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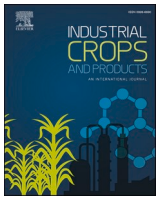

Exploring the potential of P solubilizing rhizobacteria for enhanced yield and quality in turmeric (*Curcuma longa* L.)

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A B S T R A C T

The study focused on isolation and characterization of a bevy of phosphorus solubilizing bacteria (PSB) and exploring the effects of the most promising PSB on yield and quality of turmeric. For this purpose, several bacterial strains isolated from the rhizosphere of turmeric grown across India were assessed for their capacity to solubilize tri-calcium phosphate (TCP) and di-calcium phosphate (DCP) under *in vitro* condition and in liquid medium. The soluble P turnover by the promising PSB was then investigated in soil *per se* and their effects were further validated in turmeric under both green house and field conditions for two successive years. The results revealed that *Bacillus safensis* (NCBI-MT192800), *B. marisflavi* (NCBI-MT 192801), *B. cereus* (NCBI- MT192803) and *Pseudomonas aeruginosa* (NCBI-MZ 540872) released significantly greater levels of labile P in the liquid medium due to their capacity to produce appreciable amounts of organic acids (gluconic, α ketogluconic, succinic, oxalic and tartaric). Green house studies on turmeric indicated that soil available P was significantly higher in the treatments involving PSB suggesting enhanced P solubilization and the treatment with *B. safensis* + 75 % P as rock phosphate (RP) increased rhizome yield by 129.0 % compared to the treatment with 100 % P applied as RP. The positive effects of PSB were also reflected in field experiments, wherein the treatments with combined application of *B. safensis* with 50 % or 75 % or 100 % P significantly increased soil available P by 143.0 – 246.0 %, rhizome yield by 29.0 – 120.0 %, P uptake by 51.0 – 223.0 % and curcumin content by 30.0 – 32.5 % compared to control (100 % P as RP). The use of *B. safensis* ensured 25.0 % reduction in exogenous inorganic P application while simultaneously enhancing turmeric yield and quality in P deficient soil.

1. Introduction

Turmeric (*Curcuma longa* L.; Family: Zingiberaceae) is an important spice crop cultivated in many tropical regions of the world like India, Japan, Korea, Philippines, Malaysia, Myanmar, Sri Lanka, Thailand, Vietnam, China, Central America and the Caribbean Islands. It is a rhizomatous crop with great demand in global food, pharmaceuticals and preservatives industry (Jabborova et al., 2021). Besides carbohydrates (69.4 %), fat (5.1 %), protein (6.3 %) and minerals (3.5 %), the major constituents of the rhizomes are volatile oil (5.0–6.0 %), oleoresin (7.9–10.4 %), curcumin or diferuloylmethane (2.5–6.0 %) consisting of curcumin I or curcumin (94.0 %), curcumin II or demethoxycurcumin (6.0 %) and curcumin III or bis-demethoxycurcumin (0.3 %). Turmeric is well known for its antiseptic, antidiabetic, antimutagenic, anticarcinogenic, antioxidant, antidepressant and chemotherapeutic properties, besides being anti-inflammatory, anti-tumor forming and anti-malarial (Chempakam and Parthasarathy, 2008).

Turmeric is grown in a wide range of soils and requires ample supply of organic manures and nutrients for profitable yield. The maximum nutrient uptake by the crop occurs during the active growth phase (fourth and fifth month), suggesting that expedited application of nutrients especially nitrogen (N), phosphorus (P), and potassium (K) is critical (Srinivasan et al., 2000). Among these three, P is a major component in key metabolic pathways like nutrient uptake, biological oxidation, and energy metabolism (Nesme et al., 2018) and severely limits turmeric growth and yield in deficient soils. The mean total P content in soil is 0.05 % (w/w), of which only 0.1 % is available to plants (Lambers and Plaxton, 2018) owing to poor solubility, precipitation and fixation reactions. Consequently, such pervasive P deficiency has reduced yields by ~30 % around the world, and 30–40 % of arable soils in the world are P deficient (von Uexküll and Mutert, 1995). Application of inorganic fertilizers appears to be the most common strategy to

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counter P deficiency in soil and approximately 52.3 b tons of P fertilizers are applied every year to maintain optimum P levels in soil–plant systems (FAO, 2017). However, out of this only about 20.0 % is used by crop plants (Alori et al., 2017) and 80.0–90.0 % is once again converted to insoluble forms by precipitation and fixation reactions (Trolove et al., 2003) warranting exogenous P application to crops. However, numerous studies have reported that repeated application negatively impacts soil quality and lessens P availability (Bhattacharyya et al., 2015). Also, heavy application of inorganic P fertilizer causes a cascade of problems, like enhanced production cost, rapid exhaustion of nonrenewable P resources and eutrophication (Cordell et al., 2009). Therefore, a sustainable approach would be to exploit the intrinsic capacity of certain group of microorganisms to convert insoluble P compounds (Ca-P, Al-P, Fe-P *etc.*) to readily available forms. Also, insoluble inorganic phosphates and insoluble/soluble organic phosphates especially phytate constituting 80.0 % of soil P require P solubilizing bacteria (PSB) to transform into plant available forms (Wan et al., 2020). Some of these include bacteria belonging to the genera *Pseudomonas*, *Agrobacterium* sp., *Bacillus* sp., *Thiobacillus* sp., *Burkholderia*, *Enterobacter*, *Paenibacillus* sp., *Gluconacetobacter* sp., *Acinetobacter* sp., etc (Alori et al., 2017). Considering the fact that P bioavailability is directly correlated to the capacity of soil system to supply P to the target crops, these special group of bacteria/PSB assume great importance for sustainable yield in P limited soils (Zhang et al., 2021). Some authors have reported that insoluble P can be dissolved by low molecular weight organic acids and also by extracellular enzymes (*e.g.*, phosphatase and phytase) synthesized and secreted by PSB (Tan et al., 2013). Microbial P solubilization through secretion of low molecular weight organic acids involve acidification, chelation and exchange reactions (Delvasto et al., 2009). The organic acids generate protons and temporarily decrease the soil pH to as low as 2 (Illmer and Schinner, 1995; Pérez et al., 2007) forming a P -rich micro niche. Besides, these acids produce anions that form complexes with positively charged ions like Al^{3+}, Ca^{2+}, Fe^{3+} etc, thereby releasing the P fixed by these ions (Mardad et al., 2013). Another mechanism suggested is competition with PO_4^{3-} ions for absorption sites, thereby enhancing their availability in soil (Mander et al., 2012).

Though studies on nutrient management regimes in turmeric are available (Srinivasan et al., 2016), there are very few studies that have focused on PSB for enhancing the soil available P pool for enhanced yield and quality of turmeric. Also, there are very few studies that have focused on the nature and quantity of organic acids secreted by these PSB. Therefore, the present study focused on testing rhizobacterial strains isolated from major turmeric growing tracts of India for their capability to solubilize soil P. The most promising PSB were then evaluated for their effects on yield and quality of turmeric, both in the green house and field for two successive years. The major objective of the study was to evaluate the most promising PSB for their effects on the growth and yield of turmeric (*C. longa*). Another important objective was to discern the effects of PSB on the quality of turmeric rhizomes measured in terms of P uptake, curcumin, volatile oil and oleoresin content. A secondary objective was to elucidate the type and quantity of organic acids secreted by PSB and to determine the inter-relationships between organic acid secreted, the type of insoluble P compounds and P released. We hypothesized that organic acids secretion will vary with the nature of P compounds in the immediate vicinity of the bacteria and apart from gluconic acid, other organic acids were also crucial for P solubilization. Consequently, this would affect the rate of P mineralized in soil and subsequently the yield and quality of turmeric. Apparently, the focus was to evolve a robust P solubilizing rhizobacterial strain with positive impacts on yield and quality for integration into environmentally sound and efficient nutrient management plans for turmeric cropping in P deficient soil systems.

2. Materials and methods

2.1. Soil sampling and isolation of bacteria

Soil samples were collected from the rhizosphere of turmeric from different states of India *viz.*, Kerala, Karnataka, Tamil Nadu, Andhra Pradesh state and Telangana. Details on sampling points are described in Praveena et al. (2022). Rhizobacteria were isolated using the serial dilution technique (up to 10^{-10}). The suspension from different dilutions was pour-plated in nutrient agar medium and incubated at 28.0 ± 2.0 °C for 2–4 days. The isolated rhizobacteria were tested for P solubilization by plate assay using Pikovskaya's and NBRIP media (Nautiyal, 1999) and the colonies were selected on the basis of the development of a clear halo zone around the bacterial colony (Chen and Liu, 2019). Selected isolates were sub-cultured and maintained on Nutrient agar slants. All the isolates were also stored in 40.0 % glycerol stocks and maintained at − 20.0 °C.

2.2. Qualitative study for P solubilization

P solubilization capacity of the selected bacterial isolates was tested by using the medium specified by Sahu and Jana (2000). The medium (glucose-10.0 g, $Ca_3(PO_4)_2$-5.0 g, $(NH_4)_2SO_4$-0.50 g, KCl-0.30 g, $MgSO_4 \cdot 7\ H_2O$ − 0.30 g, $MnSO_4 \cdot 4\ H_2O$- 0.030 g, $FeSO_4 \cdot 7\ H_2O$- 0.030 g, NaCl-0.30 g, yeast extract powder-0.50 g, agar-20.0 g, distilled water-1000 mL, pH 7.0–7.5) was autoclaved and poured into sterile Petri plates. One loop full of the 48.0 h old bacterial culture was spot inoculated on the Petri plate and incubated at 26.0 ± 2.0 °C for 7 days. The plates showing halo zone of clearance around the bacterial colony indicated P solubilization. Four replicates were maintained for each culture. The P solubilization efficiency was expressed as the zone of clearance (mm) around the bacterial colony.

2.3. Quantitative study for P solubilization

2.3.1. Liquid medium

Four bacterial isolates *Bacillus safensis* (NCBI-MT192800), *B. marisflavi* (NCBI-MT 192801), *B. cereus* (NCBI- MT192803) and *Pseudomonas aeruginosa* (NCBI-MZ 540872) that exhibited maximum solubilization efficiency in the qualitative study were selected for the quantitative study along with two non solubilizers [*Phytobacter diazotrophicus* (NCBI-MT 192799) and *C. firmus* (NCBI- MZ 540873)] (Table S1). For quantitative study, modified NBRIP broth (Nautiyal, 1999) consisting of glucose − 10.0 g; $MgCl_2\ 6\ H_2O$ – 5.0 g; $MgSO_4\ 7\ H_2O$ - 0.25 g; KCl - 0.20 g and $(NH_4)_2SO_4$ - 0.10 g; distilled water- 1.0 L and supplemented with either $CaHPO_4$ or $Ca_3(PO_4)_2$ @ 1000 mg P L^{-1} was used. Exactly 10.0 mL of the medium was taken in 30.0 mL screw cap vials and autoclaved (121 ℃ for 20 min). To this medium 1.0 mL suspension of 24 h grown shortlisted bacterial culture was transferred and kept in an incubator shaker (26.0 ± 2.0 °C) at 180 rpm. On 1st day after incubation (DAI), the mixture was transferred aseptically to centrifuge tubes and centrifuged at 10,000 rpm for 20 min at 4.0 °C. Then, the supernatant was sequentially filtered using Whatman No. 1 & 42 and collected in test tubes. After filtration, P in 2.0 mL of the filtrate was determined by the ascorbic acid method (Kuo, 1996). Uninoculated vials with only P source served as control and six replicates were maintained for each treatment. The assay was repeated for further time intervals *viz.*, 2nd day, 3rd day, 5th day, 10th day, 15th day, 20th day and 25th DAI. Since P release by the shortlisted bacteria was found to decrease beyond 10 DAI (Fig. 1), the study was terminated on the 25 DAI. An identical set for each treatment for each sampling day was maintained to measure the pH.

2.3.2. Soil medium

This study was conducted with the promising P solubilizers to study the solubilization pattern of P in soil *per se*. P was added as $Ca_3\ (PO_4)_2$

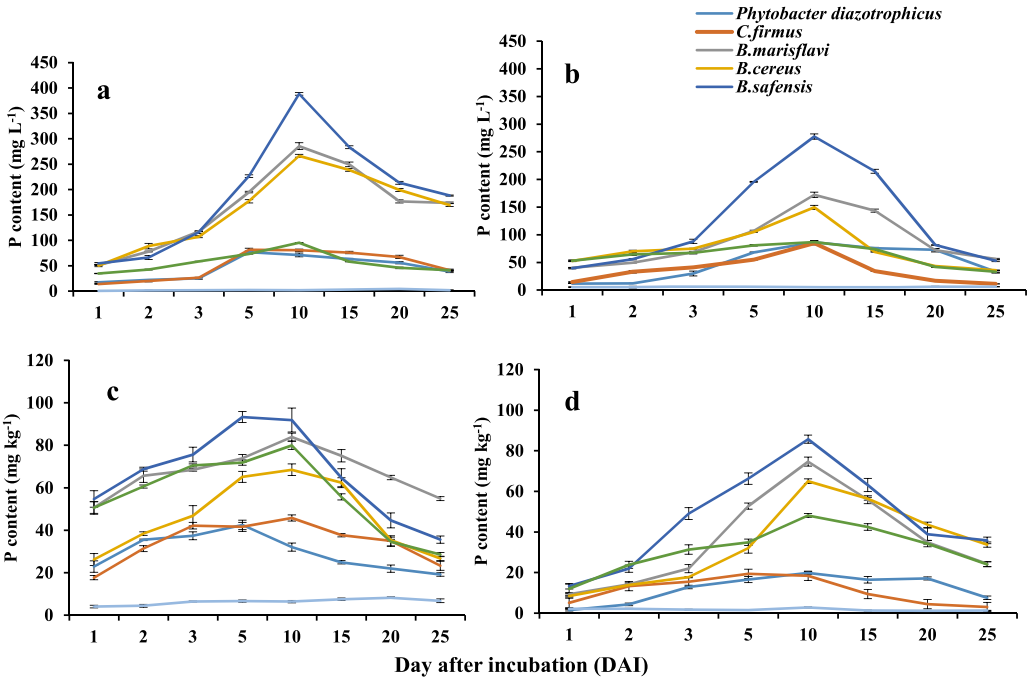

Fig.1. P content in (a) liquid medium added with di-calcium phosphate (DCP) (b) liquid medium added with tri-calcium phosphate (TCP) (c) soil amended with DCP and (d) soil amended with TCP at different days after incubation; Vertical bar at each data point indicates standard error.

(TCP) or $CaHPO_4$ (DCP) @ 1000 μg g^{-1} to 1000 g soil, mixed well and 5.0 g of this mixture was transferred to 30 mL glass vials. The P spiked soils in glass vials were saturated with distilled water and then autoclaved at 121 °C for 20 mins. To the sterilized mixture 1.0 mL of 24 h grown culture of bacterial isolate was inoculated and the vials were maintained at room temperature (26 ± 2 °C) and field capacity moisture regime. A similar set of vials with soil + $Ca_3(PO_4)_2$ or $CaHPO_4$ at similar rate, but without bacterial isolate was maintained as control. Available P was estimated on 1st, 2nd, 3rd, 5th, 10th, 15th, 20th and 25th DAI by destructive sampling. Six replicates were maintained for each treatment for each sampling day. The soil used was low in available P (0.91 mg kg^{-1}) with an initial pH of 5.5. An identical set encompassing each treatment for each sampling day was maintained to measure the pH. For measuring the P solubilization efficiency of the bacteria in liquid and soil, the peak P release levels across different DAI were considered. The P solubilization efficiency (%) of the bacteria was calculated as [(Solubilized P by bacteria – (Solubilized P in control)/ Total P added] x 100.

2.4. P release kinetics in soil

The P solubilization potentials in soil (P_o) were estimated using the Exponential model (Stanford and Smith, 1972), which assumes a first order kinetic equation to describe the net P solubilization with time. The cumulative soluble P in the soil and the soluble P at each DAI was used to determine the net solubilization over time using the single exponential (non-linear) expression:

$$P_{cum} = P_o\ [(1\text{-}\exp\ (\text{-}kt))]$$

Where, P_{cum} is the cumulative P solubilized (mg kg^{-1} soil) with time (t) in days, P_o is the potential P solubilization (mg kg^{-1} soil), and k is the solubilization rate constant (mg day^{-1}). Equations were fitted with each data point for every time interval and treatments and parameters were estimated using nonlinear curve fitting *via* Marquardt iteration. The net solubilization rate at any time (t) is the first derivative of the resulting regression line for the above equation.

2.5. Estimation of organic acids

Maximum P release in liquid medium was observed on the 10th DAI, irrespective of the PSB strain and the type of insoluble substrate used. Hence, assay for organic acids was determined on the 10th DAI. The shortlisted bacterial isolates were grown separately in 10.0 mL NBRIP broth (Nautiyal, 1999) in screw cap vials. The liquid medium was supplemented separately with TCP and DCP at 1000 mg L^{-1}. For each bacterial treatment and P substrate three replicates were maintained along with an un inoculated control. The inoculated vials were maintained at 26.0 ± 2.0 °C for 10.0 days at 180 rpm in an incubatory shaker. After 10 days of incubation, the media with bacteria was transferred aseptically into centrifuge tubes and centrifuged at 10,000 rpm for 20.0 min at 4.0 °C. Then 5.0 mL of the supernatant were pipetted out and double filtered by passing through Whatman1 filter paper and the filtrate thus obtained was collected in 10.0 mL screw cap vials. For HPLC analysis double filtered supernatant was made cell-free by using 0.45 μm PTFE membrane syringe filters. The cell free filtrate (20.0 μL) was then used for HPLC analysis.

Detection and quantification of organic acids was done on High-Performance Liquid Chromatogram equipped with SPD-M30A (Photodiode Array Detector), LC-20AP preparative pump, Shimadzu CBM-20A/CBM-20Alite system controller and Discovery® HS C18 HPLC column with 5 μM particle sizes and L x ID 25 cm × 4.6 mm. The mobile phase used for the analysis was 0.1 % ortho-phosphoric acid with a gradient flow rate (HPLC elution-profile program: Time 0–8 min - flow rate (0.40 mL/min), 8–14 min - flow rate (0.50 mL/min), 14–25 min - flow rate (1.20 mL/min). Eluates were detected at λ 210 nm and peak area and retention time of samples were compared with those of standards of organic acids (Vyas and Gulati, 2009). The organic acids were quantified by reference to the peak areas obtained for the authentic standards of citric, oxalic, gluconic, α ketogluconic acid, lactic, malic, malonic, succinic, tartaric, fumaric, formic and ascorbic acids. Each replicate was analyzed in a single run on HPLC for six bacterial isolates and two insoluble substrates of P.

2.6. Green house evaluation of promising PSB for soil P solubilization and growth promotion in turmeric

This study involved three shortlisted PSB *viz.*, *B. safensis, B. cereus* and *B. marisflavi*. The isolates were applied as single inoculum or combined with chemical P applied as rock phosphate (RP) at three levels *viz.*, 0 %, 75 % and 100 % of recommended dose (RD). Sparingly soluble RP containing 18.0 % total $P_2 0_5$ was used a P source because it is the common P fertilizer used for turmeric in acid soils (Srinivasan et al., 2000). The treatments adopted in the study are described in Table 5. The treatments were set in a completely randomized design with 12 replicates. For the study, earthen pots (capacity 15.0 kg) were filled with 12.0 kg sieved soil (Sandy clay loam Ustic Humitoprept; < 2.0 mm; 0.97 mg kg^{-1} Bray P) and sand in the ratio 1:1 for treatments without P. The recommended dose of P for turmeric is 50 kg ha^{-1} (Srinivasan et al., 2016). For treatments involving two different doses of P, the pots were filled initially with 7.0 kg soil. The two P doses were then prepared by mixing RP @ 6.25 g per 10 kg soil: sand mixture (100 % P) and @ 4.68 g RP per 10 kg soil: sand mixture (75 % P) and from this mixture, 5.0 kg was used to fill the remaining portion of pots for obtaining 100 % and 75 % P treatments, respectively. The seed rhizomes (~ 25–30.0 g; variety IISR- Prathibha with minimum two sprouts) were washed with sterile water, air-dried and planted in shallow pits at 3.0–3.5 cm depth and later covered with soil: sand mixture. For treatments involving bacteria, the rhizomes were soaked for 1.0 h in the bacterial suspension (1×10^7 CFU mL^{-1}) containing 1.0 % starch solution and shade dried prior to planting. Bacteria was also applied by drenching @ 1.0 L pot^{-1} (10^7 CFU mL^{-1}) at 30, 60 and 90 days after planting (DAP). For preparation of bacterial cultures, the freshly grown culture was inoculated in 250 mL nutrient broth and incubated in a rotary shaker (150 rpm) at 26 ± 2℃ for 48 h. After incubation, cells were harvested by centrifugation at 3000 rpm for 20 min and re-suspended in sterile distilled water to give a final concentration 10^7 CFU mL^{-1}. For mixed inoculation, an equal volume containing 10^7 CFU mL^{-1} of each bacterium was mixed (1:1) and used for drenching. Observation on sprouting was recorded 20 days after planting (DAP) and plant growth parameters like, number of tillers, shoot length, number of leaves, dry weight of shoot and root were recorded at 60 DAP by destructive sampling. Soil samples were also collected on 60 DAP. The rhizome yield pot^{-1} was recorded after the harvest of the crop at 240 DAP. The greenhouse experiment was conducted twice (2019 & 2020) to confirm the results.

2.7. Field experiments to evaluate promising PSB for soil P solubilization and growth promotion in turmeric

The field experiments were conducted at the Experimental Farm (11°35' N 75°49' E) of the ICAR- Indian Institute of Spices Research (IISR) Kozhikode, Kerala State, India. The experiment was initiated in May 2020 and was repeated in 2021. Due to nutrient exhaustive nature of turmeric, the experiment was not repeated in the same area but was taken up in a new site within the same location. The crop was grown on raised beds of size 3 × 1 × 0.30 m (l × b × h) and a spacing of 40 cm was maintained between the beds. The soil of the study site is a clay loam Ustic Humitropept. The initial properties of soil are pH – 5.12; organic C – 14.2 g kg^{-1}; mineral N – 105 mg kg^{-1}; Bray P – 1.34 mg kg^{-1}; Exchangeable K – 164 mg kg^{-1}. Seed rhizomes (20–30 g) of turmeric (variety: IISR Prathibha) with sprouts were planted in shallow pits with a spacing of 20 × 25 cm with a plant population of 40 bed^{-1}. Treatment details are given in Table 6. Farmyard manure (FYM) was given @ 15 kg bed^{-1} to all the treatments since turmeric is a heavy feeder of nutrients and application of FYM is imperative before sowing. Similar to greenhouse trials, RP containing 18.0 % total $P_2 0_5$ was used as the P source. Only the best PSB *viz.*, *B. safensis* was included for field experimentation and was given both as seed treatment and as soil drench. In case of seed treatment, prior to sowing, the turmeric rhizomes were treated with 1.0 % starch solution containing the *B. safensis* suspension (10^8 cfu mL^{-1}) for one hour, shade dried for 24 h and planted. In case of soil drench, the suspension (10^8 cfu mL^{-1}) was applied at the rate of 2 L bed^{-1} at 45 and 90 DAP. Observations on germination was recorded 45 days after planting (DAP) and on plant growth parameters like number of tillers, shoot length, number of leaves, dry weight of shoot and root were recorded 90 DAP by destructive sampling. Soil samples were also collected from each treatment at 90 DAP and available P was estimated using the Bray method. The rhizome yield (Mg ha^{-1}) was recorded after the harvest of the crop at 240 DAP and expressed as dry weight (Table 6).

Table 1
P release kinetics mediated by PSB in soil spiked with Tri-calcium phosphate and Di-calcium phosphate.

PSB isolates	Tri-calcium phosphate			Di-calcium phosphate		
	P_0^a (mg kg^{-1})	Rate constant (mg d^{-1})	R^2	P_0 (mg kg^{-1})	Rate constant (mg d^{-1})	R^2
P. diazotrophicus	15.62	0.37	0.58*	30.3	1.83	NS
C. firmus	11.76	1.18	NS	37.6	0.89	0.44*
B. marisflavi	47.57	0.34	0.47*	70.5	1.28	0.40*
B. cereus	48.96	0.24	0.71*	51.3	0.78	0.27*
B. safensis	57.74	0.46	0.48*	67.9	1.78	NS
P. aeruginosa	37.37	0.52	0.62*	57.3	2.31	NS
Control	1.77	24.2	NS	7.2	0.64	0.78*

[a]P_0- P mineralization potential (mg kg^{-1}); * indicates significance at $P < 0.05$

2.8. Estimation of plant P concentration and quality parameters

The plant samples were drawn at 60 DAP (Green house experiments) and rhizome samples were drawn during harvest (field experiments) and oven-dried at 60∘C. The dried samples were then ground to pass a 0.5 mm sieve using a Wiley mill. These samples were digested with triacid mixture (HNO_3: H_2SO_4: $HClO_4$ in the ratio of 9:2:1) and total P was estimated using the vanadomolybdate method (Jackson, 1973). To estimate curcumin content, the rhizomes were first washed thoroughly in running water, cooked in boiling water for one hour and sun dried to 8.0 % moisture. These rhizomes were then ground to powder (0.5 mm) and curcumin (sum of curcumin I–III) was extracted using EtOH and absorbance measured (425 nm) using a spectrophotometer (ASTA, 1997a). Oil was extracted by the modified Clevenger method (ASTA, 1997b) and oleoresin was measured by cold percolation with acetone and calculated gravimetrically (AOAC, 1975).

2.9. Estimation of soil physio-chemical and microbial parameters

Soil pH was measured in a 1:2.5 soil: water suspension and available P was estimated using the Bray method (Kuo, 1996). Soil microbial biomass (SMB) measured in terms of soil microbial–C (MBC), –N (MBN) and –P (MBP) in the soil were determined by fumigation-extraction (Vance et al., 1987) using kEC of 0.45 (Joergensen, 1996), kEN of 0.54 (Joergensen and Mueller, 1996) and kEP of 0.40 (Brookes et al., 1982), respectively. Dehydrogenase was assayed using 2,3,5-triphenyltetrazolium chloride as the substrate (Casida et al., 1964) and acid phosphatase (AcP) using p-nitrophenyl phosphate as the substrate (Tabatabai and Bremner, 1969).

2.10. Statistics

One- way ANOVA was used to determine the significance of treatment effects and in case the F values were significant, *post hoc* comparisons of means were made using the Least Significance Test (LSD). Simple correlations between two parameters were determined using Pearson's test. The inter-relationships between total solubilized P, pH and concentration of organic acid in the liquid medium amended with TCP and DCP were determined using Principal component analysis

Table 2
Organic acid production (μg mL^{-1}) by PSB, P released (μg mL^{-1}) and pH of the liquid medium spiked with tri -calcium phosphate on 10th day of incubation.

	B. safensis	*P. aeruginosa*	*B. cereus*	*B. marisflavi*	*P. diazotrophicus*	*C. firmus*	*Control*
Citric acid	120.8 ± 7.37	ND[a]	ND	ND	ND	ND	ND
Oxalic acid	227.0 ± 6.0	204.8 ± 6.7	285.4 ± 8.2	222.9 ± 4.2	65.3 ± 5.5	105.0 ± 0.7	ND
Gluconic acid	824.7 ± 19.2	449.2 ± 3.7	409.8 ± 4.7	751.9 ± 23.5	232.5 ± 2.81	159.5 ± 13.6	ND
α Ketogluconic acid	301.4 ± 14.70	254.8 ± 8.6	208.9 ± 5.2	238.2 ± 14.7	125.0 ± 5.7	131.7 ± 17.2	ND
Lactic acid	287.1 ± 7.8	118.0 ± 5.0	ND	ND	52.9 ± 7.0	110.4 ± 2.0	ND
Malic acid	ND	ND	102.3 ± 28.5	ND	ND	ND	ND
Malonic acid	ND	ND	ND	ND	ND	ND	ND
Succinic acid	212.7 ± 24.7	342.8 ± 12.9	403.9 ± 24.3	520.1 ± 31.9	ND	79.7 ± 4.2	ND
Tartaric acid	163.8 ± 18.5	203.5 ± 7.9	136.13 ± 7.0	133.6 ± 9.6	85.4 ± 4.0	108.9 ± 5.4	ND
Fumaric acid	ND	ND	ND	ND	ND	ND	ND
Formic acid	ND	ND	42.61 ± 20.0	42.63 ± 3.37	ND	ND	ND
Ascorbic acid	ND	ND	ND	ND	ND	ND	ND
Total acids	2336.1 ± 16.5	1612.4 ± 4.9	1546.4 ± 10.3	1909.4 ± 11.4	561.17 ± 4.1	695.2 ± 1.4	ND
P released	277.5 ± 4.9	86.8 ± 2.7	149.5 ± 3.5	172.3 ± 4.7	86.4 ± 2.3	85.0 ± 0.8	5.2 ± 0.4
pH	3.72 ± 0.57	5.20 ± 0.28	4.15 ± 0.02	4.28 ± 0.14	5.09 ± 0.15	5.19 ± 0.29	7.15 ± 0.01

[a] ND- Not detectable

Table 3
Organic acid production (μg mL^{-1}) by PSB, P released (μg mL^{-1}) and pH of the liquid medium spiked with di-calcium phosphate on 10th day of incubation.

	B. safensis	*P. aeruginosa*	*B. cereus*	*B. marisflavi*	*P. diazotrophicus*	*C. firmus*	*Control*
Citric acid	ND[a]	ND	ND	ND	ND	ND	ND
Oxalic acid	179.6 ± 5.9	170.4 ± 9.6	171.6 ± 11.4	80.1 ± 1.9	394.8 ± 20.3	236.3 ± 30.0	ND
Gluconic acid	704.8 ± 10.2	248.6 ± 1.6	554.2 ± 25.8	440.7 ± 22.4	260.2 ± 7.0	212.5 ± 6.0	ND
α Ketogluconic acid	482.7 ± 2.7	464.7 ± 26.0	269.5 ± 13.6	279.3 ± 5.1	141.0 ± 15.6	107.9 ± 3.3	
Lactic acid	105.3 ± 18.8	ND	ND	ND	37.8 ± 1.7	ND	ND
Malic acid	ND	ND	ND	37.4 ± 0.6	ND	ND	ND
Malonic acid	ND	ND	ND	ND	ND	ND	ND
Succinic acid	807.5 ± 23.7	382.6 ± 16.4	1221.4 ± 11.5	785.4 ± 15.1	ND	278.7 ± 19.1	ND
Tartaric acid	151.8 ± 9.1	332.8 ± 19.5	123.3 ± 8.4	138.2 ± 3.05	74.1 ± 5.2	118.5 ± 2.2	ND
Fumaric acid	ND	ND	ND	ND	ND	ND	ND
Formic acid	ND	ND	ND	35.06 ± 1.72	ND	ND	ND
Ascorbic acid	ND	ND	ND	ND	ND	ND	ND
Total acids	2431.7	1599.1	1758.7	2377.39	1036.35	987.12	ND
P released	388.5 ± 1.5	95.3 ± 0.3	266.5 ± 2.9	285.5 ± 6.8	71.3 ± 3.3	80.6 ± 2.4	2.1 ± 0.3
pH	3.87 ± 0.52	5.27 ± 0.24	4.47 ± 0.27	4.16 ± 0.08	5.24 ± 0.05	5.74 ± 0.20	6.83 ± 0.2

[a] ND- Not detectable

Table 4
Loadings of organic acids, soluble P and pH on the factors identified by Principal Components Analysis. The parameters are grouped according to the maximum fittings to principal components (correlation coefficients > 0.50[a]; n = 21).

	Di-calcium phosphate		Tri-calcium phosphate
	Rotated loadings on one or two principal components[b]		
	PC1	PC2	PC1
Citric acid	–	–	**0.628**
Oxalic acid	**0.552**	-0.091	**0.507**
Gluconic acid	**0.952**[a]	0.016	**0.953**
α Ketogluconic acid	**0.592**	-0.025	**0.946**
Lactic acid	**0.882**	-0.033	**0.553**
Malic acid	-0.218	**0.923**	0.154
Succinic acid	0.191	**0.915**	**0.578**
Tartaric acid	-0.030	0.099	**0.783**
Formic acid	0.106	0.026	0.328
Total acids	**0.670**	**0.547**	**0.999**
P released	**0.639**	**0.621**	**0.949**
pH	**-0.680**	**-0.542**	**-0.897**
Explained variance (%)	33.2	24.4	91.7

[a] Loadings with absolute values > 0.50 are in bold; [b] Only PCs causing > 20 % of the total variance were included

(PCA) with Varimax rotation. PCA is usually employed to reveal unexpected relationships among the parameters and hence permits explanations that would otherwise not be possible (Flury and Riedwyl, 1988). Only PCs with > 1.0 Eigen values and causing > 20 % of the total variance were considered for interpretation. All statistical analyses were performed using IBM SPSS v. 25 for Windows and Grapes1.0 (Gopinath et al., 2021).

3. Results

3.1. Shortlisting of promising P solubilizing bacteria

Out of 100 bacterial isolates obtained from the rhizosphere of turmeric, 9 could solubilize P under *in vitro* conditions. Out of this, four isolates *B. safensis*, *B. cereus*, *P. aeruginosa* and *B. marisflavi* with high *in vitro* P solubilization capacity (Table S1 and Fig S1) were selected for further investigation.

3.2. Quantitative study for P solubilization

3.2.1. P release in liquid medium

The shortlisted PSB (*B. safensis*, *B. marisflavi* and *B. cereus*) consistently released greatest P from TCP at all days of incubation (Fig. 1b). Regardless of the PSB strain used, P release peaked on the 10th DAI followed by a gradual decrease up to the 25th DAI. Highest P solubilization was observed with *B. safensis* (277.5 mg L^{-1}), followed by *B. marisflavi* (172.3 mg L^{-1}) and *B. cereus* (149.5 mg L^{-1}), while P solubilized was significantly ($P < 0.05$) lower with *C. firmus* and *P. diazotrophicus* (86.4 mg L^{-1} and 85.0 mg L^{-1}, respectively). Across the treatments, P solubilized from TCP by the P solubilizing bacteria ranged from 8.2 % to 27.2 %. P solubilization by the PSB from DCP were almost identical to those observed with TCP at different DAI (Fig. 1a). Irrespective of the PSB employed, peak P solubilization was observed on the

Table 5
Effect of shortlisted PSB applied alone and in combination with varying doses of rock phosphate on turmeric growth parameters at 60 days after planting in the green house (2021).

Treatments	No. of tillers	Height (cm)	No. of leaves	Dry root weight (g pot^{-1})	Dry shoot weight (g pot^{-1})	Soil available P (mg kg^{-1})
T1–100 % P[a]	2.8 ± 0.3[bc]	29.2 ± 2.6[d]	3.5 ± 0.3[d]	0.8 ± 0.1[c]	8.3 ± 0.1[d]	7.5 ± 0.1[c]
T2 – 75 % P[a]	2.7 ± 0.3[bc]	27.7 ± 2.6[d]	4.3 ± 0.3 [cd]	0.9 ± 0.04[c]	8.3 ± 0.10[d]	6.0 ± 0.1[d]
T3 - *B. safensis*[b] + 75 % P	4.0 ± 0.2[a]	45.8 ± 1.7[ab]	6.5 ± 0.7[ab]	1.5 ± 0.10[a]	13.5 ± 0.2[a]	8.6 ± 0.3[b]
T4 - *B. cereus* + 75 % P	4.0 ± 0.4[a]	41.5 ± 1.3[bc]	6.3 ± 0.5[ab]	1.1 ± 0.04[b]	11.2 ± 0.1[b]	4.0 ± 0.1 [f]
T5 - *B. marisflavi* + 75 % P	3.5 ± 0.3[ab]	39.1 ± 0.8[bc]	5.5 ± 0.7[bc]	0.79 ± 0.03[c]	8.6 ± 0.1[c]	4.1 ± 0.1 [f]
T6 - *B. safensis* + *B. cereus* + 75 % P	4.0 ± 0.4[a]	48.5 ± 0.9[a]	7.3 ± 0.5[a]	1.5 ± 0.04[a]	13.8 ± 0.1[a]	9.1 ± 0.1[a]
T7 - *B. safensis* + *B. marisflavi* + 75 % P	4.0 ± 0.4[a]	41.5 ± 1.5[bc]	6.3 ± 0.5[ab]	1.3 ± 0.04[a]	11.8 ± 0.1[b]	8.8 ± 0.1[b]
T8 - *B. cereus* + *B. marisflavi* + 75 % P	3.5 ± 0.3[ab]	42.1 ± 0.5[bc]	5.5 ± 0.2[bc]	0.85 ± 0.1[c]	9.1 ± 0.1[c]	5.2 ± 0.1[e]
T9 - *B. safensis* alone	3.7 ± 0.3[a]	41.7 ± 1.8[bc]	5.8 ± 0.5[b]	0.89 ± 0.1[c]	9.8 ± 0.2[c]	2.1 ± 0.3 [h]
T10 - *B. cereus* alone	3.7 ± 0.3[a]	40.7 ± 1.9[bc]	5.3 ± 0.3[bc]	0.90 ± 0.1[c]	9.2 ± 0.1[c]	1.3 ± 0.1[j]
T11 - *B. marisflavi* alone	2.7 ± 0.3[bc]	27.7 ± 2.6[d]	4.3 ± 0.3 [cd]	0.90 ± 0.1[c]	8.3 ± 0.1[d]	1.2 ± 0.1[j]
T12 - *B. safensis* + *B. cereus*	3.7 ± 0.3[a]	38.7 ± 2.2[c]	5.8 ± 0.5[b]	0.87 ± 0.1[c]	9.8 ± 0.2[c]	2.6 ± 0.1 [g]
T13 - *B. safensis* + *B. marisflavi*	3.5 ± 0.3[ab]	29.3 ± 1.4[d]	4.3 ± 0.3 [cd]	0.79 ± 0.1[c]	8.4 ± 0.1[c]	1.9 ± 0.1[i]
T14 - *B. cereus* + *B. marisflavi*	2.7 ± 0.3[bc]	28.3 ± 2.6[d]	4.3 ± 0.3 [cd]	0.83 ± 0.1[c]	7.4 ± 0.10[d]	1.8 ± 0.1[i]
T15 - Control (without P and PSB)	2.2 ± 0.3[c]	28.7 ± 1.7[d]	3.8 ± 0.3[d]	0.80 ± 0.1[c]	7.9 ± 0.1[e]	0.96 ± 0.1[j]

Values are means of 12 replications ± SE; In a column, means followed by the same letter are not significantly different at $P < 0.05$; a100 % P (applied as basal dose @ 6.25 g rock phosphate 10–1 kg soil; 75 % P (applied as basal dose @ 4.68 g rock phosphate 10–1 kg soil); b Rhizome treatment, spraying and drenching 30, 60 and 90 days after planting

Table 6
Effect of shortlisted PSB applied alone and in combination with varying doses of rock phosphate on yield and quality of turmeric under field condition (mean of 2 years).

Treatments	Rhizome yield (Dry weight) (Mg ha^{-1})	Soil available P (mg kg^{-1})	Rhizome quality parameters			
			P uptake (kg ha^{-1})	Curcumin (%)	Oleoresin (%)	Oil (%)
T1- Control	2.89[e]	2.00 ± 0.01[e]	5.67[de]	3.6 ± 0.21[b]	10.0 ± 0.58[c]	2.5 ± 0.07[d]
T2- *Bacillus safensis*[a]	4.08[d]	1.03 ± 0.01 [cd]	7.47[d]	5.0 ± 0.09[a]	13.1 ± 0.33[ab]	3.8 ± 0.49[bc]
T3- *B. safensis* + 50 % P[b]	6.36[b]	3.28 ± 0.02[b]	20.69[a]	5.3 ± 0.09[a]	14.0 ± 0.31[a]	3.8 ± 0.42[bc]
T4- *B. safensis* + 75 % P[b]	7.32[a]	4.67 ± 0.03[a]	17.56[b]	5.3 ± 0.17[a]	14.6 ± 0.47[a]	5.3 ± 0.24[a]
T5- *B. safensis* + 100 % P[b]	4.88[c]	4.15 ± 0.33[a]	9.69[c]	5.2 ± 0.18[a]	13.5 ± 0.60[ab]	4.4 ± 0.24[b]
T6–100 % P	3.32[e]	1.35 ± 0.18[c]	6.38[de]	4.0 ± 0.11[b]	11.9 ± 0.30[b]	3.2 ± 0.13 [cd]

Values are means ± SE; In a column, means followed by the same letter are not significantly different at $P < 0.05$; a Rhizome treatment, spraying and drenching 60 and 90 days after planting; b 100 % P (*i.e.* 50 kg ha-1; applied as basal dose @ 130 g rock phosphate/bed); b 75 % P (applied as basal dose @ 97.5 g rock phosphate /bed); b 50 % P (applied as basal dose @ 65 g rock phosphate /bed)

10th DAI followed by a gradual decrease. In general, P solubilized on the 10th DAI was significantly higher ($P < 0.05$) with *B. safensis* (388.5 mg L^{-1}) followed by *B. marisflavi* (285.5 mg L^{-1}) and *B. cereus* (266.5 mg L^{-1}), while it was markedly lower with *C. firmus* and *P. diazotrophicus* (80.5 mg L^{-1} and 71.2 mg L^{-1}, respectively). The P solubilized from DCP by the PSB was higher than from TCP and it ranged from 9.32 % to 38.64 %. The P solubilized by the PSB at different time intervals were markedly higher with DCP compared to TCP.

3.2.2. P release in soil

Similar to liquid medium, release of P in soil from TCP peaked on 10th DAI and subsequently registered a steady decline (Fig. 1). *B. safensis* consistently registered maximum P release followed by *B. marisflavi* and *B. cereus*. Even after 25th DAI, the P solubilizers released significantly ($P < 0.05$) greater P into the soil (24.0–36.0 mg kg^{-1}) compared to the low P solubilizers (3.0–7.5 mg kg^{-1}). Data on P release kinetics (Table 1) suggested that the P solubilization potential was significantly ($P < 0.05$) greater in soils inoculated with high P solubilizers *viz*., *B. safensis* (57.7 mg kg^{-1}), followed by *B. cereus* (48.96 mg kg^{-1}), *B. marisflavi* (47.57 mg kg^{-1}) and *P. aeruginosa* (37.4 mg kg^{-1}). In soils spiked with DCP, *B. safensis* registered peak P release on the 5th DAI (93.3 mg kg^{-1}), while the other high P solubilizers like *B. marisflavi* (84.0 mg kg^{-1}), *B. cereus* (68.5 mg kg^{-1}) and *P. aeruginosa* (80.0 mg kg^{-1}) registered peak P release on the 10th DAI (Fig. 1). Similar to TCP, P release potential (Table 1) was significantly ($P < 0.05$) greater in soils inoculated with high P solubilizers and was in the order of *B. marisflavi* (70.5 mg kg^{-1}), *B. safensis* (68.0 mg kg^{-1}), *P. aeruginosa* (57.3 mg kg^{-1}) and *B. cereus* (51.3 mg kg^{-1}). In general, irrespective of the P solubilization capacity of the PSB, the P solubilization potential was greater in soils spiked with DCP compared to TCP. The P release rates (mg d^{-1}) from both TCP and DCP in soil did not exactly reflect the P solubilization capacity of the bacteria.

3.3. Organic acid production

During TCP solubilization, the major acids produced were gluconic, α ketogluconic, oxalic, succinic and tartaric acids (Table 2). However, the rate and type of organic acid production varied markedly among the bacterial strains (Fig S3 and S4). In general, the total organic acid production during solubilization of TCP was significantly higher ($P < 0.05$) in case of high P solubilizers (1546.4–2336.0 μg mL^{-1}) compared to low P solubilizers (561.2–695.2 μg mL^{-1}). In medium spiked with TCP, the major share of the total organic acid production was contributed by gluconic acid in case of *B. safensis, B. marisflavi* and *P. aeruginosa* (35.0 %, 39.0 % and 28.0 %, respectively), while both gluconic acid (26.5 %) and succinic acid (26.1 %) contributed to the major share in case of *B. cereus* (Fig S2a). All the high P solubilizers also secreted appreciable amounts of oxalic, tartaric and α ketogluconic acids, though it was conspicuously low in case of low P solubilizers. Similar to TCP, the major acids secreted during DCP solubilization were gluconic, succinic, α ketogluconic, oxalic, and tartaric acids (Table 3). However, in case of DCP, succinic acid contributed to the major share of total organic acid

production (33.0 %) in case of both *B. safensis*, and *B. marisflavi* (Fig. S2b).

3.4. Inter-relationships between organic acids, pH and P

The inter-relationships between organic acids, P released and pH of the medium amended with TCP and DCP was determined using PCA (Table 4). The PCA from TCP data provided only one PC (PC1, total variance 91.7 %) involving positive loadings of citric, oxalic, gluconic, α ketogluconic, lactic, malonic, succinic acid, tartaric acid, soluble P and high negative loading of pH. The positive loadings of organic acids combined with high positive loading of P released suggested the positive effects of these acids on P solubilization from TCP. The PCA of DCP data provided two principal components (Table 4). PC1 accounting for 33.2 % of the total variance was positively loaded by oxalic, gluconic, α ketogluconic, lactic acids and P released. PC2 accounting for 24.4 % of the total variance was loaded mainly by malic and succinic acids and P released. Both PC1 and PC2 had strong negative loadings of pH suggesting that enhanced solubilization was achieved due to organic acid secretion by PSB. This was clearly distinct in the case of high P solubilizers, wherein the pH of the medium decreased to as low as 3.72–5.20 during TCP solubilization and 3.87–4.16 during DCP solubilization on the 10th DAI (Fig.2).

3.5. Green house evaluation of promising PSB for soil P solubilization and growth promotion

Application of PSB significantly ($P < 0.05$) promoted turmeric growth as evidenced from the enhanced number of tillers, shoot length, number of leaves, dry weight of shoot and root when compared to untreated plants at 60 DAP (Table 5). Among the treatments, T6 (*B. safensis* + *B. cereus* + 75 % P) significantly ($P < 0.05$) increased the number of tillers (31.3 %), shoot length (43.0 %), number of leaves (41.4 %), dry root weight (23.7 %), dry shoot weight (62.2 %) and soil available P (44.8 %) compared to application of 75 % P (T2). Application of *B. safensis* + 75 % P (T3) and the combined application of other two shortlisted PSB with 75 % P (T4 and T5) significantly increased growth parameters compared to sole application of 100 % and 75 % P (T1 and T2, respectively). The treatments *B. safensis* + 75 % P (T3) and *B. safensis* + *B. cereus* + 75 % P (T6) recorded the maximum yield (Fig.3), suggesting an increase by 118.0 % and 108.0 %, respectively compared to sole application of 100 % P (T2). When compared to the initial soil P (0.96 mg kg^{-1}), the available P content increased significantly ($P < 0.05$) in soils treated with PSB. Among the PSB treatments, available P was found to be significantly higher in the treatments with *B. safensis* + *B. cereus* + 75 % P (T6), *B. safensis* + *B. marisflavi* + 75 % P (T7) and *B. safensis* + 75 % P (T3) (Table 5). Soil AcP activity was

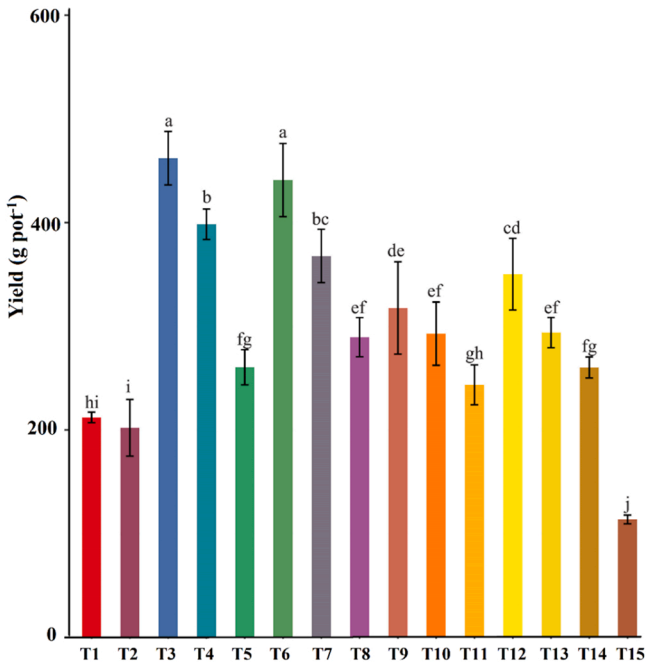

Fig. 3. Effect of shortlisted PSB applied alone and in combination with varying levels of P as rock phosphate on yield of turmeric in the greenhouse (mean of two years), Bars indicate standard error; different letters indicate significant differences at P < 0.05 (LSD).[T1 - 100 % P, T2 – 75 % P, T3 - *B. safensis* + 75 % P, T4 - *B. cereus* + 75 % P, T5 - *B. marisflavi* + 75 % P, T6 - *B. safensis* + *B. cereus* + 75 % P, T7 - *B. safensis* + *B. marisflavi* + 75 % P, T8 - *B. cereus* + *B. marisflavi* + 75 % P, T9 - *B. safensis* alone, T10 - *B. cereus* alone, T11 - *B. marisflavi* alone, T12 - *B. safensis* + *B. cereus*, T13 - *B. safensis* + *B. marisflavi*, T14 - *B. cereus* + *B. marisflavi*,T15 - Control (without P and PSB).

significantly ($P < 0.05$) higher in all treatments with PSB (Figs.6) and 73.6- 76.0 % enhanced activity was registered in the treatments with *B. safensis* + *B. marisflavi* + 75 % P (T7) and *B. cereus* + 75 % P (T4).

3.6. Field experiments using promising PSB for soil P solubilization and growth promotion

Among the treatments, the one with 75 % P + *B. safensis* (T3) registered significantly ($P < 0.05$) higher rhizome yield and the increase was greater by 46.5 % and 58.0 % compared to control (T6–100 % P) and absolute control (T7–100 % P), respectively (Table 6, Fig. 5). With regard to soil available P, the levels were significantly ($P < 0.05$) higher in all the treatments involving combined application of *B. safensis*

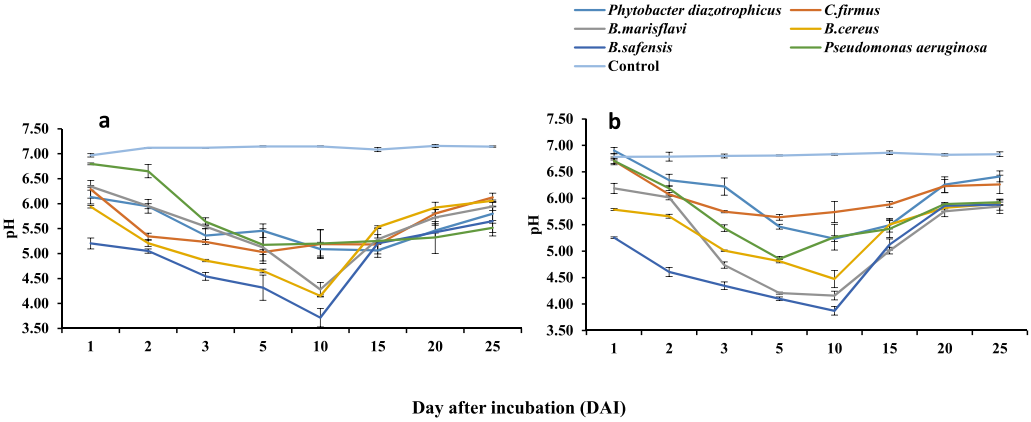

Fig. 2. pH in liquid medium (a) amended with tri-calcium phosphate (TCP) (b) amended with di-calcium phosphate (DCP) at different days after incubation. Vertical bar at each data point indicates standard error.

Fig. 4. Effect of shortlisted PSB applied alone and in combination with varying levels of P as rock phosphate on growth parameters of field grown turmeric plants at 60 days after planting (2021) [T1-Control; T2- *Bacillus safensis*; T3- *B. safensis* + 50 % P; T4- *B. safensis* + 75 % P; T5- *B. safensis* + 100 % P; T6- 100 % P].

with 50 % or 75 % or 100 % P (Table 6). In fact, compared to 100 % P treatment (T6) with soil available P of 1.35 mg kg^{-1}, the available P levels increased significantly by 246.0 %, 207.0 % and 143.0 % in treatments with 75 % P + *B. safensis*, 100 % P + *B. safensis* and 50 % P + *B. safensis*, respectively. MBC, MBN and MBP levels (Table S2) were also significantly greater in treatments with *B. safensis* combined with 75 % P (T4), registering 39.0 %, 27.0 % and 57.0 % increase, respectively compared to the treatment with 100 % P (T6). Higher microbial activity in *B. safensis* treatment was confirmed with the observation on dehydrogenase, which registered marked greater levels in T4 (Table S2). The corresponding increase in AcP activity was 26.5 % higher compared to T6 (Fig.6).

3.7. Rhizome quality parameters

The P uptake calculated using rhizome P concentration and yield (dry weight) indicated significantly ($P < 0.05$) enhanced P accumulation in all treatments with PSB (Table 6). Compared to the treatment with 100 % P (T6), the P uptake levels increased by 51.0 %, 174.0 % and 223.0 % in treatments *B. safensis* + 100 % P (T5), *B. safensis* + 75 % P (T4) and *B. safensis* + 50 % P (T3), respectively. The curcumin content increased by 30.0– 32.5 % in treatments with only *B safensis* (T2) and those with *B. safensis* + 50.0 or 75.0 or 100.0 % P (T3-T5) compared to control (T6) and treatments with combined application of *B. safensis* with different levels of P (T2-T5) consistently registered maximum curcumin content (5.0 – 5.3 %) (Table 6). Oleoresin content increased by 13.4–23.0 % and oil content by 18.7–66.0 % in treatments with *B. safensis* + 50.0 or 75.0 or 100.0 % P (T3-T5) compared to control (T6). Similar to curcumin content, the oleoresin and oil contents were significantly higher in the treatments with *B. safensis* + 75.0 P (Table 6).

4. Discussion

The rate and type of organic acid production varied markedly with the PSB strains and the P substrates used. In presence of TCP, gluconic acid contributed the major part of total organic acid production in case of *B. safensis*, whereas succinic acid was the dominant acid produced by *B. cereus* and *B. marisflavi*. This suggested that the type and amount of

Fig. 5. Effect of shortlisted PSB applied alone and in combination with varying levels of P as rock phosphate on yield of turmeric clump under field condition (2021) [T1-Control; T2- *Bacillus safensis*; T3- *B. safensis* + 50 % P; T4- *B. safensis* + 75 % P; T5- *B. safensis* + 100 % P; T6- 100 % P].

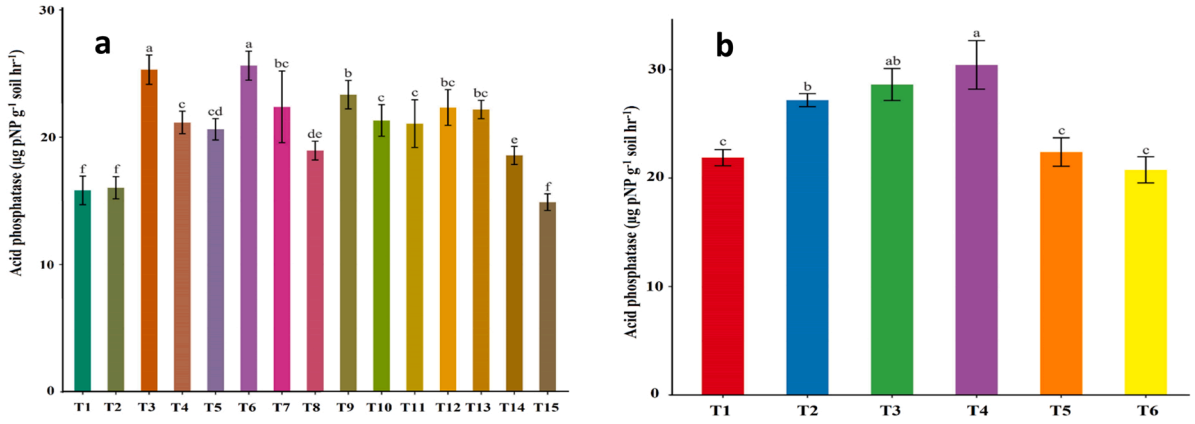

Fig.6. Effect of shortlisted PSB applied alone and in combination with varying levels of P as rock phosphate on acid phosphatase activity in soils in (a) green house and (b) field, Bars indicate standard error; different letters indicate significant differences at $P < 0.05$ (LSD)[(a) Greenhouse study: T1 - 100 % P, T2 – 75 % P, T3 - *B. safensis* + 75 % P, T4 - *B. cereus* + 75 % P,T5 - *B. marisflavi* + 75 % P, T6 - *B. safensis* + *B. cereus* + 75 % P, T7 - *B. safensis* + *B. marisflavi* + 75 % P, T8 - *B. cereus* + *B. marisflavi* + 75 % P, T9 - *B. safensis* alone, T10 - *B. cereus* alone, T11 - *B. marisflavi* alone, T12 - *B. safensis* + *B. cereus,* T13 - *B. safensis* + *B. marisflavi,* T14 - *B. cereus* + *B. marisflavi*,T15 - Control (without P and PSB); (b) Field study: T1-Control; T2- *Bacillus safensis*; T3- *B. safensis* + 50 % P; T4- *B. safensis* + 75 % P; T5- *B. safensis* + 100 % P; T6- 100 % P].

organic acids secreted are variable and play different roles during the solubilizing process (Archana et al., 2012). Gluconic acid was the major acid secreted by several PSB during TCP solubilization followed by α ketogluconic or succinic acids. The study also revealed the involvement of other organic acids *viz*., oxalic, tartaric, malonic, succinic and tartaric acid during TCP and DCP solubilization. Our observations are consistent with the reports that besides gluconic and, other acids like oxalic, citric, malic, malonic acids *etc.* also play important roles during P release, suggesting that the total acids secreted by the bacteria is more important during solubilization than the individual acids (Serna Posso et al., 2017). This was corroborated by the highly significant correlation ($P < 0.01$; $n = 21$; at 10 DAI) between P released and total acids in medium spiked with TCP ($r = 0.92$) and DCP ($r = 0.84$). PCA also strongly supported the combined influence of organic acids on P solubilization. Likewise, the significant negative loading of pH suggested that organic acid secretion by the bacteria decreased the pH and consequently enhanced P release from the insoluble P sources. This was evident in the medium with high P solubilizer like *B. safensis*, which registered high total acid production accompanied by decreased pH and therefore released greater amounts of P from both TCP and DCP (Fig S5 and S6). Unlike liquid medium, there was very little change in the pH of the soil at different DAI, though P solubilized were significantly higher in case of the high P solubilizers (*B. safensis*, *B. marisflavi, B. cereus* and *P. aeruginosa*). This supported the finding that there was no correlation between P solubilized by bacteria and pH of the soil (Asea et al., 1988). Under such circumstances, P solubilization in soils by bacteria can be explained by the theory proposed by Illmer and Schinner (1995), involving simultaneous cation assimilation and proton excretion. Like for instance, simultaneous assimilation of NH_4^+ within microbial cells and excretion of H^+ induces P solubilization even in the absence of organic acids (Sharma et al., 2013). Another mechanism suggested by Rodríguez and Fraga (1999) is the release of H^+ to the outer surface in exchange for cation uptake or with the help of H^+ translocation ATPase. Therefore, these mechanisms were probably more effective in P solubilization from RP in soil than strict

acid dissolution. However, it is possible that the organic acids were produced by the bacteria at the interface between the biofilm and P substrate surface, causing a temporary pH drop in this micro niche (Delvasto et al., 2009).

The three promising P solubilizers (*B. safensis*, *B. cereus* and *B marisflavi*) were then evaluated in the green house and field using turmeric as the test crop. In the green house, *B. safensis* and *B. cereus* treated plants showed significantly ($P < 0.05$) higher growth and enhanced soil available P compared to *B marisflavi*. Turmeric yield was also significantly higher in treatments with combined application of PSB and RP especially *B. cereus* + 75 % P and *B. safensis* + *B. marisflavi* + 75 % P. Under field conditions, soil available P, rhizome yield and quality were significantly ($P < 0.05$) enhanced in all the treatments with *B. safensis*. It was apparent that inoculation of *B. safensis* with lowered levels of RP markedly increased SMB and AcP activity triggering enhanced soil P solubilization and enhanced P uptake. Release of enzymes, especially the non-specific acid phosphatases (phosphomonoesterases or phosphatases) which can be either acid or alkaline phosphomonoesterases is considered to be the prime mechanism of P solubilization by bacteria in soil (Jorquera et al., 2011). They dephosphorylate the phosphor-ester or phosphoanhydride bond of organic compounds thereby releasing P and among the phosphatases, AcP was found to be the major player in acidic and neutral soils (Rodríguez and Fraga, 1999). Apparently, *B. safensis* was found to be the most promising PSB due to its ability to enhance SMB and dehydrogenase levels and subsequently synthesize greater amounts of AcP, which in turn helped in mineralizing significant amounts of P. In fact, available P levels in soil increased by a staggering 143.0–246.0 % in treatments combining *B. safensis* and graded levels of RP, clearly suggesting that *B. safensis* was a powerful P solubilizer even under field conditions. Among the various PSB treatments, significantly greater levels of soil available P and rhizome yield and quality in the treatment with 75% P + *B. safensis* also suggested a 25.0 % decrease in RP amendment in the presence of *B. safensis*. Similar promising effects of PSB in combination with chemical fertilizers have been observed in several crops (Soumare et al., 2020). Consistent with yield, the rhizome quality measured in terms of curcumin, oleoresin and volatile oil was also markedly improved due to *B. safensis* inoculation and significant enhancement was registered in the treatment with 75 % P + *B. safensis*. While earlier studies on PSB in turmeric are very few, our earlier study has clearly revealed the robust biocontrol potential of this *B. safensis* strain (NCBI- MT192800) for protection against a wide array of fungal phytopathogens infecting turmeric (Praveena et al., 2022). Therefore, considering its broad-spectrum biocontrol activity and the present results on its remarkable soil P solubilizing ability including the positive influence on yield and quality, we suggest using *B. safensis* combinedly with reduced doses of inorganic P for enhanced turmeric yield and quality in P deficient soils.

5. Conclusions

The promising PSB registered greater P solubilization potential and efficiency in soil and the combined influence of all the organic acids was found to be critical during solubilization in liquid medium. The *B. safensis* strain (NCBI-MT192800) had all the trappings of a promising PSB due to higher solubilization efficiency and positive impacts on turmeric yield and quality making it a promising candidate for integration into crop nutrient management plans in P deficient soil systems. Future studies focusing on the machinations of P release by this strain in different soil types and agro-ecological regions will provide clear insights and nuanced understanding on the significance of PGPR additions into turmeric based cropping systems.

CRediT authorship contribution statement

R. Dinesh: Visualization, Supervision, Writing – original draft, Editing. **V. Srinivasan:** Investigation, Data curation, Formal analysis. **R. Praveena:** Conceptualization, Investigation, Resources, Editing. **K.P Subila**: Investigation, Validation. **Priya George:** Investigation. **Akshaya Das:** Investigation. **O. Shajina:** Investigation. **K. Anees:** Investigation. **N. K. Leela:** Validation. **P. Haritha:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.115826.

References

Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front. Microbiol. 8, 971. https://doi.org/10.3389/fmicb.2017.00971. ⟨https://doi⟩.

AOAC (Association of Official Agricultural Chemists), and Horwitz, W., 1975. Official methods of analysis (Vol. 222). Washington, DC, Association of Official Analytical Chemists.

Archana, G., Buch, A., Kumar, G.N., 2012. Pivotal role of organic acid secretion by rhizobacteria in plant growth promotion. In: Satyanarayana, T., Johri, B.N., Prakash, A. (Eds.), Microorganisms in Sustainable Agriculture and Biotechnology. Springer, Netherlands, pp. 35–53.

Asea, P.E.A., Kucey, R.M.N., Stewart, J.W.B., 1988. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol. Biochem. 20, 459–464. https://doi.org/10.1016/0038-0717(88)90058-2.

ASTA, Steam Volatile Oil (Modified Clevenger Method), Method 5.0, Official Analytical Methods, 4th edition. American Spice Trade Association, Washington DC, pp. 17.

ASTA, Curcumin content of Turmeric Spice & Oleoresins, Method 18.0, Official Analytical Methods, 4th ed. American Spice Trade Association, Washington DC, pp. 81.

Bhattacharyya, P., Nayak, A.K., Shahid, M., Tripathi, R., et al., 2015. Effects of 42-year long-term fertilizer management on soil phosphorus availability, fractionation, adsorption-desorption isotherm and plant uptake in flooded tropical rice. Crop J. 3, 387–395, 10.1016/j.cj.2015. 03.009.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319–329. https://doi.org/10.1016/0038-0717(82)90001-3.

Casida Jr., L.E., Klein, D.A., Santoro, R., 1964. Soil dehydrogenase activity. Soil Sci. 98, 371–378. https://doi.org/10.1097/00010694-196412000-00004.

Chempakam, B., Parthasarathy, V.A., 2008. Turmeric. In: Parthasarathy, V.A., Chempakam, B., Zachariah, T.J. (Eds.), Chemistry of Spices. CABI, Oxfordshire, UK, pp. 97–123.

Chen, Q., Liu, S., 2019. Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in Reclamation Soil in Shanxi, China. Front. Microbiol. 10, 2171. https://doi.org/10.3389/fmicb.2019.02171.

Cordell, D., Drangert, J.O., White, S., 2009. The story of phosphorus: global food security and food for thought. Glob. Environ. Chang 19, 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009.

Delvasto, P., Ballester, A., Muñoz, J.A., González, F., Blázquez, M.L., Igual, J.M., Valverde, A., García-Balboa, C., 2009. Mobilization of phosphorus from iron ore by the bacterium *Burkholderia caribensis* FeGL03. Miner. Eng. 22, 1–9. https://doi.org/10.1016/j.mineng.2008.03.001.

FAO. 2017. The Future of Food and Agriculture – Trends and Challenges. Rome. The future of food and agriculture | FAO | Food and Agriculture Organization of the United Nations (fao.org) (accessed on 21/07/2022).

Flury, B., Riedwyl, H., 1988. Multivariate statistics. A Practical Approach. Chapman and Hall,, London.

Gopinath, et al., 2021. grapesAgri1: collection of shiny apps for data analysis in agriculture. J. Open Source Softw. 6 (63), 3437. https://doi.org/10.21105/joss.03437.

Illmer, P., Schinner, F., 1995. Solubilization of inorganic calcium phosphates – solubilization mechanisms. Soil Biol. Biochem. 27, 257–263. https://doi.org/10.1016/0038-0717(94)00190-C.

Jabborova, D., Sulaymanov, K., Sayyed, R.Z., Alotaibi, S.H., Enakiev, Y., Azimov, A., Jabbarov, Z., Ansari, M.J., Fahad, S., Danish, S., Datta, R., 2021. Mineral fertilizers improves the quality of turmeric and soil. Sustainability 13, 9437 https:// doi.org/10.3390/su13169437.

Jackson, M.L., 1973. Plant tissue analysis- mineral constituents. In: Soil Chemical Analysis. Prentice-Hall, New Delhi, India, pp. 326–338.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. Soil Biol. Biochem 28, 25–31 https://doi.org/10.1016/0038-0717 (95)00102-6.

Joergensen, R.G., Mueller, T., 1996. The fumigation–extraction method to estimate soil microbial biomass: calibration of the kEN value. Soil Biol. Biochem. 28, 33–37. https://doi.org/10.1016/0038-0717(95)00101-8.

Jorquera, M.A., Crowley, D.E., Marschner, P., Greiner, R., et al., 2011. Identification of β-propeller phytase-encoding genes in culturable Paenibacillus and Bacillus spp. from the rhizosphere of pasture plants on volcanic soils. FEMS Microbiol. Ecol. 75, 163–172. https://doi.org/10.1111/j.1574-6941.2010.00995.x.

Kuo, S., 1996. Phosphorus. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis, Part 3, Chemical Methods. SSSA, Madison, WI, pp. 869–919.

Lambers, H., Plaxton, W.C., 2018. Phosphorus: back to the roots. Annu. Plant Rev. 48, 3–22 https://doi:10.1002/9781118958841.ch1.

Mander, C., Wakelin, S., Young, S., Condron, L., O'Callaghan, M., 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biol. Biochem. 44, 93–101. https://doi.org/10.1016/j.soilbio.2011.09.009.

Mardad, I., Serrano, A., Soukri, A., 2013. Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. Afr. J. Microbiol. Res 7, 626–635. https://doi.org/10.5897/AJMR12.1431.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 170, 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.

Nesme, T., Metson, G.S., Bennett, E.M., 2018. Global phosphorus flows through agricultural trade. Glob. Environ. Change 50, 133–141.

Pérez, E., Sulbarán, M., Ball, M.M., Yarzábal, L.A., 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol. Biochem. 39, 2905–2914. https://doi.org/10.1016/j.soilbio.2007.06.017.

Praveena, R., Srekha, K., Revathy, R., Srinivasan, V., et al., 2022. New rhizobacteria strains with effective antimycotic compounds against rhizome rot pathogens and identification of genes encoding antimicrobial peptides. Rhizosphere 22, 100515. https://doi.org/10.1016/j.rhisph.2022.100515.

Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17, 319–339, 2. https://doi.org/10.1016/S0734-9750(99)00014-2.

Sahu, S.N., Jana, B.B., 2000. Enhancement of the fertilizer value of rock phosphate engineered through phosphate-solubilizing bacteria. Ecol. Eng. 15, 27–39. https://doi.org/10.1016/S0925-8574(99)00013-0.

Serna Posso, E.J., Sánchez-de Prager, M., Cisneros-Rojas, C.A., 2017. Organic acids production by rhizosphere microorganisms isolated from a Typic Melanudands and its effects on the inorganic phosphates solubilization. Acta Agron. 66, 241–247. https://doi.org/10.15446/acag.v66n2.56148.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer 2, 587–600 https://doi.org/10.1186/ 2193-1801-2-587.

Soumare, A., Boubekri, K., Lyamlouli, K., Hafidi, M., et al., 2020. From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: Status and Needs. Front. Bioeng. Biotechnol. 7, 425. https://doi.org/10.3389/fbioe.2019.00425.

Srinivasan, V., Sadanandan, A.K., Hamza, S., 2000. Efficiency of rock phosphate sources on ginger and turmeric in an Ustic Humitoprept. J. Indian Soc. Soil Sci. 48, 532–536.

Srinivasan, V., Thankamani, C.K., Dinesh, R., Kandiannan, K., Zachariah, T.J., Leela, N. K., Hamza, S., Shajina, O., Ansha, O., 2016. Nutrient management systems in turmeric: Effects on soil quality, rhizome yield, and quality. Ind. Crops Prod. 85, 241–250.

Stanford, G., Smith, S.J., 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. 36, 465–472. https://doi.org/10.2136/sssaj1972.03615995003600030029x.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil. Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.

Tan, S., Yang, C., Mei, X., Shen, S., et al., 2013. The effect of organic acids from tomato root exudates on rhizosphere colonization of *Bacillus amyloliquefaciens* T-5. Appl. Soil. Ecol. 64, 15–22. https://doi.org/10.1016/j.apsoil.2012.10.011.

Trolove, S.N., Hedley, M.J., Kirk, G.J.D., Bolan, N.S., Loganathan, P., 2003. Progress in selected areas of rhizosphere research on P acquisition. Aust. J. Soil Res. 41, 471–499.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil. Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant. Soil. 171, 1–15.

Vyas, P., Gulati, A., 2009. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent Pseudomonas. BMC Microbiol 9, 174. https://doi.org/10.1186/1471-2180-9-174.

Wan, W., Qin, Y., Wu, H., Zuo, W., et al., 2020. Isolation and characterization of phosphorus solubilizing bacteria with multiple phosphorus sources utilizing capability and their potential for lead immobilization in soil. Front. Microbiol. 11, 752. https://doi.org/10.3389/fmicb.2020.00752.

Zhang, Y., Li, Y., Wang, S., Umbreen, S., Zhou, C., 2021. Soil phosphorus fractionation and its association with soil phosphate-solubilizing bacteria in a chronosequence of vegetation restoration. Ecol. Eng. 164, 106208 https://doi.org/10.1016/j.ecoleng.2021.106208.