



Effect of Phosphate Solubilising Bacteria on Tomato Growth and Biological Control of *Pythium* sp., the Causal Agent of Tomato Root Rot Disease under Pot Grown Conditions

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Authors' contributions

This work was carried out through the collaboration of all authors. Authors DTD and HF conceptualised the study. Authors DTD, HLM, GVTT and CS developed the methodology. Authors DTD, HLM and HF performed the data analysis. Author DTD prepared the original draft of the manuscript. Authors CS, VDT and HF reviewed and edited the manuscript. Authors VDT and HF supervised the study. All authors read and approved the final manuscript.

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Abstract

Poorly soluble phosphates may become more available to plants through the activity of phosphate-solubilising bacteria, potentially improving growth where P is limiting. Four strains, identified as *Bacillus* sp., *Paenibacillus* sp., *Curtobacterium* sp. and *Burkholderia* sp., were examined for their ability to release phosphorus from rock phosphates of different origins and to affect tomato growth with or without *Pythium* sp., the organism associated with root rot. Initially, the strains were tested in solid and liquid NBRIP media containing Algerian, Senegalese, Malian, Moroccan or Mexican rock phosphate. Solubilisation indices on agar ranged from 1.92 to 3.21, with the highest overall value recorded for *Bacillus* sp. Algerian and Senegalese phosphate gave the greatest indices (3.55 and 3.48, respectively). In liquid culture, soluble P varied from 95.56 mg/L with *Burkholderia* sp. to 335.54 mg/L with *Curtobacterium* sp. Release of soluble P was highest from Senegalese phosphate, followed by the Malian, Algerian, Mexican and Moroccan sources. A greenhouse pot study then assessed tomato growth in Senegalese rock-phosphate-amended soil after bacterial inoculation, either with or without *Pythium* sp. Effects on plant height, stem diameter and dry biomass differed between strains. For certain growth measures, *Bacillus* sp., *Curtobacterium* sp. and *Burkholderia* sp. performed better than the negative control. These results suggest a possible contribution of selected strains to phosphate solubilisation and tomato growth, although validation under field conditions remains necessary.

Keywords: Tomato; *Bacillus* sp.; *Paenibacillus* sp.; *Curtobacterium* sp.; *Burkholderia* sp.; root rot disease; *Pythium* sp.

1. Introduction

With global output estimated at 192 million tonnes in 2023, tomato (*Solanum esculentum* L.) ranks as the world's most widely produced vegetable and, after potato, among the most frequently consumed (FAO, 2024). Tomatoes contain lycopene, an antioxidant reported to slow the growth of cancerous cells (Bhowmik et al., 2012; Gunaseeli et al., 2024). They are also recommended by nutritionists and dietitians as part of weight-management programmes (Keswani, 2015). Regular tomato consumption has been associated with a lower risk of cancers affecting the lungs, prostate, stomach, cervix, breast, oral cavity, colorectum, oesophagus and pancreas, among other sites. In addition to being eaten fresh, tomatoes are processed into canned products, sauces, juice, ketchup, stews and soups (Lenucci et al., 2006).

In Cameroon, tomato production rose from 15,800 tonnes in 1992 to more than 1.2 million tonnes in 2023 (Tassah and Kouam, 2025). To improve yields, smallholder growers frequently apply chemical fertilisers, including phosphate fertilisers, despite their cost and undesirable environmental effects. Tomato cultivation is also threatened by pathogens, notably *Pythium* sp., which is associated with root rot (Gunaseeli et al., 2024).

After nitrogen (N), phosphorus (P) is regarded as a key nutrient for plant growth (Bakki et al., 2024). Its biochemical functions encompass photosynthesis, respiration, energy transfer and storage, and the division and enlargement of cells. Phosphorus also contributes to plants' ability to withstand winter conditions and, in some species, to resist disease (Amit et al., 2012). Although soils contain substantial quantities of both organic and inorganic P, much of this nutrient is inaccessible to roots. Inorganic P is commonly retained in poorly soluble mineral complexes, some of which form following repeated applications of chemical fertilisers (Tchouomo et al., 2023). Organic matter likewise contains immobilised P and has been reported to account for 20–80% of soil P (Richardson, 1994). Consequently, despite the total P reservoir, only approximately 0.1% occurs in a soluble form available for uptake (Zhou et al., 1992). Converting insoluble soil phosphates into soluble forms through microbial activity is therefore important in both agricultural and natural systems. Greenhouse and field investigations have documented increased P availability and improved plant growth after inoculation with phosphate-solubilising microorganisms (PSMs) (Reyes et al., 2002; Zaidi et al., 2003). Numerous fungi and bacteria possess the capacity to mobilise inorganic phosphates, making PSMs relevant to the more sustainable use of phosphate fertilisers. Phosphate-solubilising bacteria (PSB) may also enhance biological nitrogen fixation and thus plant N supply. Their plant-growth-promoting effects additionally involve greater accessibility of trace elements and the synthesis of growth-promoting substances (Ahmad et al., 2008; Zaidi et al., 2009). In a greenhouse study of tomato, Yoon et al. (2013) reported that inoculation with *Pantoea agglomerans* and *Burkholderia anthina* increased plant height, root length, shoot and root dry mass, P uptake and soil-available P relative to the control. The ability of *Bacillus* strains to solubilise mineral phosphates, including dicalcium phosphate, tricalcium phosphate and rock phosphate, has also been described (Ahmad et al., 2022; Bouizgarne et al., 2023). Han et al. (2005) and Crowley et al. (2006) reported enhanced growth of eggplant, pepper and cucumber following co-inoculation with *Bacillus mucilaginosus* and the phosphate-solubilising *Bacillus megaterium*. Rhizosphere isolates from the genera *Pseudomonas* and *Bacillus* are frequently recognised as plant-growth-promoting rhizobacteria (PGPR), partly because their contribution to plant P availability can support crop health and productivity (Bouizgarne, 2013; Bakki et al., 2024). Accordingly, inoculation with P-solubilising rhizobacteria has attracted interest as a means of improving crop P uptake (Azaroual et al., 2020).

This study investigated four previously isolated Cameroonian rhizosphere PSB strains—*Bacillus* sp., *Paenibacillus* sp., *Curtobacterium* sp. and *Burkholderia* sp.—to determine their ability to solubilise rock phosphates from five origins in solid and liquid media. Their effects on tomato growth and their potential for biological control of *Pythium* sp., the organism associated with tomato root rot, were also examined in greenhouse pots.

2. Materials and Methods

2.1 Bacterial Strains

Four bacterial strains were obtained from the culture collection maintained by the Laboratory of Plant Biology and Physiology, Faculty of Science, University of Douala. They belonged to *Bacillus* sp., *Paenibacillus* sp., *Curtobacterium* sp. and *Burkholderia*

sp. and had originally been isolated from different agroecological zones of Cameroon. Identification was performed by partial sequencing of the 16S rRNA gene.

2.2 Seeds of Tomato Plant

Tomato seeds of the Rio Grande + variety were obtained from a seed-selling establishment. This improved standard variety is principally cultivated during the cool, dry season (MINADER, 2024).

2.3 Characterisation of Strains on Plates Supplemented with Different Types of Rock Phosphate Origins

2.3.1 Rock Phosphates

Five rock phosphates, whose mineral compositions are presented in Table 1, were selected for the experiment: Tilemsi phosphate from Mali, Gafsa phosphate from Mexico, and phosphates originating from Algeria, Senegal and Morocco. Before use, each material underwent four washes in warm water to remove soluble constituents, with successive intervals of 1 h, 24 h, 1 h and 24 h. The washed phosphates were homogenised after drying at 60 °C until the water had completely evaporated.

2.3.2 Preparation of Bacterial Inoculum and Assessing of their Capacity to Solubilise Rock Phosphate on Agar Plate

2.3.2.1 Preparation and Evaluation of the Concentration of the Inocula

A pure colony from each strain was transferred separately into 50 mL of nutrient broth (NB; 5 g peptone, 1 g beef extract, 2 g yeast extract, 5 g sodium chloride and 1000 mL distilled water; pH 7.0). Following incubation for 3 days at 28 °C with shaking at 150 rpm, cells were collected by centrifugation at 10.000 g for 10 min at 4 °C. They were washed three times under the same centrifugation conditions using sterile 0.85% NaCl and then resuspended in the same saline solution. Suspension optical density (OD) was adjusted to 0.2 at 620 nm. Cell concentration was estimated by serially diluting 1 mL of this suspension to 10^{-7} and plating 200 μ L of the 10^{-7} dilution, in duplicate, on nutrient agar (NA; 5 g peptone, 1 g beef extract, 2 g yeast extract, 5 g sodium chloride, 15 g agar and 1000 mL distilled water; pH 7.0). Colonies were counted after 4 days at 28 °C to determine colony-forming units (CFU) per mL. The resulting inoculum concentrations ranged from 1.2 to 2.03×10^8 CFU/mL.

Table 1. Mineral composition of the different rock phosphates used

Origin	Chemical nutrient (%)								mg/kg		
	Total P ₂ O ₅	Available P	K	Ca	Mg	Na	Fe	Al	Mn	Zn	Cu
Mali	30	12.98	0.056	28.19	0.131	0.232	3.844	0.80	8360	87	51
Mexico	28	8.87	0.219	25.94	0.222	0.358	0.442	0.58	788	103	18
Morocco	13	9.33	0.093	28.83	1.93	0.552	0.267	0.42	96	219	38
Algeria	29	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Senegal	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

Nd: Not determined

2.3.2.2 Assessing of the Strains to Solubilise Rock Phosphate

Rock-phosphate solubilisation was evaluated on modified National Botanical Research Institute's Phosphate (NBRIP) agar, following Fankem et al. (2014, 2015a). Per litre of distilled water, the medium contained 20 g glucose, 5 g MgCl₂·6H₂O, 0.25 g MgSO₄·7H₂O, 0.2 g KCl, 0.1 g (NH₄)₂SO₄ and 5 g.L⁻¹ of one of the five rock phosphates (Malian, Moroccan, Senegalese, Algerian or Mexican). Bromocresol green (0.5%) was included to facilitate observation, and the pH was brought to 7.5 with 1 M KOH. A 3 μ L aliquot of each bacterial suspension was deposited at a single point in a compartmentalised Petri dish. After sealing, plates were maintained at 28 °C for 5 days. A yellow halo around a colony was taken as evidence of phosphate solubilisation, and the solubilisation index (SI) was determined according to Edi-Premono et al. (1996).

2.3.2.3 Assessing of Phosphate Solubilising Bacteria to Solubilise Rock Phosphate in Liquid Medium

Soluble P release was quantified in liquid NBRIP medium (Nautiyal, 1999), each preparation containing one of the five rock phosphates from Algeria, Senegal, Mali, Morocco or Mexico at 5 g.L⁻¹. Erlenmeyer flasks received 25 mL of medium and the corresponding rock phosphate (0.25 g), after which the pH was set to 7 ± 0.2 . Once sterilised and cooled, the medium was inoculated with 200 μ L of a microbial suspension containing approximately 15×10^8 CFU/mL, as described above. Control vessels instead received 200 μ L of uninoculated medium. Three replicates were prepared for each rock-phosphate type with the four bacterial strains and controls, giving 75 experimental units in total. The flasks were shaken at 150 rpm for 7 days at 28 °C. Cultures were then centrifuged at 5000 rpm and 4 °C; 9 mL of the resulting medium was used for pH measurement and 10 mL for colorimetric estimation of soluble phosphate using the procedure of Murphy and Riley (1962), as cited by Fankem (2007).

A calibration curve produced from known concentrations of soluble phosphorus supplied as KH₂PO₄ was used to calculate P concentrations in the medium. The calculation followed the original relation: mg/L P = (mg/L $\times$ solution volume)/sampling volume.

2.3.2.4 Isolation, Purification and Identification of *Pythium* sp.

Pythium sp. was isolated by the commonly used baiting technique, which employs susceptible material such as potato and lemon (Tristan, 2007). Potato and lemon samples were surface-sterilised with sodium hypochlorite, rinsed three times in sterile distilled water, and immersed in lidded buckets containing sampled soil mixed with sterile distilled water. Four replicates were established.

After 7 days, potentially infected tissue was collected from the flotation line using a sterile scalpel. Each sample was transferred to a sterile Petri dish containing water agar alone to limit contamination by other fungi and bacteria. Four replicate dishes were then incubated for 4 days at 28 °C.

The recovered isolate was purified on potato dextrose agar (PDA), prepared with 200 g potato, 20 g sugar and 20 g agar per litre at pH 6 (Bouneghou, 2011). Identification involved comparison with a reference strain for hyphal morphology, colour, appearance, growth rate and whether colonies were distributed homogeneously or heterogeneously across the Petri dish. A laboratory pathogenicity test was not undertaken because the intended assessment of PSB-mediated control of the tomato root-rot organism was to be conducted in the greenhouse. The reference strain also assisted morphological assessment of *Pythium* sp.

2.3.2.5 Preparation of Inoculum of *Pythium* sp.

To prepare the *Pythium* sp. inoculum, the isolate was subcultured on potato dextrose agar and incubated for at least 1 week at 20 °C, following Besnard and Davet (1993). Cultures were subsequently transferred into sterile distilled water and held for 3–4 days at 20 °C to obtain a sporangial suspension (El-Tarabily et al., 2003). In the greenhouse, sterile soil received the root-rot organism at 120 sporangia per mL. Sporangia were enumerated microscopically using a Malassez counting chamber by counting those within its individual channels.

2.3.2.6 Sterilisation and Germination of Tomato Seeds

Tomato seeds were immersed in 70% alcohol for 1 min and rinsed twice with sterile distilled water. They were then treated with 3% sodium hypochlorite for 2 min, rinsed four times with sterile distilled water and kept in sterile Petri dishes for 6 days to allow germination.

2.3.2.7 Pot Experiment

The pot trial used soil previously characterised by Fankem et al. (2015b), with the following properties: pH H₂O, 5.5; pH KCl, 4.4; nitrogen, 0.06%; available P, 7.54 ppm; organic matter, 0.59%; organic carbon, 0.34%; iron, 1.75 ppm; aluminium, 0.29 méq/g; calcium, 1.04 méq/g; magnesium, 1.60 méq/g; potassium, 0.15 méq/g; and sodium, 0.07 méq/g. Available P was therefore low (7.54 ppm). Plants were grown in a greenhouse under natural photoperiod conditions, with supplemental lighting providing a 12 h day length and temperatures of 23–26 °C.

The experimental arrangement comprised six treatments, each implemented with and without *Pythium* sp.: four single-strain bacterial inoculations and two controls. The bacterial treatments were designated A (*Bacillus* sp.), B (*Paenibacillus* sp.), C (*Burkholderia* sp.) and D (*Curtobacterium* sp.). Six replicates per treatment combination provided 72 experimental units. Senegalese rock phosphate was supplied to all pots except those assigned to the positive control at 80 kg.ha⁻¹, equivalent to 0.3625 g per 0.8836 dm³, to increase soil phosphate; its reported characteristics are shown in Table 1. One pregerminated tomato seed was placed in a small depression in each inoculated pot, treated with 1 mL of bacterial suspension and lightly covered with soil. Positive-control pots (Cont+) received soluble KH₂PO₄ at 350 mg P/g soil (Fankem et al., 2015b), whereas negative-control pots (Cont-) were supplied with Senegalese rock phosphate but no bacterial inoculum. To increase PSM abundance in the sterile soil, a second bacterial inoculation was given 13 days after the first. The trial continued for 46 days, with each pot receiving 200–300 mL of water daily. At 25 days after planting, when the plants had gained sufficient vigour, *Pythium* sp. was applied at the stem base between the root and shoot, following Senan et al. (2008). Plant height, leaf number and stem-base diameter were measured on that day and subsequently at weekly intervals over the following 3 weeks. On day 46, the plants were harvested and separated into shoots and roots. These fractions were dried at 60 °C to constant mass for determination of shoot, root and total dry biomass.

2.4 Statistical Analysis

The data were analysed in SigmaPlot version 12.0. Treatment differences were examined by analysis of variance, followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons.

3. Results

3.1 Capacity of Strains to Solubilise Rock Phosphate on Agar Plate and in Liquid Medium

3.1.1 Capacity of Strains to Solubilise Rock Phosphate on Agar Plate

When phosphate solubilisation was assessed on agar (Fig. 1), *Bacillus* sp. produced the largest solubilisation index (SI; 3.21). Across the four isolates, SI values extended from 1.92 to 3.21; the indices for *Burkholderia* sp., *Paenibacillus* sp. and *Curtobacterium* sp. were 2.20, 2.01 and 1.92, respectively.

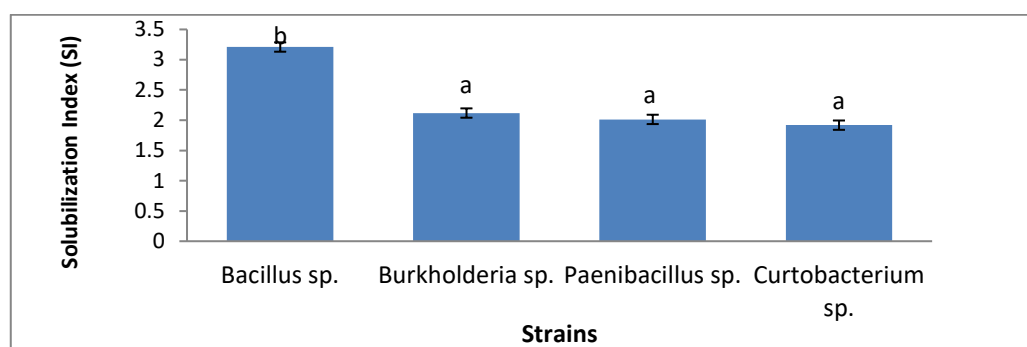


Fig. 1. Capacity of strains to solubilise in solid medium
Different letters indicate significant differences at p -value ($p < 0.05$)

3.1.2 Capacity of Solubilisation of Different Rock Phosphates on Agar Plate

As illustrated in Fig. 2, rock-phosphate origin influenced solubilisation on agar. The highest SI values were obtained for Algerian (3.55) and Senegalese (3.48) phosphates, followed by Malian phosphate (2.92). The values for Mexican and Moroccan materials were lower, at 0.85 and 0.77, respectively.

3.1.3 Assessing of the Capacity of Strains to Solubilise Different Rock Phosphates in Liquid Medium

Measurements in liquid NBRIP medium (Fig. 3) showed a different strain ranking from that observed on agar. *Curtobacterium* sp. released the most soluble P (335.54 mg/L), closely followed by *Paenibacillus* sp. (333.57 mg/L). *Bacillus* sp. and *Burkholderia* sp. yielded 166.03 and 95.56 mg/L, respectively.

3.1.4 Capacity of Solubilisation of Different Rock Phosphates in Liquid Medium

In the liquid-medium assay (Fig. 4), Senegalese rock phosphate yielded the greatest soluble P concentration (252.16 mg/L). It was followed, in descending order, by the Malian (234.68 mg/L), Algerian (203.16 mg/L), Mexican (160.57 mg/L) and Moroccan (130.61 mg/L) sources, a ranking different from that observed on agar.

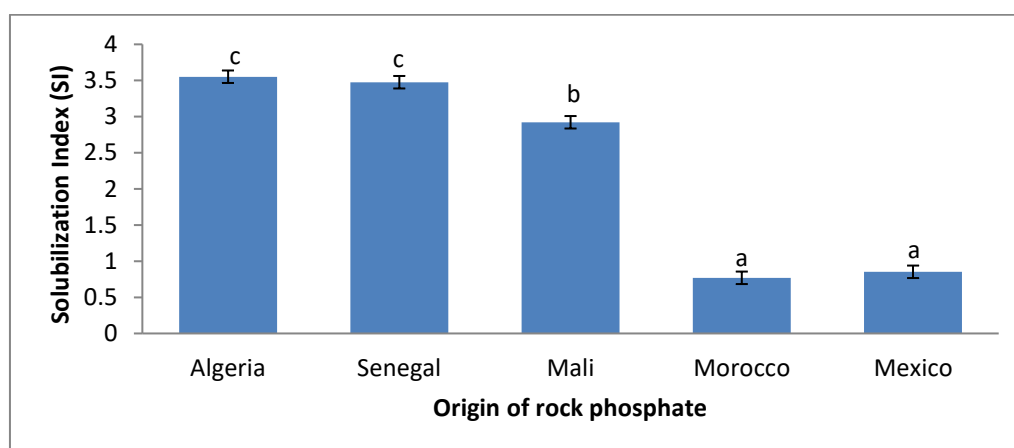


Fig. 2. Capacity of solubilisation of different rock phosphates in solid medium
Different letters indicate significant differences at p -value ($p < 0.05$)

3.2 Effect of Inoculation by Phosphate Solubilising Bacteria on Tomato Plant Growth in Presence of the Causal Agent of Tomato Root Rot Disease *Pythium* sp. under Pot Grown Conditions

The greenhouse trial was designed to identify strains that might support tomato growth while limiting the effects of *Pythium* sp., the organism associated with root rot.

3.2.1 Effect of PSB after 25 days without *Pythium* sp.

At 25 days after planting, no statistically significant treatment differences were observed in plant height, leaf number or stem-base diameter among inoculated plants and controls (Table 2).

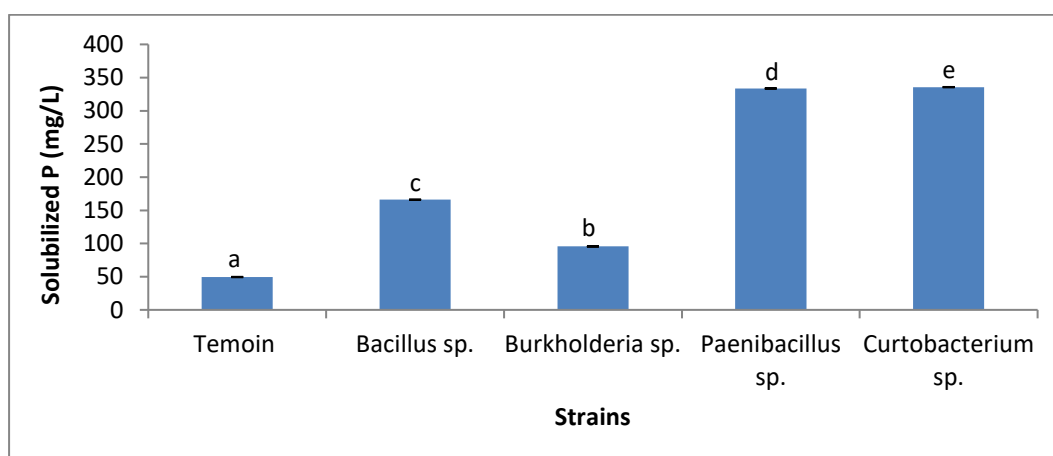


Fig. 3. Capacity of strains to solubilise in liquid medium
Different letters indicate significant differences at p -value ($p < 0.05$)

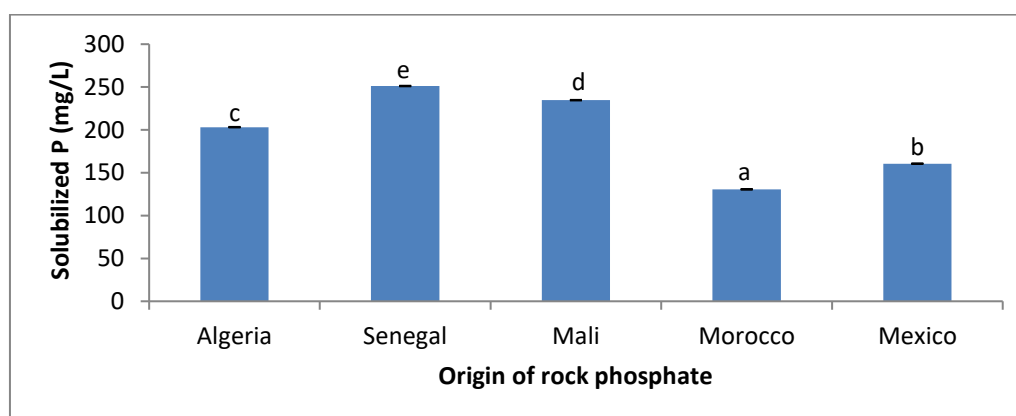


Fig. 4. Capacity of solubilisation of different origin of rock phosphates in liquid medium
Different letters indicate significant differences at p -value ($p < 0.05$)

Table 2. Growth parameters obtained after 25 days without *Pythium* sp.

Treatment	Plant height (cm)	Number of leaves	Stem base diameter (cm)
Control (-)	9.82 ± 2.43 a	6.50 ± 0.91 a	0.27 ± 0.08 a
Control (+)	14.72 ± 2.57 a	7.08 ± 1.24 a	0.36 ± 0.07 a
<i>Bacillus</i> sp.	11.30 ± 1.51 a	6.67 ± 1.30 a	0.27 ± 0.07 a
<i>Burkholderia</i> sp.	10.97 ± 2.59 a	6.55 ± 1.04 a	0.26 ± 0.06 a
<i>Paenibacillus</i> sp.	9.68 ± 3.03 a	6.08 ± 0.79 a	0.24 ± 0.07 a
<i>Curtobacterium</i> sp.	12.54 ± 1.09 a	6.75 ± 0.62 a	0.29 ± 0.06 a

The same letter in the same column indicates no significant difference at p -value ($p < 0.05$)

3.2.2 Effect of PSB on Tomato Plant in Presence of *Pythium* sp.

3.2.2.1 Effect of PSB on Plant Height in Presence of *Pythium* sp.

At 7 days after *Pythium* sp. inoculation, several bacterial treatments had greater plant height than the negative controls (Table 3). Heights were 17.30 cm for *Curtobacterium* sp. with *Pythium* sp. and 16.37 cm without it; 15.93 cm for *Burkholderia* sp. with *Pythium* sp.; and 16.70 cm for *Bacillus* sp. with *Pythium* sp. The corresponding negative controls measured 12.63 cm in the presence and 15.22 cm in the absence of *Pythium* sp. Nevertheless, the highest values occurred in the positive controls, at 21.58 cm with *Pythium* sp. and 22.22 cm without it.

At 14 days after pathogen inoculation, plant heights under all four bacterial treatments exceeded that of the negative control with *Pythium* sp. (19.37 cm). Measurements for *Curtobacterium* sp. were 25.22 cm with and 23.87 cm without *Pythium* sp.; those for *Burkholderia* sp. were 22.95 and 23.15 cm, respectively. *Bacillus* sp. treatments measured 23.80 cm with and 21.70 cm without *Pythium* sp., while *Paenibacillus* sp. with *Pythium* sp. reached 21.10 cm. Positive-control plants remained tallest, measuring 28.77 cm with and 29.48 cm without *Pythium* sp.

Table 3. Growth parameters (plant height and number of leaves) obtained each week after inoculation with *Pythium* sp.

Treatment	Plant height (cm)			Number of leaves		
	7 days	14 days	21 days	7 days	14 days	21 days
Control (-)	15.22 ± 2.99 a	21.83 ± 4.53 abc	31.65 ± 5.32 a	8.00 ± 0.63 a	8.83 ± 0.98 b	10.50 ± 1.52 a
Control (-) with <i>Pythium</i> sp.	12.63 ± 3.57 a	19.37 ± 4.68 a	28.58 ± 6.44 a	7.67 ± 1.37 a	7.83 ± 0.75 b	9.50 ± 1.38 a
Control (+)	22.22 ± 3.46 c	29.48 ± 5.04 c	37.05 ± 7.02 b	9.00 ± 1.55 a	10.00 ± 1.27 b	11.17 ± 2.23 a
Control (+) with <i>Pythium</i> sp.	21.58 ± 4.39 bc	28.77 ± 7.23 bc	37.57 ± 8.63 b	8.00 ± 2.28 a	9.17 ± 2.14 b	10.50 ± 2.07 a
<i>Bacillus</i> sp.	14.93 ± 2.71 a	21.78 ± 4.10 abc	31.60 ± 5.24 a	7.50 ± 1.38 a	7.67 ± 0.82 b	9.00 ± 1.10 a
<i>Bacillus</i> sp. + <i>Pythium</i> sp.	16.70 ± 1.64 abc	23.80 ± 3.97 abc	33.58 ± 6.91 a	8.33 ± 1.86 a	8.50 ± 0.84 b	9.83 ± 2.23 a
<i>Burkholderia</i> sp.	15.13 ± 1.70 a	23.15 ± 2.68 abc	33.83 ± 4.52 a	7.67 ± 1.03 a	8.00 ± 1.27 b	9.17 ± 1.17 a
<i>Burkholderia</i> sp. + <i>Pythium</i> sp.	15.93 ± 4.10 ab	22.95 ± 4.51 abc	31.50 ± 7.23 a	7.67 ± 1.75 a	8.33 ± 2.25 b	9.83 ± 2.79 a
<i>Paenibacillus</i> sp.	13.30 ± 2.76 a	20.05 ± 3.82 ab	29.37 ± 4.81 a	7.17 ± 1.72 a	7.33 ± 1.97 b	8.83 ± 2.23 a
<i>Paenibacillus</i> sp. + <i>Pythium</i> sp.	14.23 ± 5.01 a	21.10 ± 7.15 abc	30.25 ± 10.00 a	6.67 ± 1.03 a	7.00 ± 1.41 a	8.83 ± 2.32 a
<i>Curtobacterium</i> sp.	16.37 ± 2.30 abc	23.87 ± 3.50 abc	31.95 ± 5.70 a	7.67 ± 0.82 a	7.83 ± 0.98 b	8.33 ± 1.51 a
<i>Curtobacterium</i> sp. + <i>Pythium</i> sp.	17.30 ± 1.01 abc	25.22 ± 1.96 abc	35.52 ± 2.40 b	8.00 ± 1.10 a	7.67 ± 1.03 b	9.50 ± 1.52 a

The same letter in the same column indicates no significant difference at p -value ($p < 0.05$)

Table 4. Growth parameters (stem base diameter and dry weight) obtained each week after inoculation with *Pythium* sp.

Treatment	Stem base diameter (cm)			Dry weight (g)		
	7 days	14 days	21 days	Root	Shoot	Total
Control (-)	0.34 ± 0.04 abc	0.46 ± 0.08 abc	0.54 ± 0.07 abc	0.31 ± 0.17 abc	1.08 ± 0.42 abc	1.40 ± 0.59 ab
Control (-) with <i>Pythium</i> sp.	0.31 ± 0.08 a	0.41 ± 0.09 a	0.50 ± 0.10 ab	0.29 ± 0.16 abc	1.00 ± 0.54 abc	1.29 ± 0.70 ab
Control (+)	0.45 ± 0.09 c	0.59 ± 0.08 c	0.68 ± 0.12 c	0.46 ± 0.25 c	1.59 ± 0.98 bc	2.10 ± 1.23 b
Control (+) with <i>Pythium</i> sp.	0.44 ± 0.11 bc	0.55 ± 0.13 bc	0.68 ± 0.13 bc	0.44 ± 0.27 bc	1.62 ± 0.83 c	2.10 ± 1.06 ab
<i>Bacillus</i> sp.	0.31 ± 0.06 a	0.40 ± 0.06 a	0.47 ± 0.06 a	0.20 ± 0.06 abc	0.71 ± 0.27 abc	0.90 ± 0.31 ab
<i>Bacillus</i> sp. + <i>Pythium</i> sp.	0.31 ± 0.05 a	0.41 ± 0.04 ab	0.49 ± 0.07 ab	0.17 ± 0.08 ab	0.77 ± 0.33 abc	0.94 ± 0.39 ab
<i>Burkholderia</i> sp.	0.33 ± 0.03 abc	0.43 ± 0.04 ab	0.51 ± 0.03 abc	0.26 ± 0.11 abc	0.71 ± 0.21 abc	0.97 ± 0.27 ab
<i>Burkholderia</i> sp. + <i>Pythium</i> sp.	0.32 ± 0.08 ab	0.41 ± 0.08 a	0.48 ± 0.09 ab	0.13 ± 0.06 a	0.70 ± 0.29 abc	0.83 ± 0.34 a
<i>Paenibacillus</i> sp.	0.31 ± 0.04 a	0.38 ± 0.04 a	0.45 ± 0.07 a	0.14 ± 0.08 a	0.49 ± 0.19 a	0.64 ± 0.25 a
<i>Paenibacillus</i> sp. + <i>Pythium</i> sp.	0.31 ± 0.08 a	0.38 ± 0.07 a	0.44 ± 0.11 a	0.18 ± 0.12 ab	0.67 ± 0.46 ab	0.85 ± 0.57 a
<i>Curtobacterium</i> sp.	0.36 ± 0.03 abc	0.45 ± 0.58 abc	0.53 ± 0.08 abc	0.23 ± 0.09 abc	0.72 ± 0.25 abc	0.95 ± 0.34 ab
<i>Curtobacterium</i> sp. + <i>Pythium</i> sp.	0.35 ± 0.03 abc	0.41 ± 0.02 ab	0.49 ± 0.04 ab	0.24 ± 0.07 abc	0.85 ± 0.12 abc	1.09 ± 0.17 ab

The same letter in the same column indicates no significant difference at p -value ($p < 0.05$)

By 21 days after inoculation, the greatest plant height among the bacterial treatments occurred with *Curtobacterium* sp. in the presence of *Pythium* sp. (35.52 cm). This exceeded the negative control both with (28.58 cm) and without (31.65 cm) *Pythium* sp., with a significant difference reported between treatments. According to the statistical comparison, the positive controls with and without *Pythium* sp. did not differ appreciably from each other.

3.2.2.2 Effect of PSB on Number of Leaves of Tomato Plant in Presence of *Pythium* sp.

No statistically significant differences in leaf number were reported among the treatments at 7, 14 or 21 days after inoculation (Table 3).

3.2.2.3 Effect of PSB on Stem Base Diameter in Presence of *Pythium* sp.

Seven days after inoculation (Table 4), the stem-base diameters recorded for *Curtobacterium* sp. were 0.35 cm with and 0.36 cm without *Pythium* sp. *Burkholderia* sp. without *Pythium* sp. produced a diameter of 0.33 cm. These measurements exceeded the 0.31 cm recorded for the negative control with *Pythium* sp., although the highest values were observed in the positive controls (0.44 cm with and 0.45 cm without *Pythium* sp.).

At 14 days after inoculation, stem-base diameter remained greatest in the positive-control treatments: 0.55 cm with *Pythium* sp. and 0.59 cm without it.

The positive controls likewise had the largest stem-base diameters at 21 days: 0.68 cm with *Pythium* sp. and 0.68 cm without it.

3.2.2.4 Effect of PSB on Dry Weight of Tomato Plant in Presence of *Pythium* sp.

For root dry mass (Table 4), the positive controls produced the highest measurements, at 0.44 g with *Pythium* sp. and 0.46 g without it.

Shoot dry mass was also greatest in the positive controls (1.62 g with *Pythium* sp. and 1.59 g without it).

The same pattern was observed for total dry mass, which reached 2.10 g with *Pythium* sp. and 2.10 g without it in the positive controls.

4. Discussion

Across the bacterial isolates, SI values ranged between 1.92 and 3.21. For comparison, Babana et al. (2013) and Fankem et al. (2014) reported PSB indices of 1.00–3.60 and 1.44–4.74, respectively. In the comparison of rock-phosphate sources discussed here, Senegalese phosphate was described as the most readily solubilised (SI = 2.85), ahead of Malian (SI = 2.68), Algerian (SI = 1.53), Mexican (SI = 1.38) and Moroccan (SI = 0.79) phosphates. Earlier work similarly found Malian phosphate to be more readily solubilised than the Algerian and Moroccan materials (Fankem et al., 2014; Sani et al., 2019). Differences in mineral nutrient composition, particularly the proportion of available P, may explain variation between phosphate sources.

Quantifying the P released into liquid medium provided an additional means of comparing the phosphate-solubilising ability of the bacterial isolates. The concentration of soluble P served as a measure of the microorganisms' solubilisation activity. Consistent with the observations on agar, Moroccan rock phosphate was relatively resistant to solubilisation in liquid medium.

The two assay formats did not identify the same leading isolate. *Curtobacterium* sp. released 335.54 mg/L soluble P in liquid medium, compared with 166.03 mg/L for *Bacillus* sp., although *Bacillus* sp. performed better on agar. Baig et al. (2010) likewise described differences between qualitative and quantitative assessments. This supports Babana's (2003) recommendation that both approaches be used when selecting the most effective isolates.

In the greenhouse, three of the four isolates—*Bacillus* sp., *Curtobacterium* sp. and *Burkholderia* sp.—performed comparatively well relative to the negative control when Senegalese rock phosphate was supplied. The resilience of *Bacillus* species under adverse conditions has been attributed to their Gram-positive, spore-forming nature (Bouizgarne, 2013). The tomato rhizosphere has been proposed as a source of adapted PGPR capable of phosphate solubilisation and antagonism towards plant pathogens (Bakki et al., 2024). Screening microorganisms for multiple PGPR characteristics is consequently considered promising (Sun et al., 2026). Improvements in plant growth associated with PSB have been described by Gamalero et al. (2004), Yahya et al. (2021) and Bouizgarne et al. (2023). In *Bacillus*, growth promotion may involve auxin production and enhanced accessibility of P (Deepa et al., 2010), alongside mechanisms such as nitrogen fixation, the solubilisation of P and iron, phytohormone modulation and improved tolerance of abiotic stress (Bakki et al., 2024).

Plant-growth-promoting rhizobacteria may increase P supply to roots by mobilising phosphate in the rhizosphere (Richardson, 2001; Fankem et al., 2015a; Fankem et al., 2015b). Plants, in turn, provide these microorganisms with photosynthesis-derived compounds (Pang et al., 2021). Pal (1998) documented increased available P in associations between *Bacillus* sp. and five plant species, while Adesemoye et al. (2008) reported a 31% rise in tomato dry biomass following treatment with *Bacillus subtilis*. In the present work, *Bacillus* sp., *Burkholderia* sp. and *Curtobacterium* sp. also appeared comparatively effective against *Pythium* sp., the tomato root-rot organism. One proposed explanation is that PSB release antagonistic compounds, including siderophores, that may restrict *Pythium* sp. growth. Selvakumar et al. (2008) described the capacity of *Bacillus* sp. to produce indoleacetic acid and siderophores

with antifungal activity, an interpretation also discussed by Cazorla et al. (2007). Wani et al. (2007) likewise reported phosphate solubilisation together with indoleacetic acid and siderophore production by *Bacillus* sp.

5. Conclusion

The four bacterial isolates exhibited different capacities to solubilise rock phosphate, depending on the assay medium and the origin of the phosphate. *Bacillus* sp. gave the largest overall SI on agar, whereas *Curtobacterium* sp. and *Paenibacillus* sp. produced higher soluble P concentrations in liquid culture. Solubilisation was generally greater for the Senegalese and Algerian sources than for Moroccan and Mexican phosphates. In greenhouse pots amended with Senegalese phosphate, selected PSB influenced tomato growth under the conditions examined. Some growth measures were higher than those of the negative control for *Bacillus* sp., *Curtobacterium* sp. and *Burkholderia* sp., including when *Pythium* sp. was present. The positive control nevertheless produced the greatest growth values overall, suggesting an advantage of readily soluble P over rock phosphate in this experiment. The results therefore indicate potential for certain strains to improve P availability and support tomato growth, but their reliability, efficacy against root rot and usefulness under field conditions require further investigation.

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Competing Interests

Authors have declared that no competing interests exist.

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