4 °C for short-term use, while glycerol stocks were preserved at –20 °C for long-term storage.

Screening of phosphorus-solubilising and mineralising bacterial isolates for multiple PGP traits

The purified isolates were functionally screened for multiple PGP traits. The phosphorus-solubilising and mineralising abilities of the isolates were assessed by inoculation onto Pikovskaya's medium containing DCP and TCP, phytate medium, lecithin medium and Sperber's medium, followed by incubation for 72 hr. The potassium solubilisation ability of the isolates was determined by inoculating the isolates on the Aleksandrov media with insoluble potassium aluminium silicate and incubation of 48 hr (16). The isolates were inoculating on the tris minimal media supplemented with 0.1 % of zinc oxide and were incubated for 48 hr to estimate their zinc solubilisation ability (17).

The presence of halo zones around the bacterial colonies indicated positive solubilisation and mineralisation activity. The quantitative measure of the effectiveness was calculated as solubilising index (SI) by measuring the diameter of these clear zones to the size of the colony (15).

$$\text{Solubilisation Index (SI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}} \quad \text{(Eqn. 1)}$$

Quantitative estimation of indole-3-acetic acid (IAA) production was performed by inoculating bacterial isolates into tryptic soy broth with and without 0.1 % L-tryptophan, followed by incubation for 7 days at 30 °C. The cultures were centrifuged at 10000 rpm for 10 min and 2 mL of Salkowski reagent and two drops of orthophosphoric acid were added to the supernatant collected for IAA production (19). After 30 min of incubation, development of pink colour indicated positive IAA production, with the intensity of colour being quantitatively measured in a spectrophotometer against a standard curve of IAA at 540 nm.

Hydrogen cyanide (HCN) production was assessed by inoculating the bacterial isolates onto nutrient agar plates supplemented with 4.4 g L^{-1} glycine. A filter paper soaked in alkaline picric acid was placed on the inner side of the plate lid and the plates were incubated for 7 days. The development of reddish-brown colour on the filter paper was considered a positive indication of HCN production (20).

Ammonia production by the bacterial isolates was evaluated using peptone water. The isolates to be screened were inoculated in the peptone water and incubated for 4 days at 28 °C (21). After incubation, cultures were centrifuged and Nessler's reagent was added to the supernatant to assay ammonia production. A change in colour from yellow to dark brown indicated a positive reaction.

Scoring of bacterial isolates

The rhizobacterial isolates were assessed for eleven PGP traits pertaining to plant growth promotion. Based on solubilisation indices and other PGP traits, isolates were selected using a scoring system in which no, low, moderate and high activities were assigned scores of 0, 1, 2 and 3, respectively and a bonitur scale was generated (22, 23).

In vitro compatibility studies among isolates for consortium development

Compatibility of bacterial isolates with each other was evaluated by the cross-streak method (24). After incubation for a week the cultures were checked for the development of inhibition zones which indicates the incompatibility within the test isolates.

Consortia formulation and *in vitro* germination assay

For consortium formulation, a loopful of each potential and compatible bacterial cultures was inoculated into nutrient broth and incubated for 3–5 days. Then the culture was centrifuged, the pellet was washed with phosphate buffered saline (PBS) and it was made up to 25 mL with PBS (23). The optical density of the suspension was adjusted to an OD_{600} of 1.0, corresponding to approximately 1×10^7 CFU mL^{-1}. Seeds were surface sterilised with 2 % sodium hypochlorite for 1 min, followed by 70 % ethanol for 1 min and subsequently rinsed thoroughly with

sterile distilled water. The sterilised seeds were soaked overnight in the bacterial consortia containing three organisms in equal proportions (OD_{600} = 1.0), placed on water agar plates and incubated at 28 °C for 7 days. Data on germination percentage, shoot length, root length, seedling length and vigor indices (I and II) were recorded (23).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds kept for germination}} \quad \text{(Eqn. 2)}$$

$$\text{Vigor index I} = \frac{\text{Germination (\%)}}{\text{Seeding length (cm)}} \times 100 \quad \text{(Eqn. 3)}$$

$$\text{Vigor index II} = \frac{\text{Germination (\%)}}{\text{Dry weight of seedlings (g)}} \quad \text{(Eqn. 4)}$$

Molecular identification of bacteria isolates

Genomic DNA from selected bacterial isolates was isolated using the Gsure bacterial DNA isolation kit (GCC Biotech, India) according to the manufacturer's instructions. Amplification of the 16S rRNA gene was performed using a Bio-Rad T100 thermocycler (Bio-Rad, USA) with two primer pairs: (i) 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 907R (5′-CCGTCAATTCCTTTRAGTTT-3′) and (ii) 785F (5′-GGATTAGATACCCTGGTA-3′) and 1492R (5′-CGGTTACCTTGT-TACGACTT-3′), developed in-house for 16S rRNA amplification. PCR amplification was carried out in a 20 µL reaction mixture containing 2 µL of template DNA, 1.6 µL dNTPs, 2 µL of 10× buffer, 0.2 µL Taq DNA polymerase and appropriate primers. The cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 30 sec, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min.

Statistical analysis

Statistical analysis of triplicate data was performed using OPSTAT software (hau.ernet.in/about/opstat.php) through analysis of variance (ANOVA). Mean values were compared using the least significant difference (LSD) test at a significance level of $p \leq 0.05$ (25).

Results and Discussion

Isolation of phosphorous solubilising and mineralising bacteria

From the five soil samples, a total of 45 distinct phosphorous-solubilising and mineralising bacterial isolates were obtained and purified based on colony morphology, including shape, margin, elevation, appearance and colour and designated as AP-1 to AP-45 (Table 1 and Fig. 1). Among these, 13 isolates were obtained on Pikovskaya's (TCP), 11 on Pikovskaya's (DCP), 9 on phytate, 7 on lecithin and 5 on Sperber's medium. Several researchers isolated P solubilising and mineralising bacteria from the rhizospheric soil using Pikovskaya's agar media (26, 27). The clear zones on Pikovskaya's medium formed due to acidification mechanisms that solubilise tricalcium or dicalcium phosphate (28, 29). Isolates on phytate agar exhibited halo formation, confirming phytate hydrolysis which may harbor phytases, known to release phosphorus from organic inositol phosphates (30). Lecithin-utilising isolates indicated the presence of phospholipase-producing bacteria capable of releasing P from phospholipid complexes (31).

Characterising phosphorous solubilising and mineralising bacteria for plant growth promoting properties

Phosphorus solubilisation and mineralising ability

Phosphorus has a crucial role in plant growth, metabolism and yield. Plant growth-promoting rhizobacteria isolates possess the ability to solubilise the insoluble forms of phosphorus and convert them into plant-available forms through various biochemical mechanisms (2). In the present study, among the 45 isolates screened, 28 isolates produced distinct halo zones on Pikovskaya's (DCP), 31 isolates on Pikovskaya's (TCP), 18 on Sperber's Medium indicating phosphorus solubilising activity. When tested for phosphorous mineralisation, 25 isolates on phytate agar and 21 isolates on lecithin agar showed halo zone. The solubilisation index (SI) varied widely among isolates, indicating different phosphorus solubilisation capabilities. Among them, isolates AP-06, AP-10, AP-14, AP-22, AP-30, AP-33 and AP-42 exhibited high solubilisation on DCP and TCP media, with SI values ranging from 3.0 to 3.9. In Sperber's medium, isolate AP-33 (2.5) exhibited highest solubilisation indices.

Phytate solubilisation index was high in isolates AP-18, AP-34 and AP-42 with SI values between 2.6 and 2.9. On lecithin medium, isolates AP-20 (2.7) and AP-12 (2.6) showed the highest solubilisation indices. Among the isolates, AP-01, AP-04, AP-11, AP-30 and AP-34 were multifunctional phosphorus solubilising bacteria (PSB), indicating their role in solubilising both mineral-bound and organic forms of phosphorus, potentially contributing to improve phosphorus bioavailability in soils (Table 2 and Fig. 2). These findings align with earlier reports where the majority of rhizobacterial isolates showed moderate to high phosphorus solubilisation potential (32). The phosphorus solubilisation observed in these isolates can be attributed to the production of organic acids like gluconic, citric, oxalic and lactic acids, which reduce the pH of the surrounding environment and convert insoluble phosphorus into bioavailable forms. Enzymes such as acid phosphatase and phytase also play a significant role in organic phosphorus mineralisation, thereby contributing to the overall solubilisation process (33).

Potassium solubilisation ability of bacterial isolates

Potassium (K) is the key element in many physiological and biochemical systems that define plant health, productivity and resilience. Among the 45 isolates screened, 12 exhibited the ability to solubilise K. Among these, isolate AP-04 demonstrated the highest potassium solubilisation with an SI of 2.80 followed by AP-40 (2.71), AP-26 (2.47) and AP-42 (2.45), which showed considerable potassium-solubilising potential (Table 2 and Fig. 2). These findings are in line with studies that isolated potassium solubilising bacteria (KSB) from the rhizospheric soils of wheat and identified strains like *Bacillus subtilis* and *Bacillus velezensis* with high SI that suggested their ability to convert in-soluble potassium into usable forms in plants (34).

Zinc solubilisation ability of bacterial isolates

Zinc (Zn) is an important micronutrient needed for enzyme functioning, protein synthesis and overall metabolic processes in plants. However, zinc often becomes fixed in alkaline soils and hence its solubilisation is a key characteristic among the PGPR. In this study, 28 isolates exhibited halo zones that showed the presence of zinc solubilisation. The SI values ranged from 1.10 to 2.89. The solubilisation of zinc was highest in AP-39 (2.89), followed by AP-38 (2.82), AP-15 (2.78) and AP-05 (2.77) indicating their high

Table 1. Isolation and characterisation of phosphorous solubilising and mineralising bacteria from different soil samples

Sl No.	Isolate ID	Source of sample	Medium used	Shape	Colour	Texture	Elevation	Margin	Opacity
1	AP-01	Rajendranagar	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Opaque
2	AP-02	Rajgir	Phytate	Irregular	White	Shiny	Raised	Undulate	Opaque
3	AP-03	Nalgonda	Pikovskaya's (DCP)	Circular	White	Smooth	Flat	Entire	Translucent
4	AP-04	Nallamalla	Pikovskaya's (TCP)	Irregular	White	Shiny	Raised	Undulate	Opaque
5	AP-05	Rajendranagar	Phytate	Irregular	Cream	Rough	Flat	Entire	Opaque
6	AP-06	Rajgir	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Translucent
7	AP-07	Nalgonda	Phytate	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
8	AP-08	Nallamalla	Pikovskaya's (DCP)	Circular	White	Smooth	Flat	Entire	Opaque
9	AP-09	Nallamalla Forest	Pikovskaya's (DCP)	Irregular	White	Shiny	Raised	Undulate	Translucent
10	AP-10	Nalgonda	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Opaque
11	AP-11	Nallamalla	Phytate	Circular	White	Smooth	Flat	Entire	Opaque
12	AP-12	Nallamalla Forest	Pikovskaya's (DCP)	Circular	White	Smooth	Flat	Entire	Translucent
13	AP-13	Rajendranagar	Sperber's	Circular	White	Smooth	Flat	Entire	Opaque
14	AP-14	Nallamalla	Pikovskaya's (TCP)	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
15	AP-15	Nallamalla Forest	Phytate	Irregular	White	Shiny	Raised	Undulate	Translucent
16	AP-16	Rajendranagar	Pikovskaya's (DCP)	Irregular	Cream	Rough	Flat	Entire	Opaque
17	AP-17	Rajgir	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Opaque
18	AP-18	Nallamalla Forest	Pikovskaya's (TCP)	Irregular	White	Mucoid	Raised	Undulate	Translucent
19	AP-19	Rajendranagar	Phytate	Irregular	Cream	Rough	Flat	Entire	Opaque
20	AP-20	Rajgir	Lecithin	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
21	AP-21	Nalgonda	Pikovskaya's (DCP)	Irregular	Yellow	Mucoid	Raised	Undulate	Translucent
22	AP-22	Rajendranagar	Pikovskaya's (TCP)	Irregular	Cream	Rough	Flat	Entire	Opaque
23	AP-23	Rajgir	Phytate	Circular	White	Smooth	Flat	Entire	Opaque
24	AP-24	Nalgonda	Lecithin	Circular	White	Smooth	Flat	Entire	Translucent
25	AP-25	Nallamalla	Sperber's	Circular	White	Smooth	Flat	Entire	Opaque
26	AP-26	Rajgir	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Opaque
27	AP-27	Nalgonda	Phytate	Circular	White	Smooth	Flat	Entire	Translucent
28	AP-28	Nallamalla	Lecithin	Circular	White	Smooth	Flat	Entire	Opaque
29	AP-29	Nallamalla Forest	Sperber's	Circular	White	Smooth	Flat	Entire	Opaque
30	AP-30	Nalgonda	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Translucent
31	AP-31	Nallamalla	Phytate	Circular	White	Smooth	Flat	Entire	Opaque
32	AP-32	Nallamalla Forest	Lecithin	Circular	White	Smooth	Flat	Entire	Opaque
33	AP-33	Rajendranagar	Sperber's	Irregular	White	Shiny	Raised	Undulate	Translucent
34	AP-34	Nallamalla	Pikovskaya's (TCP)	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
35	AP-35	Nallamalla Forest	Pikovskaya's (DCP)	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
36	AP-36	Rajendranagar	Lecithin	Irregular	Yellow	Mucoid	Raised	Undulate	Translucent
37	AP-37	Rajgir	Pikovskaya's (DCP)	Irregular	Yellow	Mucoid	Raised	Undulate	Opaque
38	AP-38	Nallamalla Forest	Pikovskaya's (TCP)	Irregular	White	Shiny	Raised	Undulate	Opaque
39	AP-39	Rajendranagar	Pikovskaya's (DCP)	Circular	White	Smooth	Flat	Entire	Translucent
40	AP-40	Rajgir	Lecithin	Irregular	White	Shiny	Raised	Undulate	Opaque
41	AP-41	Nalgonda	Sperber's	Circular	White	Smooth	Flat	Entire	Opaque
42	AP-42	Rajendranagar	Pikovskaya's (TCP)	Circular	White	Smooth	Flat	Entire	Opaque
43	AP-43	Rajgir	Pikovskaya's (DCP)	Irregular	White	Shiny	Raised	Undulate	Opaque
44	AP-44	Nalgonda	Lecithin	Irregular	Cream	Rough	Flat	Entire	Opaque
45	AP-45	Nallamalla	Pikovskaya's (DCP)	Circular	White	Smooth	Flat	Entire	Opaque

Table 2. Screening of phosphorous solubilising and mineralising for different plant growth promoting traits

Sl No.	Isolate ID	Pikovskaya's (DCP) SI	Pikovskaya's (TCP) SI	Sperber's SI	Phytate SI	Lecithin SI	K solubilisation (SI)	Zn solubilisation (SI)	Siderophore (SI)	IAA (µg mL^{-1})	HCN	Ammonia
1	AP-01	2.8	3.2	1.6	2.4	–	–	1.36	–	29.97	++	+++
2	AP-02	3	–	–	2.1	–	–	–	–	–	–	++
3	AP-03	2.5	3.1	–	2.6	–	–	2.42	–	–	–	+
4	AP-04	3.4	3.8	2	2.7	1.9	2.8	–	2.42	24.38	–	+++
5	AP-05	2.1	2.9	1.7	2.3	–	1.31	2.77	–	28.43	–	–
6	AP-06	3.3	3.6	–	2.2	–	–	2.11	1.58	–	–	++
7	AP-07	2.7	–	–	2	–	2	–	–	7.51	–	++
8	AP-08	1.9	–	1.8	–	2.4	–	–	–	12.2	–	–
9	AP-09	3.1	–	2.2	–	–	1.48	–	–	13.46	++	+++
10	AP-10	3.5	3.9	–	–	2.3	–	1.68	–	–	–	+
11	AP-11	1.7	2.6	1.5	2.1	–	–	2.24	–	29.01	–	++
12	AP-12	2	–	–	–	2.6	–	1.62	–	–	–	++
13	AP-13	–	2.9	2.3	–	–	1.93	1.49	1.57		+	–
14	AP-14	3.2	3.7	–	2.8	2.1	–	–	1.52	23.56	–	+
15	AP-15	2.6	–	–	2	–	–	2.78	–	–	+++	–
16	AP-16	–	3.4	–	–	2.5	–	1.89	2.96		–	+
17	AP-17	–	3	1.9	–	–	–	–	–	21.21	–	++
18	AP-18	3.4	3.6	1.4	2.9	–	–	–	1.61	13.7	–	++
19	AP-19	–	2.7	–	2.2	–	–	2.42	–		+	–
20	AP-20	2.5	–	–	–	2.7	–	–	1.38	12.4	++	++
21	AP-21	–	3.1	2.1	–	–	–	1.3	1.42	21.01	–	++
22	AP-22	3.1	3.5	–	2.5	–	–	1.41	1.61	4.21	–	–
23	AP-23	–	2.8	–	2.3	2.4	–	2.13	–	–	–	–
24	AP-24	2	–	–	–	2.2	–	1.33	–	13.91	–	+
25	AP-25	–	3	2.4	–	–	2.17	–	–	–	+	–
26	AP-26	3.3	3.8	–	2.6	2.1	2.47	–	2.45		–	++
27	AP-27	–	2.9	2	2.4	–	1.55	2.25	1.88		–	–
28	AP-28	2.1	–	–	–	2.3	–	–	2.42	23.94	++	++
29	AP-29	–	3.2	2.3	–	–	–	1.77	–	–	–	–
30	AP-30	3.4	3.7	1.7	2.7	1.7	–	1.52	–	33.66	+++	++
31	AP-31	–	2.9	–	2.1	2	–	2.33	2.16	18.21	–	–
32	AP-32	2.4	–	–	–	2.2	–	–	1.56	36.37	–	+
33	AP-33	–	3.3	2.5	–	–	–	2.29	1.22	25.52	+	–
34	AP-34	3.2	3.6	2.4	2.8	1.8	–	1.72	–		–	++
35	AP-35	–	2.8	–	2.1	–	–	–	–	13.24	–	+
36	AP-36	2.3	–	–	–	2.5	1.18	–	–		–	–
37	AP-37	–	3	2.2	–	–	–	1.97	–	–	++	++
38	AP-38	3.1	3.6	–	2.7	–	–	2.82	–	12.28	–	–
39	AP-39	–	2.7	–	2.3	2	–	2.89	–	16.61	+	–
40	AP-40	2.4	–	–	–	2.3	2.71	2.02	1.87	–	–	+
41	AP-41	–	3.1	2.4	–	–	–	1.1	–	–	–	++
42	AP-42	3.5	3.8	–	2.9	1.1	2.45	1.66	3	18.11	–	–
43	AP-43	–	–	–	2.2	2.1	–	–	–	–	–	+
44	AP-44	2.2	–	–	–	2.3	1.59	1.78	–	–	++	–
45	AP-45	–	3	–	2.1	–	–	–	–	15.19	–	–
SEM (±)		0.07	0.08	0.07	0.09	0.08	0.06	0.07	0.05	1.12	–	–
C.D(p≤0.05)		0.023	0.24	0.21	0.27	0.23	0.18	0.2	0.15	3.35	–	–

(–) indicates negative result, Colour intensity high (+++), Colour intensity medium (++), Colour intensity low (+).

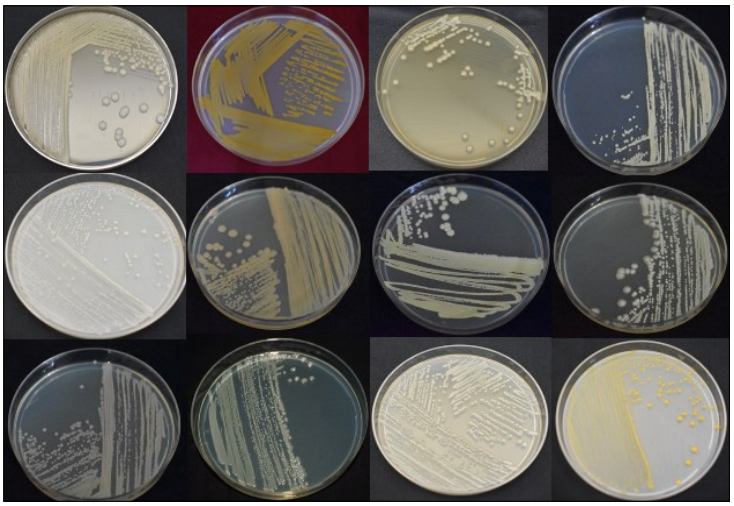

Fig. 1. Purified colonies of phosphorous solubilising and mineralising bacteria.

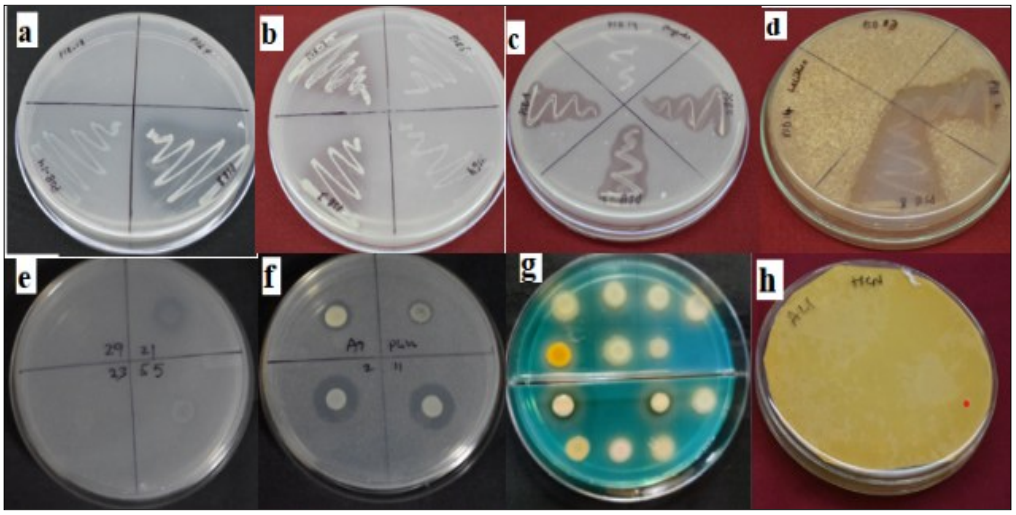

Fig. 2. Screening of bacterial isolates for plant growth promoting traits. (a) Solubilisation of DCP; (b) solubilisation of tricalcium phosphate (TCP); (c) mineralisation of phytate; (d) mineralisation of dicalcium phosphate (DCP); (e) solubilisation of potassium; (f) solubilisation of zinc; (g) siderophore production; (h) hydrogen cyanide production.

Zn solubilising potential (Table 2 and Fig. 2). Several reports on P solubilising rhizobacteria with Zn solubilising ability and their capability of solubilising different zinc compounds and hence enhancing zinc availability to plants were reported (35).

Siderophore production by bacterial isolates

Siderophore synthesis is an important PGP trait which increases iron availability in iron limiting environments. Siderophores chelate Fe^{3+} and transport it into microbial or plant cell, enhancing nutrient uptake and making plant pathogens vulnerable. In this study, 17 isolates exhibited siderophore production ability. Isolates AP-42 (3.00), AP-16 (2.96) and AP-26 (2.45) recorded the highest iron-chelating potential (Table 2 and Fig. 2). These results are consistent with the studies that isolated siderophore producing bacteria in soils (36). The siderophores produced by the bacterial isolates in this study indicates the possibility of these isolates positively affecting plant growth by increasing iron availability.

Indole-3-acetic acid production by bacterial isolates

Indole-3-acetic acid is a key plant hormone involved in root elongation, cell division and nutrient uptake. Many PGPR are known to produce IAA, thus contributing to enhanced plant growth (37). In the present study, 24 isolates had positive IAA production. The most potential IAA producers included AP-01, AP-11, AP-30, AP-31 and AP-32 (Table 2). Several studies have reported the ability of rhizobacteria to produce IAA and also demonstrated their potential to enhance plant growth in rice (38). The ability of the isolates to produce IAA in the study suggests their potential role in enhanced plant growth by promoting root development and nutrient uptake.

Hydrogen cyanide production

Hydrogen cyanide production is a well-documented characteristic of the PGPs, enabling them to inhibit pathogenic fungi by inhibiting the action of cytochrome c oxidase in the respiratory chain. Thirteen of the 45 isolates screened had dark brown colouration on the filter paper, which is a sign of HCN production (Table 2 and Fig. 2). These results are in line with reports that indicated only a subset of rhizobacterial isolates are capable of producing HCN. *Pseudomonas*, *Bacillus* genera have been found to be significant producers of HCN contributing to their effectiveness as biocontrol agent in agriculture (39).

Ammonia production

Ammonia production is a common PGPR trait that contributes to nitrogen availability in the rhizosphere and helps suppress phytopathogens through pH modulation and oxidative stress induction. In the present study, 26 isolates exhibited ammonia production, highest ammonia producing included AP-01, AP-04 and AP-09 (Table 2). Plant growth-promoting rhizobacteria like

Pseudomonas, *Enterobacter*, *Klebsiella* and *Bacillus* are proficient in ammonia production. These bacteria have a role in enhancing nitrogen availability to plants and suppresses phytopathogens through local pH changes and oxidative stress induction in fungi (40).

Selection of potential bacterial isolates

Plant growth-promoting traits were the major categories used for evaluating and scoring bacterial isolates. Each isolate was evaluated for key functional traits, including nutrient solubilisation and mineralisation of diverse phosphorus sources (DCP, TCP, Sperber's medium, phytate and lecithin), potassium and zinc solubilisation, phytohormone production (IAA) and biocontrol traits (siderophore, ammonia and HCN production). A scoring method was adopted to evaluate the PGP traits of bacterial isolates. The scoring included 0 for no detectable activity, 1 for moderate activity, 2 for high activity and 3 for very high activity. Based on cumulative scores, the top-performing isolates were identified. The highest scoring isolates in the PGP trait category were AP-04 and AP-30 with a total score of 21 and 19 respectively, followed by AP-20 and AP-26 with a score of 18 (Fig. 3). These isolates showed consistently strong performance across multiple traits including phosphorus solubilisation, siderophore production and plant hormone synthesis. A similar isolate-based scoring and selection approach was used where the bacterial isolates were evaluated based on PGP attributes to identify strains with potential for plant growth promotion (22).

Compatibility of phosphorous solubilising and mineralising bacterial isolates

Ensuring the compatibility of bacterial isolates is critical when applying PGP bacteria as consortia to enhance plant growth. This test ensures that the isolates can coexist and function synergistically. Based on the above screening tests, a total of 9 best performing bacterial isolates were selected and tested for compatibility. Based on the growth of the isolates, it was observed that there was no inhibition zone and all the isolates were compatible with each other. From the selected isolates, three consortium groups were formed in such a way that each group has 3 isolates C1 (AP-01, AP-34, AP-42), C2 (AP-04, AP-26, AP-30) and C3 (AP-14, AP-18, AP-10) that when combined together have all the PGP traits. A similar compatibility assessment by inoculating a bacterial isolate at the centre of a nutrient agar plate, with other isolates streaked at right angles to the original culture (41). The results showed that all isolates were compatible, as evidenced by the merging of bacterial growth at the intersections. Reports suggest that beneficial interactions between plant-associated bacteria could coexist without antagonistic effects, promoting plant growth when applied together as consortia and improve nutrient uptake and stress resistance in plants (42).

Evaluation of the effect of consortia on germination of rice under *in vitro* conditions

The influence of phosphorous solubilising and mineralising bacterial consortia (C1, C2 and C3) on rice (ISM variety) seed germination, shoot length, root length was assessed under controlled *in vitro* conditions (Fig. 4). Among the treatments, consortium C2 recorded the highest germination percentage (100 %), along with the greatest root length (9.45 ± 0.31 cm), shoot length (6.70 ± 0.22 cm) and total seedling length (16.16 ± 0.52 cm). Vigour index I (1616 ± 42.6) and vigour index II (5.47 ± 0.19) were also highest in C2, indicating a significant improvement over the control. The percentage increase in seedling length, germination percentage, vigour index I and II is presented in Fig. 5. C1 also showed enhanced results, C3 showed slightly lower performance compared to C1 and C2 but higher than the control. The improved seed germination and seedling vigour observed in the consortia-treated seeds, especially, can be attributed to the synergistic interactions among the microbial members of the consortium. These bacteria likely possess diverse PGP traits such as

Table 3. Identity of phosphorous solubilising, mineralising and accumulating bacterial isolates (16S rRNA gene sequencing)

S. No	Isolate ID	Identified strain	Accession number
1	AP-4 (IIRRPA25-1)	*Bacillus* sp.	PV639401
2	AP-26 (IIRRPA25-2)	*Priestia megaterium*	PV639408
3	AP-30 (IIRRPA25-3)	*Bacillus subtilis*	PV639451

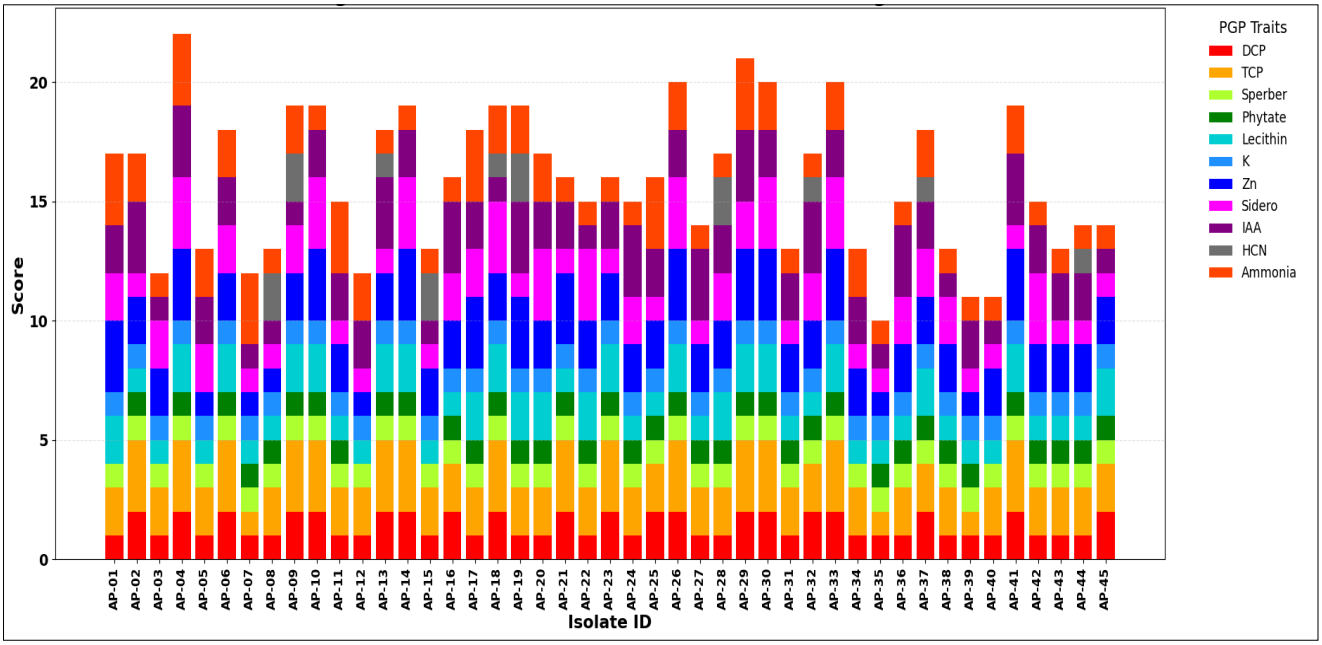

Fig. 3. Scoring of phosphorous solubilising and mineralising bacterial isolates based on plant growth promoting traits.

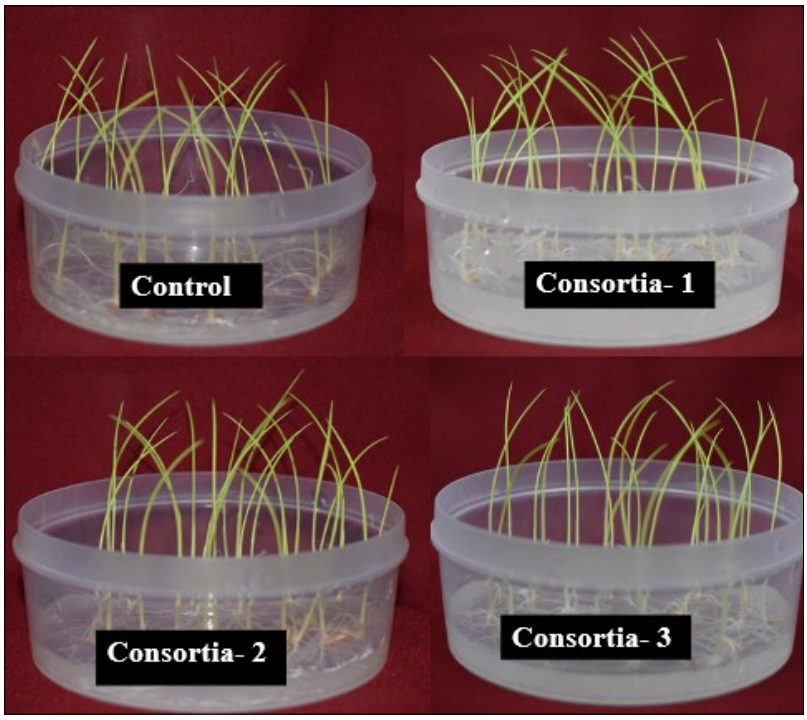

Fig. 4. *In vitro* germination assay of rice seeds with un-inoculated (control) and inoculated (C1, C2, C3) treatment.

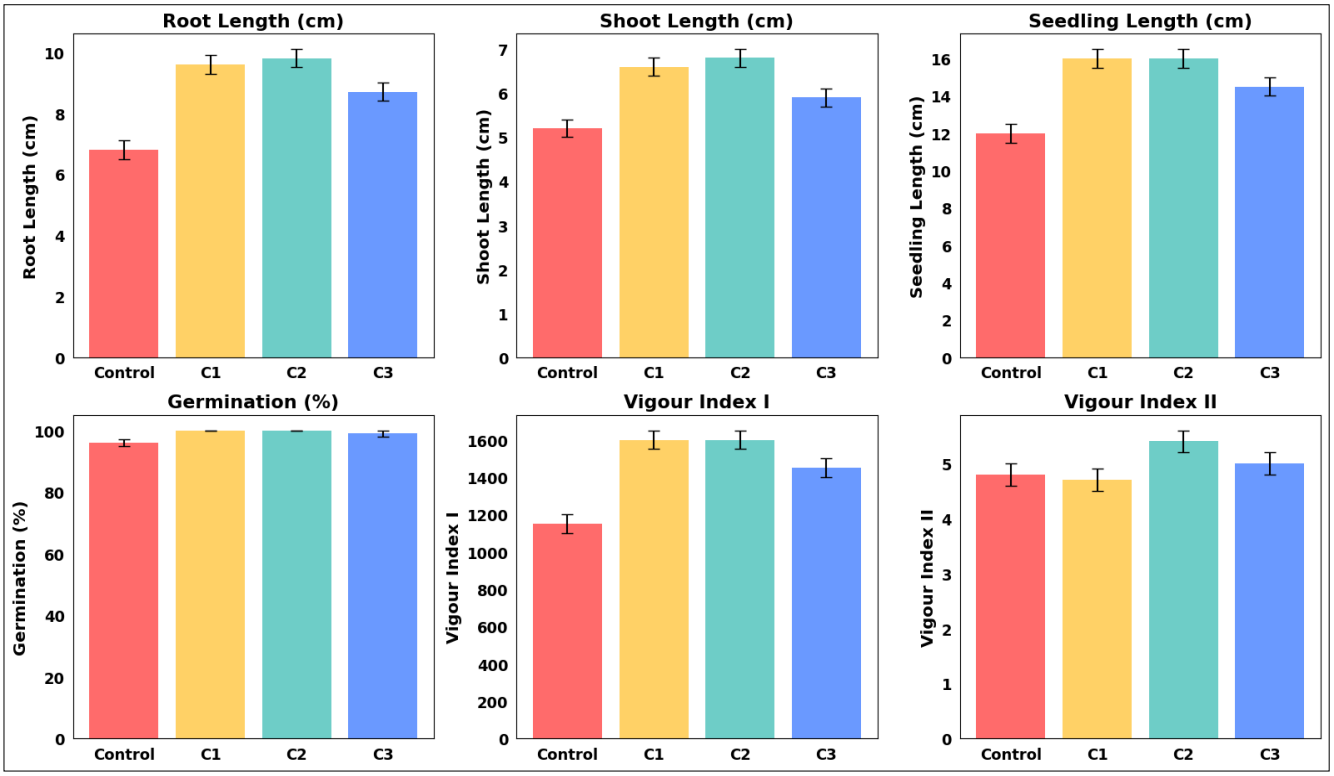

Fig. 5. Effect of bacterial consortia inoculations on growth parameters of rice under *in vitro* conditions.

phosphorus solubilisation, phytohormones production like IAA and siderophore production all of which contribute to enhanced seedling development. Similar enhanced rice growth parameters under *in vitro*, greenhouse and field trials when treated with rhizobacterial consortia (43, 44).

Molecular identification of bacteria isolates

Identification based on 16S rRNA revealed that the consortial partners in C2 i.e., A4 (IIRRPA25-1) has similarity with *Bacillus* sp., A26 (IIRRPA25-2) showed similarity with *Priestia megaterium*, while A30 (IIRRPA25-3) showed similarity with *B. subtilis* (Table 3). *Bacillus* species play a crucial role in enhancing rice productivity through multiple mechanisms, including nutrient solubilisation, biotic or abiotic stress mitigation and phytohormone production (45). *Priestia megaterium* and *B. subtilis* in addition to their well-known P solubilising activity has been demonstrated to improve rice growth under drought conditions by enhancing nutrient uptake and inducing systemic tolerance (46). Several studies indicate that different *Bacillus* strains including *Bacillus* sp., *P. megaterium* and *B. subtilis* exhibit distinct yet complimentary PGP traits such as P solubilisation and production of siderophore which collectively enhance soil fertility and rice resilience (47). The present study

had demonstrated that the isolates *Bacillus* sp., *P. megaterium* and *B. subtilis* in the consortium function synergistically to improve rice seed germination and seedling growth.

Conclusion

The present investigation isolated 45 phosphorus solubilising and mineralising bacteria which were screened for PGP traits. Among the 45 isolates screened, several exhibited multifaceted PGP activities including P solubilisation and mineralisation, K and Zn solubilisation and production of siderophores, IAA, ammonia and HCN. Based on scoring, nine highly efficient strains were further grouped into three compatible consortia. The *in vitro* evaluation of the consortia revealed that C2 was the most effective in enhancing plant growth in rice when compared with other consortia and control treatments. The bacterial partners in C2 were further identified as *Bacillus* sp., *P. megaterium* and *B. subtilis*. These findings suggest that phosphorus-solubilising and mineralising PGP bacterial consortia play an important role not only in mobilising soil phosphorus but also in enhancing rice seedling growth, highlighting their potential application as bioinoculants for sustainable rice cultivation.

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Authors' contributions

PA performed sample collection, laboratory work and wrote the original draft. PCL initiated the idea, performed analysis and supervised the experimental process. PCL, SBG, TC and RS were responsible for conceptualisation and revised the manuscript. All the authors reviewed the findings and endorsed the final draft of the paper. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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