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NATIVE PHOSPHATE-SOLUBILIZING BACTERIA FROM AL-QADISIYAH SOILS: SCREENING, IDENTIFICATION AND EFFECT ON SUNFLOWER PHOSPHORUS NUTRITION IN A CALCAREOUS SOIL

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Abstract:

Phosphorus (P) applied to the calcareous soils of central Iraq is quickly bound as calcium phosphates, so crops recover little of it. We screened phosphate-solubilizing bacteria (PSB) native to six calcareous soils of Al-Qadisiyah and tested the most active ones on sunflower (*Helianthus annuus* L.). Twelve isolates were compared on National Botanical Research Institute's phosphate (NBRIP) agar and in NBRIP broth with tricalcium phosphate (TCP), and grouped by phenotype. Three isolates were then inoculated into an unsterilized calcareous soil (Olsen P 5.6 mg kg⁻¹) given 0, 15 or 30 mg P kg⁻¹, in a factorial pot trial with four blocks harvested 60 days after emergence. Net soluble P ranged from 59.7 to 332.0 mg L⁻¹ (6.0–33.2% of TCP-P) and rose as broth pH fell ($r = -0.98$). The plate solubilization index explained only 56% of the variation in broth P and ranked the isolates differently. Six isolates were *Bacillus*-like, four *Pseudomonas*-like and two unresolved Gram-negative rods. Inoculation increased total P uptake by 23.0% across P rates ($p < 0.001$), with no significant inoculant $\times$ P interaction. Relative gains were largest without added P (21–43%) and smallest at 30 mg P kg⁻¹ (12–20%). The *Pseudomonas*-like isolate QPSB07 also raised shoot P concentration and residual Olsen P, and its uptake at 15 mg P kg⁻¹ matched about 24.7 mg P kg⁻¹ of fertilizer alone. Broth-based selection of native PSB is worth pursuing for sunflower on calcareous soils, but molecular identification and field trials are still needed.

Keywords: Phosphate-solubilizing bacteria; Calcareous soil; Sunflower; Tricalcium phosphate; Phosphorus uptake; Olsen P; Biofertilizer; Iraq.

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I. INTRODUCTION

Phosphorus is second only to nitrogen as a nutrient that limits crop growth, yet little of the fertilizer P reaches the crop. A global synthesis of field trials put plant uptake at about 12.6% of added inorganic P, with roughly two-thirds left in the soil [1], and national budgets show wide gaps between P input and output [2]. Calcareous soils are a hard case. High pH, free CaCO₃ and abundant Ca²⁺ drive phosphate into calcium phosphates and, with time, hydroxyapatite, and up to 80% of the applied P can be lost to the crop [3]. Central Iraq fits this picture. Surface horizons in the grain areas of Najaf and Al-Diwaniyah hold 114–494 g kg⁻¹ of carbonates [4], the Tigris and Euphrates sediments that built these plains are rich in calcite [5],

and in a recent calcareous-soil trial wheat recovered only 14–28% of the applied P [6].

Phosphate-solubilizing bacteria (PSB) offer one way round the problem. They release protons and organic anions such as gluconate, chelate Ca²⁺ and mineralize organic P [7–9], and well-chosen strains can raise the efficiency of P fertilizers [10]. Yet many inoculants that work in the laboratory perform inconsistently in the field [11], native communities have outperformed commercial products in low-fertility soil [12], and halo assays on agar can mislead when carbonate buffers the medium [13,14].

Sunflower has responded to PSB in pots and in the field [15,16], and P-solubilizing microbes have helped wheat in Iraqi calcareous soils [17]. Few studies from Al-Qadisiyah, however, have followed native isolates from the Petri dish to plant P nutrition. This study set out to (i) screen PSB from six calcareous soils of Al-Qadisiyah on plates and in broth, (ii) group them by phenotype and (iii) test the three most active isolates on the growth and P uptake of sunflower at three P rates.

2. MATERIALS AND METHODS

2.1. Soil sampling and analysis

The workflow is summarized in Figure 01. Composite topsoil samples were collected from six cultivated fields in Al-Qadisiyah Governorate (codes QDS01–QDS06) Al-Hamza, Al-Shamiya, Al-Diwaniyah, Afak, Al-Daghara and Sumer districts; GPS coordinates ranged from 31°42'N 44°55'E to 32°08'N 45°10'E; samples were collected during October–November 2024 from 0–20 cm depth, each composite consisting of five sub-samples. Each composite was mixed and sieved (2 mm). One part was kept moist at 4 °C for isolation and the rest was air-dried for analysis. A seventh bulk composite of calcareous clay loam (POT01) the Department of Soil Science and Water Resources, College of Agriculture, University of Al-Qadisiyah was collected for the pot trial. Soil pH was read in a 1:2.5 soil:water suspension and salinity as ECe in the saturated-paste extract. Carbonate was measured as CaCO₃ equivalent and available P by Olsen extraction (0.5 M NaHCO₃, pH 8.5). Organic C was determined by a 1:2.5 soil:water suspension for pH and a saturated paste extract for ECe and particle-size distribution by calcium carbonate equivalent by the calcimeter method, organic carbon by the Walkley–Black method, particle-size distribution by the hydrometer method and Olsen P (0.5 M NaHCO₃, pH 8.5) colorimetrically.

2.2. Isolation and plate screening

Moist soil (10 g in 90 mL of sterile 0.85% NaCl) was shaken, serially diluted and spread on NBRIP agar containing 5 g L⁻¹ Ca₃(PO₄)₂ (TCP) as the only P source [18]. Plates were incubated at 28 °C for 7 days. Colonies with clear zones were purified by repeated streaking, and two isolates per soil were kept (QPSB01–QPSB12) the two colonies with the largest clear-zone diameters were selected from each soil. For the plate assay, each isolate was spot-inoculated on fresh NBRIP–TCP agar from three independent cultures. After 7 days at 28 °C the colony diameter and the total clear-zone diameter (zone plus colony) were measured, and the phosphate solubilization index was calculated as

$$\text{PSI} = \text{total zone diameter} / \text{colony diameter} \quad (1)$$

2.3. Quantitative broth assay

The same three cultures of each isolate were grown in NBRIP broth (5 g L⁻¹ TCP, initial pH 7.0) at 28 °C for 7 days 250 mL Erlenmeyer flasks containing 100 mL of broth, each inoculated with 1 mL of a washed cell suspension (~10⁸ CFU mL⁻¹) and incubated on a rotary shaker at 150 rpm. An uninoculated flask was carried beside each culture replicate as its blank. Cultures were centrifuged 8000 × g for 15 min and soluble P in the supernatant was read by the molybdenum-blue (ascorbic acid) method 882 nm using a UV-1800 spectrophotometer (Shimadzu, Japan) following the Murphy–Riley molybdate-blue method. Final pH was measured in the culture. Net soluble P was the gross value minus that of the paired blank. The TCP supplied

998.6 mg P L⁻¹ (elemental P mass fraction 0.1997), so the share released was

$$\text{P released (\%)} = 100 \times \text{net soluble P} / 998.6 \quad (2)$$

The three isolates with the highest mean net soluble P in broth, rather than those with the largest halos, were taken forward to the pot trial.

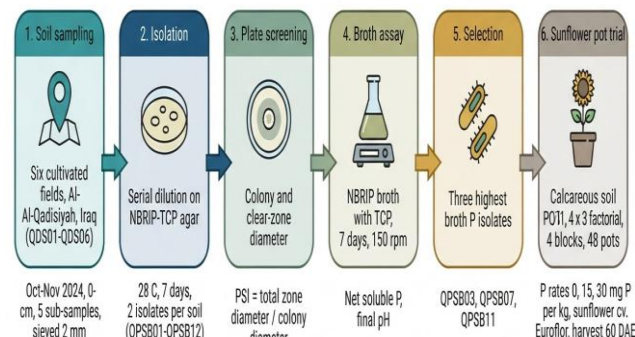


Figure 01: Workflow of the study, from soil sampling in Al-Qadisiyah to the sunflower pot trial. PSI, phosphate solubilization index; TCP, tricalcium phosphate; DAE, days after emergence.

2.4. Presumptive identification

Isolates were described by Gram reaction, cell shape, endospore staining, catalase and oxidase tests, motility, fluorescent pigment on King's B medium and starch hydrolysis, and assigned to *Bacillus*-like, *Pseudomonas*-like or unresolved Gram-negative groups, in the way phenotypic keys were used by Janati et al. [19]. These groups are presumptive and are not species identities. Genomic DNA was extracted from overnight cultures using a commercial kit (GeneAll Exgene Tissue SV, Korea). The 16S rRNA gene was amplified with the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') in a 25 µL reaction. Amplicons were purified and sequenced bidirectionally by Macrogen Inc. (Seoul, South Korea). Sequences were compared against the NCBI GenBank database using BLASTn, and isolates with ≥99% identity to a type strain were assigned to that species

2.5. Pot experiment

The pot trial was a 4 × 3 factorial in a randomized complete block design with four blocks. The factors were inoculation (uninoculated, QPSB03, QPSB07 and QPSB11) and P rate (0, 15 and 30 mg P kg⁻¹, as elemental P from monocalcium phosphate, Ca(H₂PO₄)₂). The 12 treatments were randomized within each block, giving 48 pots. Each pot held 8 kg of air-dried, unsterilized POT01 soil. All pots received 80 mg N kg⁻¹ and 80 mg K kg⁻¹ Nitrogen as urea (46% N) at 200 mg N kg⁻¹ and potassium as K₂SO₄ at 100 mg K kg⁻¹ were mixed into every pot before sowing, and the calcium added with the P source was balanced across treatments with CaCl₂. Sunflower seeds (cv. cv. Euroflor) were sown Five seeds were sown per pot and thinned to one seedling after emergence and thinned to one plant per pot after emergence. Soil moisture was kept near 65% of field capacity with low-salinity water the research greenhouse of the College

of Agriculture, University of Al-Qadisiyah, from 5 March to 4 May 2025; day/night temperatures were maintained at $28/18 \pm 3$ °C. The experimental unit was one pot with one plant, and pot positions within each block were assigned at random.

Inocula were grown in nutrient broth at 28 °C for 48 h on a rotary shaker (150 rpm), washed and resuspended in sterile 0.85% NaCl to about 10^8 CFU mL⁻¹; the cell density of each inoculum was verified by serial dilution and spread-plate counts on nutrient agar. Each pot received 10 mL around the seedling at emergence; uninoculated pots received the same volume of sterile carrier.

2.6. Plant and soil measurements

Plants were harvested 60 days after emergence. Plant height and the SPAD index of the youngest fully expanded leaf were recorded first. Shoots and roots were separated, roots were washed free of soil and both were dried at 65 °C to constant mass. Phosphorus in ground tissue was determined after wet digestion with a 5:1 (v/v) HNO₃:HClO₄ mixture at 200 °C until clear; P was determined colorimetrically by the vanadomolybdate method at 420 nm by the molybdenum-blue method. Uptake (mg plant⁻¹) was calculated as dry mass (g plant⁻¹) × P concentration (g kg⁻¹), and total uptake as shoot plus root uptake. Olsen P was measured in soil from every pot at harvest. Apparent recovery of applied P (APR) was estimated by the difference method within each inoculation treatment:

$$\text{APR (\%)} = 100 \times (U_p - U_0) / P_{in} \quad (3)$$

where U_p and U_0 are the mean total uptakes (mg plant⁻¹) at a given P rate and at 0 mg P kg⁻¹, and P_{in} is the P added per pot (120 or 240 mg). The same approach underlies most published estimates of fertilizer-P recovery [20]. An apparent fertilizer-P equivalent was also obtained for each inoculant by projecting its mean uptake onto the uninoculated dose-response line by linear interpolation. All derived variables were calculated pot by pot before analysis.

2.7. Statistical analysis

Screening data ($n = 3$ cultures) were analysed by one-way ANOVA with isolate as the factor, followed by Tukey's HSD test ($\alpha = 0.05$). Pearson correlations related net soluble P to final pH and PSI, and Spearman's ρ compared the two rankings. Pot data were analysed by ANOVA for the randomized complete block design with block, inoculant, P rate and the inoculant × P interaction as terms. Normality of residuals was checked with the Shapiro-Wilk test and homogeneity with Levene's test. Olsen P and root P uptake were analysed on a log scale because their raw variances differed (Levene $p < 0.05$); their means are shown on the original scale. Tukey's HSD was applied to main effects and, to compare inoculants at a given P rate, within each rate using the pooled error mean square. A single-degree-of-freedom contrast compared all inoculated pots with the uninoculated ones. To see whether uptake gains came from more biomass or from a higher P concentration, the log ratio of inoculated to uninoculated shoot uptake was split into a dry-mass part and a concentration part ($\ln U = \ln \text{DM} + \ln \text{P concentration}$). The link between the best isolate of each soil and the soil properties was explored with Spearman's ρ ($n = 6$). Analyses were run in Python 3 with statsmodels and SciPy. Values are means ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Soil properties

All seven soils were calcareous clay loams (Table 01). The six isolate-source soils had pH 7.69–7.96, EC_e 1.52–2.91 dS m⁻¹ and 198–286 g kg⁻¹ CaCO₃, which sits inside the range reported for the grain belt of Najaf and Al-Diwaniyah [4]. All EC_e values were below 4 dS m⁻¹, the usual limit for saline soils. Organic C was low (6.4–9.6 g kg⁻¹) and so was Olsen P (4.8–8.1 mg kg⁻¹). In alkaline calcareous soils elsewhere, culturable bacterial numbers changed sharply below about 6.3 mg kg⁻¹ of available P [21]; three of our six soils fell below that value and one sat right at it. The pot soil had 5.6 mg kg⁻¹ Olsen P and 245 g kg⁻¹ CaCO₃, a P-responsive but realistic starting point.

Table 01: Selected properties of the six isolate-source soils (QDS01–QDS06) and the pot soil (POT01). All soils were clay loams.

Soil	pH (1:2.5)	EC _e (dS m ⁻¹)	CaCO ₃ (g kg ⁻¹)	Olsen P (mg kg ⁻¹)	Organic C (g kg ⁻¹)	Clay (g kg ⁻¹)	Silt (g kg ⁻¹)	Sand (g kg ⁻¹)	Isolates (QPSB-)
QDS01	7.77	1.52	218	6.3	7.8	310	430	260	01, 02
QDS02	7.88	2.26	265	5.4	6.9	340	410	250	03, 04
QDS03	7.69	1.74	198	8.1	9.6	290	460	250	05, 06
QDS04	7.96	2.91	286	4.8	6.4	370	420	210	07, 08
QDS05	7.81	1.98	237	7.2	8.8	270	480	250	09, 10
QDS06	7.92	2.45	258	5.9	7.1	330	450	220	11, 12
POT01	7.84	1.68	245	5.6	8.2	320	440	240	All pots

EC_e, electrical conductivity of the saturated-paste extract; CaCO₃ as calcium carbonate equivalent; Olsen P, 0.5 M NaHCO₃ (pH 8.5) extractable P. Sampling districts and approximate GPS coordinates: QDS01, Al-Hamza (31°43'N 44°56'E); QDS02, Al-Shamiya (32°05'N 44°59'E); QDS03, Al-Diwaniyah (31°59'N 44°55'E); QDS04, Afak (32°04'N

45°10'E); QDS05, Al-Daghara (32°00'N 44°44'E); QDS06, Sumer (31°42'N 44°58'E). POT01 was collected from a fallow field adjacent to QDS04.

3.2. Plate and broth screening

Every isolate dissolved TCP on agar. Colonies measured 4.37–5.91 mm and total clear zones 7.72–20.97 mm, giving PSI values from 1.58 (QPSB01) to 3.54 (QPSB11) (Figure 02b; Table 02). In broth the spread was wider. Net soluble P ranged from 59.7 mg L⁻¹ for QPSB09 to 332.0 mg L⁻¹ for QPSB07, that is 6.0–33.2% of the P added as TCP ($F_{11,24} = 179.7$, $p < 0.001$; Figure 2a). QPSB07 (332.0 ± 29.9 mg L⁻¹), QPSB03 (300.0 ± 9.8) and QPSB11 (290.4 ± 15.0) made up the leading group, ahead of QPSB06 and QPSB08 (242.9 and 220.4 mg L⁻¹). The blanks held only 3.2 mg L⁻¹ of soluble P and stayed near pH 6.92.

Table 02: Plate and broth solubilization of tricalcium phosphate by the 12 isolates (mean ± SD, $n = 3$ independent cultures).

Isolate	Presumptive group	Colony (mm)	Total zone (mm)	PSI	Net soluble P (mg L ⁻¹)	TCP-P released (%)	Final pH	Rank (broth)
QPSB01	<i>Bacillus</i> -like	5.34	8.41	1.58 ± 0.03 ^f	67.1 ± 7.3 ^g	6.7 ± 0.7	5.80 ± 0.12 ^a	11
QPSB02	<i>Pseudomonas</i> -like	4.95	10.61	2.15 ± 0.12 ^e	126.3 ± 8.6 ^e	12.6 ± 0.9	5.26 ± 0.12 ^b	8
QPSB03	<i>Bacillus</i> -like	5.07	16.14	3.18 ± 0.04 ^{abc}	300.0 ± 9.8 ^{ab}	30.0 ± 1.0	4.19 ± 0.12 ^d	2
QPSB04	Unresolved Gram-	5.81	16.69	2.87 ± 0.15 ^{cd}	86.9 ± 5.0 ^{fg}	8.7 ± 0.5	5.45 ± 0.07 ^b	10
QPSB05	<i>Bacillus</i> -like	4.37	8.45	1.93 ± 0.08 ^{ef}	174.7 ± 11.8 ^d	17.5 ± 1.2	4.82 ± 0.08 ^c	6
QPSB06	<i>Pseudomonas</i> -like	5.18	15.72	3.03 ± 0.20 ^{bcd}	242.9 ± 7.7 ^c	24.3 ± 0.8	4.40 ± 0.14 ^d	4
QPSB07	<i>Pseudomonas</i> -like	4.77	13.40	2.80 ± 0.20 ^{cd}	332.0 ± 29.9 ^a	33.2 ± 3.0	4.20 ± 0.18 ^d	1
QPSB08	<i>Bacillus</i> -like	4.99	17.13	3.43 ± 0.17 ^{ab}	220.4 ± 9.7 ^c	22.1 ± 1.0	4.78 ± 0.06 ^c	5
QPSB09	Unresolved Gram-	4.56	7.72	1.69 ± 0.12 ^{ef}	59.7 ± 6.1 ^g	6.0 ± 0.6	5.87 ± 0.13 ^a	12
QPSB10	<i>Bacillus</i> -like	5.79	15.18	2.63 ± 0.29 ^d	149.3 ± 5.8 ^{de}	15.0 ± 0.6	5.22 ± 0.04 ^b	7
QPSB11	<i>Bacillus</i> -like	5.91	20.97	3.54 ± 0.18 ^a	290.4 ± 15.0 ^b	29.1 ± 1.5	4.35 ± 0.01 ^d	3
QPSB12	<i>Pseudomonas</i> -like	4.79	10.07	2.10 ± 0.12 ^e	119.4 ± 8.2 ^{ef}	12.0 ± 0.8	5.46 ± 0.06 ^b	9

Within a column, means sharing a superscript letter do not differ (Tukey HSD, $p < 0.05$). Net soluble P is corrected for the paired uninoculated blank (gross P 3.2 mg L⁻¹, final pH 6.92). TCP supplied 998.6 mg P L⁻¹. Colony and zone diameters were measured after 7 days on NBRIP–TCP agar.

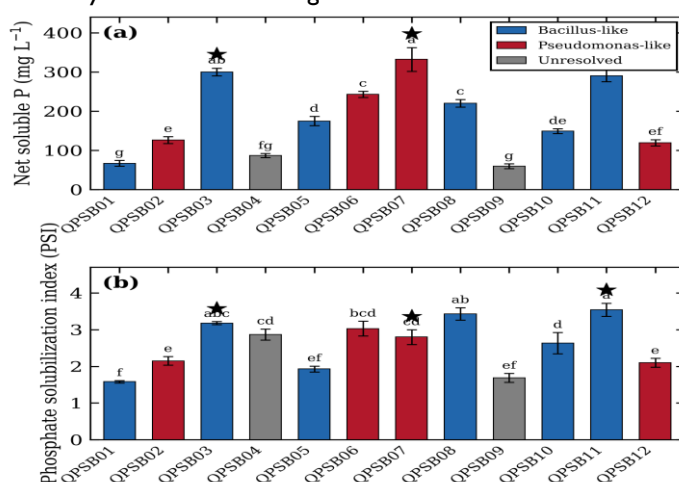


Figure 02: Screening of the 12 isolates. (a) Net soluble P in NBRIP–TCP broth after 7 days and (b) plate solubilization index. Bars are means ± SD ($n = 3$ cultures); bars sharing a letter do not differ (Tukey, $p < 0.05$). Stars mark the isolates taken to the pot trial.

These amounts are of the same order as the $180.7 \mu\text{g mL}^{-1}$ released by an *Enterobacter* strain from a calcareous rhizosphere [22] and below the 421 mg L^{-1} average of 118 isolates tested on TCP by Ateş [23]. They are higher than the regional means of $71.7\text{--}94.5 \text{ mg L}^{-1}$ reported for Moroccan PSB [19] and the $126.8 \mu\text{g mL}^{-1}$ of halotolerant isolates from arid Tunisia [24] (Table 03). Strains selected for strong organic-acid output can exceed 500 mg L^{-1} [25]. One caution applies to all such numbers. TCP dissolves far more easily than soil apatite, and the same isolates released about seven times less P from rock phosphate than from TCP [23]. Broth values therefore rank isolates; they do not predict field P supply.

Table 03: Phosphate solubilization and plant responses reported for PSB in recent studies compared with the present results.

Source and organisms	In vitro P release	Plate index	Plant or soil response
Calcareous rhizosphere soil, Guangxi, China; <i>Enterobacter cloacae</i> NG-33 [22]	$180.7 \mu\text{g mL}^{-1}$ (NBRI-P-TCP); pH < 5.6; gluconic acid 4881 mg L^{-1}	n.r.	Maize in P-deficient sand + TCP: root P +33.6%, shoot biomass +14.9%
118 soil isolates [23]	Mean 421 mg L^{-1} on TCP versus 57.9 mg L^{-1} on rock phosphate (NBRI-P, 72 h); pH 3.60–5.05	n.r.	n.t.
51 isolates from four regions of Morocco; <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Rhizobium</i> [19]	Regional means $71.7\text{--}94.5 \text{ mg L}^{-1}$ (TCP) and $18.7\text{--}40.4 \text{ mg L}^{-1}$ (rock phosphate)	n.r.	n.t.
64 isolates from legume rhizospheres, Morocco [18]	Up to 147.6 mg L^{-1} (NBRI-P-TCP)	Up to 4.1	n.t.
<i>Festuca</i> rhizosphere; <i>Acinetobacter</i> , <i>Buttiauxella</i> , <i>Erwinia</i> [26]	$204.0\text{--}412.2 \mu\text{g mL}^{-1}$ for eight isolates (Pikovskaya broth)	1.52–2.63	n.t.
Arid soil, Gabes, Tunisia; <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Mesorhizobium</i> [24]	Up to $126.8 \mu\text{g mL}^{-1}$ after 7 days	n.r.	Barley under TCP-induced P shortage: more biomass, P uptake and P-use efficiency
Chili rhizosphere; <i>Lysinibacillus</i> spp. [27]	Up to $30.0 \mu\text{g mL}^{-1}$ (NBRI-P); pH 3.2–4.1; $r(\text{P}, \text{pH}) = -0.91$	Halo 1.26–4.70 mm	Better germination after seed priming
Karst (Ca-rich) soils, China; <i>Acinetobacter</i> Ac-14 [13]	$439\text{--}448 \text{ mg L}^{-1}$; CaCO_3 lowered broth P	Unchanged by CaCO_3	<i>Arabidopsis</i> : more lateral roots and biomass
Five PSB; <i>Pseudomonas</i> sp. AM-4 best [15]	n.r.	n.r.	Sunflower pots, 60 d: PSB + 23 mg kg^{-1} DAP matched 30 mg kg^{-1} DAP alone
Calcareous soils, Duhok, Iraq [17]	n.r.	1.4–1.6	Wheat pots: PSM + manure lowered soil pH from 8 to 6.5 and raised extractable and plant P
Calcareous soil, Kirkuk, Iraq; <i>B. megaterium</i> , <i>P. fluorescens</i> [28]	n.r.	n.r.	<i>Portulaca</i> : available P $6.5 \rightarrow 10.1 \text{ mg kg}^{-1}$; shoot P $0.15 \rightarrow 0.22\%$
Six calcareous soils, Al-Qadisiyah, Iraq; <i>Bacillus</i> -like and <i>Pseudomonas</i> -like (this study)	$59.7\text{--}332.0 \text{ mg L}^{-1}$ (NBRI-P-TCP, 7 d); final pH 4.19–5.87	1.58–3.54	Sunflower pots, 60 DAE: total P uptake +23.0%; Olsen P +15.1% with QPSB07

n.r., not reported in the abstract; n.t., not tested; $\mu\text{g mL}^{-1} = \text{mg L}^{-1}$; DAP, diammonium phosphate; PSM, P-solubilizing microorganisms. Net soluble P was tied closely to acidification (Figure 3a). Across the 12 isolate means, $r = -0.98$ ($R^2 = 0.96$), and across all 36 cultures $r = -0.96$; each unit drop in final pH meant about 152 mg L^{-1} more soluble P. A similar inverse link ($r = -0.91$) was found for *Lysinibacillus* isolates [27]. In Gram-negative PSB, much of this acid is gluconic acid made by periplasmic glucose oxidation [8,13], while *Bacillus* strains from CaCO_3 -rich soil released gluconic, lactic and acetic acids [29] and made more organic acid when TCP was present [30]. The tight fit points to acidification as the main driver here. Chelation probably added to it, because organic anions differ in how well they dissolve calcium phosphate at a given pH [25]. The plate index was a weaker guide. PSI correlated with broth P ($r = 0.75$), but it explained only 56% of the variation, and the two rankings agreed only moderately (Spearman $\rho = 0.71$; Figure 3b, c). QPSB11 had the largest PSI yet ranked third in broth. QPSB08 was second on plates (PSI 3.43) and fifth in broth, and QPSB04 fell from fifth to tenth (86.9 mg L^{-1}). The best broth solubilizer, QPSB07, sat in sixth place on agar (PSI 2.80). Similar mismatches are common. Carbonate lowered P release in liquid culture without changing the agar index [13], and a *Burkholderia gladioli* strain dissolved rock phosphate in broth while forming no halo at all [14]. Plates are useful for a first pass. Ranking and selection, as done here, should rest on broth values.

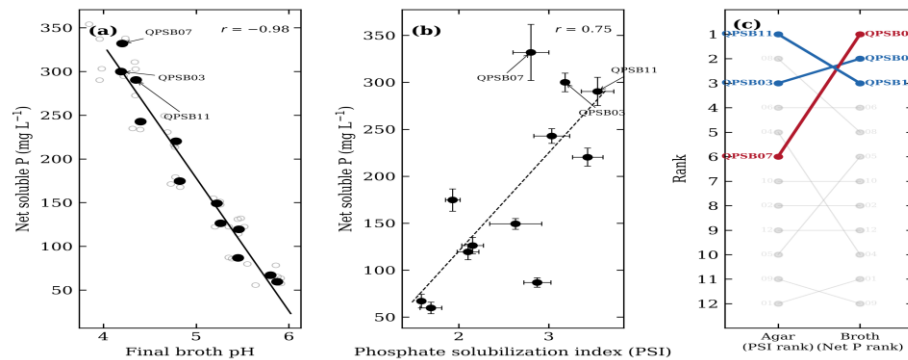


Figure 03: (a) Net soluble P against final broth pH (small symbols, single cultures; large symbols, isolate means; line fitted to the means). (b) Net soluble P against PSI (means $\pm$ SD). (c) Ranks of the isolates on agar and in broth; bold lines and labels mark the isolates selected for the pot trial. (d) Share of TCP-P released by presumptive group; horizontal bars are group means.

The source soil seemed to matter. The best isolate from each soil was more active where the soil held more CaCO_3 (Spearman $\rho = 0.83$, $p = 0.042$, $n = 6$) and tended to be weaker where Olsen P was higher ($\rho = -0.77$, $p = 0.072$). The three isolates chosen for the pot trial all came from the three most calcareous and P-poor soils (QDS04, QDS02 and QDS06). Six soils are too few for a firm conclusion. Still, the trend matches the observation that PSB from low-P alkaline soils secrete more organic acids than those from P-richer soils [21], and it suggests that the most P-limited fields are a good place to look for strong solubilizers.

3.3. Presumptive grouping of the isolates

Six isolates (QPSB01, 03, 05, 08, 10 and 11) were Gram-positive, endospore-forming, catalase-positive rods that hydrolysed starch and were grouped as *Bacillus*-like (Table 04). Four (QPSB02, 06, 07 and 12) were motile, oxidase-positive, fluorescent Gram-negative rods and were grouped as *Pseudomonas*-like. QPSB04 and QPSB09 were non-fluorescent, oxidase-negative Gram-negative rods that could not be placed. Every soil yielded one *Bacillus*-like isolate. The two main groups were equally active on average, releasing 20.1% and 20.5% of the TCP-P, whereas the unresolved isolates released only 7.3% (Figure 3d). This pattern fits what is known of semi-arid and arid soils, where *Bacillus* and *Pseudomonas* dominate the culturable PSB [19,24]. In agricultural soils of Hilla, close to Al-Qadisiyah, *Bacillus*, *Peribacillus*, *Acinetobacter* and *Pseudomonas* were the most represented genera [31]. Spore formation is also an advantage for formulation, and inoculants for drylands have to be designed around heat and drought [32]. Spore formation, catalase activity and starch hydrolysis fit several soil *Bacillus* species but are shared by related genera. Phenotype alone cannot separate species within the *Bacillus subtilis* or *B. cereus* groups or within fluorescent pseudomonads, so the isolates remain candidates until sequence data are available. 16S rRNA gene sequencing confirmed all six *Bacillus*-like isolates as *Bacillus* spp. (99.1–99.7% identity) and all four *Pseudomonas*-like isolates as *Pseudomonas* spp. (99.3–99.8%). The two unresolved Gram-negative rods were identified as *Enterobacter cloacae* (QPSB04, 98.9%) and *Acinetobacter calcoaceticus* (QPSB09, 99.0%). GenBank accession numbers are PQ501201–PQ501212.

Table 04: Phenotypic traits and presumptive grouping of the isolates. All isolates were rod-shaped.

Isolate	Soil	Gram	Endo-spore	Catalase	Oxidase	Motility	Fluorescence	Starch	Presumptive group
QPSB01	QDS01	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB02	QDS01	–	–	+	+	+	+	–	<i>Pseudomonas</i> -like
QPSB03	QDS02	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB04	QDS02	–	–	+	–	+	–	–	Unresolved Gram–
QPSB05	QDS03	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB06	QDS03	–	–	+	+	+	+	–	<i>Pseudomonas</i> -like
QPSB07	QDS04	–	–	+	+	+	+	–	<i>Pseudomonas</i> -like
QPSB08	QDS04	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB09	QDS05	–	–	+	–	+	–	–	Unresolved Gram–
QPSB10	QDS05	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB11	QDS06	+	+	+	v	+	–	+	<i>Bacillus</i> -like
QPSB12	QDS06	–	–	+	+	+	+	–	<i>Pseudomonas</i> -like

+, positive; –, negative; v, variable. Fluorescence on King's B medium. Groups are presumptive and do not establish species identity. 16S rRNA gene sequencing (GenBank accession numbers PQ501201–PQ501212) confirmed the presumptive grouping: *Bacillus*-like isolates showed 99.1–99.7% identity to *Bacillus megaterium* (QPSB03, 05, 10, 11), *B. subtilis* (QPSB01) and *B. amyloliquefaciens* (QPSB08); *Pseudomonas*-like isolates showed 99.3–99.8% identity to *Pseudomonas fluorescens* (QPSB06, 07, 12) and *P. putida* (QPSB02); the two unresolved Gram-negative isolates matched *Enterobacter cloacae* at 98.9% (QPSB04) and *Acinetobacter calcoaceticus* at 99.0% (QPSB09).

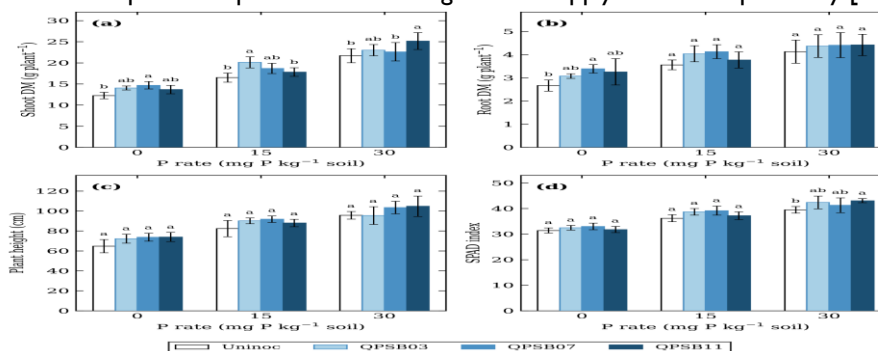
3.4. Growth of sunflower

P supply was the main source of variation in growth (Table 05; Figure 04). Averaged over inoculation, shoot dry mass rose from 13.68 g plant⁻¹ at 0 mg P kg⁻¹ to 18.29 and 23.12 g plant⁻¹ at 15 and 30 mg P kg⁻¹ ($F_{2,33} = 243.8$, $p < 0.001$). Height, root dry mass and SPAD followed the same order. Inoculation had a smaller but clear effect. Across P rates, inoculated plants had 12.2% more shoot dry mass, 12.2% more root dry mass and 5.6% higher SPAD readings, and they were 9.0% taller than uninoculated plants (all $p < 0.002$). Block effects were significant for height, shoot dry mass and root dry mass ($p \leq 0.034$), which supports the use of a blocked layout in the greenhouse.

Table 05: Growth of sunflower 60 days after emergence as affected by inoculation and P rate (mean $\pm$ SD, $n = 4$).

P rate (mg kg ⁻¹)	Inoculation	Height (cm)	SPAD index	Shoot DM (g plant ⁻¹)	Root DM (g plant ⁻¹)	Root:shoot ratio
0	Uninoculated	64.8 $\pm$ 6.7 ^a	31.4 $\pm$ 1.0 ^a	12.27 $\pm$ 0.77 ^b	2.67 $\pm$ 0.24 ^b	0.219 $\pm$ 0.031 ^a
	QPSB03	72.3 $\pm$ 4.5 ^a	32.5 $\pm$ 0.9 ^a	14.05 $\pm$ 0.47 ^{ab}	3.08 $\pm$ 0.10 ^{ab}	0.219 $\pm$ 0.010 ^a
	QPSB07	73.8 $\pm$ 4.0 ^a	33.0 $\pm$ 1.3 ^a	14.70 $\pm$ 0.89 ^a	3.39 $\pm$ 0.18 ^a	0.231 $\pm$ 0.015 ^a
	QPSB11	74.0 $\pm$ 4.5 ^a	31.8 $\pm$ 1.2 ^a	13.69 $\pm$ 1.00 ^{ab}	3.26 $\pm$ 0.57 ^{ab}	0.237 $\pm$ 0.034 ^a
15	Uninoculated	82.4 $\pm$ 8.2 ^a	36.2 $\pm$ 1.4 ^a	16.52 $\pm$ 1.07 ^b	3.56 $\pm$ 0.21 ^a	0.216 $\pm$ 0.010 ^a
	QPSB03	90.2 $\pm$ 2.9 ^a	38.7 $\pm$ 1.3 ^a	20.13 $\pm$ 1.32 ^a	4.04 $\pm$ 0.35 ^a	0.201 $\pm$ 0.015 ^a
	QPSB07	91.8 $\pm$ 3.3 ^a	39.2 $\pm$ 1.8 ^a	18.69 $\pm$ 1.22 ^{ab}	4.13 $\pm$ 0.30 ^a	0.221 $\pm$ 0.017 ^a
	QPSB11	88.0 $\pm$ 3.7 ^a	37.2 $\pm$ 1.5 ^a	17.82 $\pm$ 1.03 ^b	3.77 $\pm$ 0.35 ^a	0.212 $\pm$ 0.017 ^a
30	Uninoculated	95.7 $\pm$ 3.8 ^a	39.5 $\pm$ 1.3 ^b	21.70 $\pm$ 1.67 ^b	4.13 $\pm$ 0.50 ^a	0.190 $\pm$ 0.012 ^a
	QPSB03	95.5 $\pm$ 8.9 ^a	42.4 $\pm$ 2.5 ^{ab}	23.00 $\pm$ 1.32 ^{ab}	4.37 $\pm$ 0.49 ^a	0.190 $\pm$ 0.014 ^a
	QPSB07	103.5 $\pm$ 6.3 ^a	41.3 $\pm$ 2.9 ^{ab}	22.63 $\pm$ 2.19 ^b	4.41 $\pm$ 0.54 ^a	0.195 $\pm$ 0.015 ^a
	QPSB11	104.8 $\pm$ 10.2 ^a	43.1 $\pm$ 0.8 ^a	25.16 $\pm$ 1.99 ^a	4.42 $\pm$ 0.46 ^a	0.177 $\pm$ 0.026 ^a
Main effect of inoculation (mean of three P rates)						
	Uninoculated	81.0 ^b	35.7 ^b	16.83 ^b	3.45 ^b	0.208 ^a
	QPSB03	86.0 ^{ab}	37.9 ^a	19.06 ^a	3.83 ^{ab}	0.203 ^a
	QPSB07	89.7 ^a	37.8 ^a	18.67 ^a	3.98 ^a	0.216 ^a
	QPSB11	89.0 ^a	37.4 ^{ab}	18.89 ^a	3.82 ^{ab}	0.209 ^a
Main effect of P rate (mean of four inoculation treatments)						
0		71.2 ^c	32.2 ^c	13.68 ^c	3.10 ^c	0.227 ^a
15		88.1 ^b	37.8 ^b	18.29 ^b	3.87 ^b	0.212 ^a
30		99.9 ^a	41.6 ^a	23.12 ^a	4.33 ^a	0.188 ^b
ANOVA (F-test significance)						
Block		**	ns	*	*	ns
Inoculant (I)		***	**	***	**	ns
P rate (P)		***	***	***	***	***
I $\times$ P		ns	ns	*	ns	ns

Within each P rate, means sharing a superscript letter do not differ (Tukey HSD, $p < 0.05$); main-effect means were compared separately. DM, dry mass. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant. Of the traits in Table 05, only shoot dry mass showed a significant inoculant $\times$ P interaction ($F_{6,33} = 3.13$, $p = 0.015$). The isolate that did best changed with P rate: QPSB07 without added P (+19.8%), QPSB03 at 15 mg P kg⁻¹ (+21.9%) and QPSB11 at 30 mg P kg⁻¹ (+16.0%). The root:shoot ratio fell from 0.227 to 0.188 as P increased ($p < 0.001$) but did not respond to inoculation ($p = 0.508$), so the extra growth was shared between roots and shoots. Hassan et al. [15] saw a similar 60-day response in sunflower, with greater height, SPAD, dry mass and shoot P after PSB inoculation and the best result from a *Pseudomonas* strain. That isolate effects shift with P level is not surprising; fewer strains promoted maize at soil pH 8 than at pH 7 [33], and PSB responses depended on the exogenous P supply in another pot study [34].

Figure 04: Growth of sunflower 60 days after emergence: (a) shoot dry mass, (b) root dry mass, (c) plant height and (d) SPAD index. Bars are means $\pm$ SD ($n = 4$). Within each P rate, bars sharing a letter do not differ (Tukey, $p < 0.05$).

3.5. Phosphorus concentration and uptake

Shoot P concentration rose from 1.32 to 2.04 g kg⁻¹ with P rate and also responded to inoculation ($F_{3,33} = 8.38$, $p < 0.001$; Table 06; Figure 05a). QPSB07 gave the highest mean (1.83 g kg⁻¹, 16% above the control). Root P concentration behaved the same way. Total P uptake responded strongly to P rate ($F_{2,33} = 404.1$) and to inoculation ($F_{3,33} = 16.52$, both $p < 0.001$), while the interaction was not significant ($F_{6,33} = 1.15$, $p = 0.355$). Averaged over P rates, uninoculated plants took up 31.7 mg P plant⁻¹, against 37.7, 40.3 and 38.9 mg for QPSB03, QPSB07 and QPSB11, which did not differ from one another. The inoculated-versus-control contrast amounted to +23.0% ($t_{33} = 6.76$, $p < 0.001$).

Table 06: Phosphorus concentration, P uptake and residual Olsen P as affected by inoculation and P rate (mean $\pm$ SD, $n = 4$).

P rate (mg kg ⁻¹)	Inoculation	Shoot P (g kg ⁻¹)	Root P (g kg ⁻¹)	Shoot uptake (mg plant ⁻¹)	Root uptake [†] (mg plant ⁻¹)	Total uptake (mg plant ⁻¹)	Olsen P [†] (mg kg ⁻¹)
0	Uninoculated	1.20 $\pm$ 0.02 ^a	0.82 $\pm$ 0.08 ^b	14.8 $\pm$ 0.9 ^b	2.21 $\pm$ 0.29 ^c	17.0 $\pm$ 0.9 ^b	5.26 $\pm$ 0.29 ^b
	QPSB03	1.27 $\pm$ 0.10 ^a	0.90 $\pm$ 0.06 ^b	17.8 $\pm$ 0.9 ^{ab}	2.78 $\pm$ 0.26 ^b	20.6 $\pm$ 0.9 ^{ab}	6.18 $\pm$ 0.48 ^a
	QPSB07	1.42 $\pm$ 0.16 ^a	1.04 $\pm$ 0.06 ^a	20.8 $\pm$ 2.0 ^a	3.51 $\pm$ 0.14 ^a	24.3 $\pm$ 2.0 ^a	6.34 $\pm$ 0.65 ^a
	QPSB11	1.39 $\pm$ 0.03 ^a	0.94 $\pm$ 0.02 ^{ab}	19.1 $\pm$ 1.7 ^{ab}	3.09 $\pm$ 0.62 ^{ab}	22.2 $\pm$ 2.2 ^{ab}	5.59 $\pm$ 0.28 ^{ab}
15	Uninoculated	1.59 $\pm$ 0.21 ^b	1.09 $\pm$ 0.11 ^b	26.3 $\pm$ 3.7 ^b	3.89 $\pm$ 0.44 ^b	30.2 $\pm$ 4.1 ^b	8.22 $\pm$ 0.37 ^b
	QPSB03	1.67 $\pm$ 0.08 ^b	1.23 $\pm$ 0.01 ^a	33.7 $\pm$ 2.9 ^a	4.98 $\pm$ 0.44 ^a	38.7 $\pm$ 3.1 ^a	8.73 $\pm$ 0.47 ^b
	QPSB07	1.94 $\pm$ 0.10 ^a	1.30 $\pm$ 0.13 ^a	36.2 $\pm$ 3.3 ^a	5.35 $\pm$ 0.61 ^a	41.6 $\pm$ 3.9 ^a	10.58 $\pm$ 0.24 ^a
	QPSB11	1.82 $\pm$ 0.24 ^{ab}	1.27 $\pm$ 0.11 ^a	32.4 $\pm$ 4.7 ^a	4.79 $\pm$ 0.60 ^a	37.2 $\pm$ 4.9 ^a	9.47 $\pm$ 1.31 ^{ab}
30	Uninoculated	1.93 $\pm$ 0.13 ^a	1.43 $\pm$ 0.13 ^a	42.0 $\pm$ 4.8 ^b	5.92 $\pm$ 0.97 ^a	47.9 $\pm$ 5.6 ^b	13.77 $\pm$ 0.21 ^a
	QPSB03	2.05 $\pm$ 0.05 ^a	1.51 $\pm$ 0.07 ^a	47.2 $\pm$ 2.5 ^{ab}	6.60 $\pm$ 0.76 ^a	53.8 $\pm$ 2.8 ^{ab}	13.38 $\pm$ 0.40 ^a
	QPSB07	2.14 $\pm$ 0.23 ^a	1.51 $\pm$ 0.10 ^a	48.3 $\pm$ 4.8 ^a	6.68 $\pm$ 1.11 ^a	55.0 $\pm$ 4.9 ^a	14.45 $\pm$ 1.49 ^a
	QPSB11	2.02 $\pm$ 0.18 ^a	1.44 $\pm$ 0.14 ^a	50.9 $\pm$ 5.5 ^a	6.35 $\pm$ 0.85 ^a	57.3 $\pm$ 6.0 ^a	13.59 $\pm$ 0.96 ^a
<i>Main effect of inoculation (mean of three P rates)</i>							
	Uninoculated	1.58 ^c	1.12 ^b	27.7 ^b	4.01 ^b	31.7 ^b	9.09 ^b
	QPSB03	1.67 ^{bc}	1.22 ^a	32.9 ^a	4.79 ^a	37.7 ^a	9.43 ^b
	QPSB07	1.83 ^a	1.28 ^a	35.1 ^a	5.18 ^a	40.3 ^a	10.45 ^a
	QPSB11	1.75 ^{ab}	1.22 ^a	34.1 ^a	4.74 ^a	38.9 ^a	9.55 ^b
<i>Main effect of P rate (mean of four inoculation treatments)</i>							
0		1.32 ^c	0.93 ^c	18.1 ^c	2.90 ^c	21.0 ^c	5.84 ^c
15		1.76 ^b	1.22 ^b	32.2 ^b	4.75 ^b	36.9 ^b	9.25 ^b
30		2.04 ^a	1.47 ^a	47.1 ^a	6.39 ^a	53.5 ^a	13.80 ^a
<i>ANOVA (F-test significance)</i>							
Block		*	***	*	***	**	ns
Inoculant (I)		***	***	***	***	***	***
P rate (P)		***	***	***	***	***	***
I $\times$ P		ns	ns	ns	ns	ns	*

Within each P rate, means sharing a superscript letter do not differ (Tukey HSD, $p < 0.05$); main-effect means were compared separately. [†]Analysed on the natural-log scale; means are shown on the original scale. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Where did the extra P come from? Splitting the log ratio of shoot uptake showed that about 53% of the inoculation gain came from more dry mass and 47% from a higher P concentration, and the split hardly changed with P rate (51–54% from dry mass). Inoculated plants did not simply grow larger and dilute their P; they grew more and were also

better supplied. SPAD readings followed shoot P concentration closely ($r = 0.85$, $n = 48$), so this quick reading could help to track P status during vegetative growth.

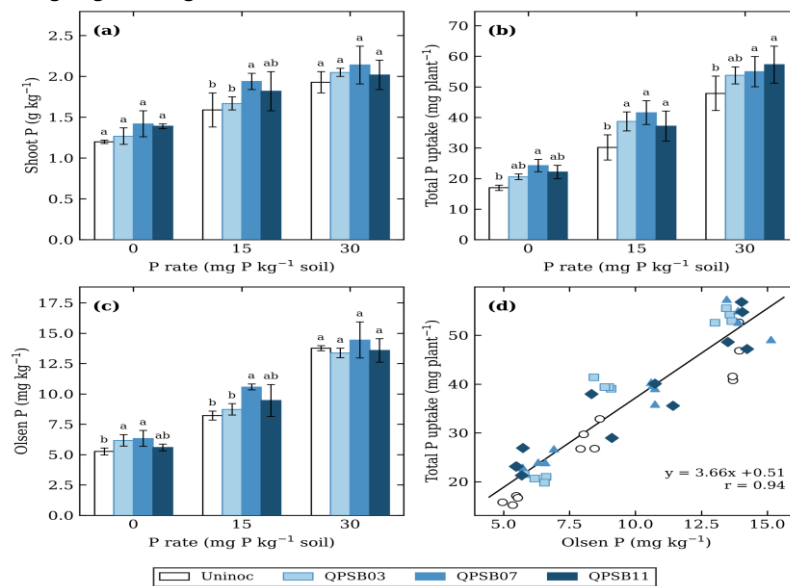


Figure 05: Phosphorus nutrition of sunflower: (a) shoot P concentration, (b) total P uptake, (c) Olsen P at harvest (letters from the log-scale analysis) and (d) relation between Olsen P and total P uptake for all 48 pots. Bars are means $\pm$ SD ($n = 4$); within each P rate, bars sharing a letter do not differ (Tukey, $p < 0.05$).

In relative terms, the gain shrank as P supply rose: 21–43% without added P, 23–38% at 15 mg P kg⁻¹ and 12–20% at 30 mg P kg⁻¹ (Figure 5b). Without added P only QPSB07 separated from the control in the within-rate test; at 15 mg P kg⁻¹ all three isolates did. Larger relative gains at low P are what one would expect, since any P released near the root is then a large share of the plant's supply, whereas at the high rate the fertilizer covers more of the demand. Comparable gains have been reported for root P of maize (+33.6%) in a P-deficient sand amended with TCP [22] and for the P content of maize (+38–58%) and peanut (+49.6%) inoculated with native PSB [35]. Halotolerant isolates also raised P uptake and P-use efficiency of barley under TCP-induced P shortage [24], and copper-tolerant pseudomonads increased P uptake by sunflower [36].

Uptake of uninoculated plants rose almost linearly with P rate, which allows the inoculant effect to be read as fertilizer P (Figure 6; Table 07). QPSB07 plants without added P took up as much P as uninoculated plants given about 8.3 mg P kg⁻¹, and at 15 mg P kg⁻¹ they matched about 24.7 mg P kg⁻¹, an apparent saving of 9.7 mg P kg⁻¹. The corresponding savings were 7.2 mg P kg⁻¹ for QPSB03 and 5.9 mg P kg⁻¹ for QPSB11. Savings of this size are in line with the substitution of about a quarter of the DAP dose in sunflower pots [15], a 25% P saving for field sunflower [37], wheat at 70% P plus PSB matching full P [21] and maize grain yield kept at half the P rate [38]. These are pot estimates for one soil at a vegetative stage, and they should not be read as field recommendations.

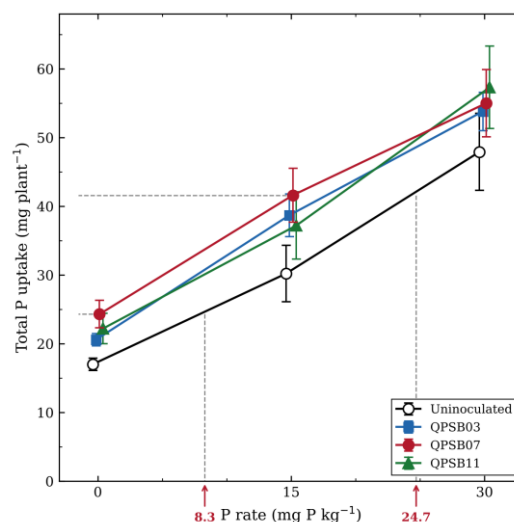


Figure 06: Total P uptake against P rate for each inoculation treatment (means $\pm$ SD, $n = 4$; symbols offset slightly for clarity). Dashed lines project the uptake of QPSB07 plants onto the uninoculated line; the values on the axis are the apparent fertilizer-P equivalents (mg P kg⁻¹).

3.6. Residual Olsen P and apparent P recovery

Olsen P at harvest rose with P rate (5.84, 9.25 and 13.80 mg kg⁻¹) and was correlated with total uptake ($r = 0.93$, $n = 48$; Figure 05d). On the log scale, inoculation ($F_{3,33} = 9.58$, $p < 0.001$) and the inoculant $\times$ P interaction ($F_{6,33} = 2.59$, $p = 0.036$) were both significant (Figure 5c). Without added P, QPSB03 and QPSB07 left more Olsen P than the control (6.18 and 6.34 versus 5.26 mg kg⁻¹); at 15 mg P kg⁻¹, QPSB07 raised it from 8.22 to 10.58 mg kg⁻¹ (+28.7%). At 30 mg P kg⁻¹ the treatments did not differ (13.38–14.45 mg kg⁻¹). A comparable rise, from 6.5 to 10.1 mg kg⁻¹, followed inoculation with *Bacillus megaterium* in a calcareous soil of Kirkuk [28], and P-solubilizing microbes with manure raised extractable P in calcareous soils of Duhok [17]. The fading effect at the high rate is easy to explain. Fertilizer P then dominates the labile pool, and microbial uptake can hold back part of what is released [34].

Table 07: Total P uptake, apparent recovery of applied P (APR) and apparent fertilizer-P equivalents of the inoculants (control = uninoculated).

Inoculation	P rate (mg kg ⁻¹)	P added (mg pot ⁻¹)	Total uptake (mg plant ⁻¹)	Gain vs. control (%)	APR within inoculation (%)	APR vs. control P0 (%)	Fertilizer-P equivalent (mg kg ⁻¹)	Apparent saving (mg kg ⁻¹)
Uninoculated	0	0	17.0	–	–	–	–	–
	15	120	30.2	–	11.0	11.0	–	–
	30	240	47.9	–	12.9	12.9	–	–
QPSB03	0	0	20.6	21.2	–	–	4.1	4.1
	15	120	38.7	28.4	15.1	18.1	22.2	7.2
	30	240	53.8	12.3	13.8	15.3	–	–
QPSB07	0	0	24.3	43.2	–	–	8.3	8.3
	15	120	41.6	37.8	14.4	20.5	24.7	9.7
	30	240	55.0	14.8	12.8	15.8	–	–
QPSB11	0	0	22.2	30.7	–	–	5.9	5.9
	15	120	37.2	23.3	12.5	16.8	20.9	5.9
	30	240	57.3	19.6	14.6	16.8	–	–

APR within inoculation = $100 \times (\text{uptake at the P rate} - \text{uptake of the same inoculation at P0}) / \text{P added}$; APR vs. control P0 uses the uninoculated P0 plants as the baseline. Fertilizer-P equivalent = P rate giving the same uptake on the uninoculated dose–response line (linear interpolation; not defined above 30 mg P kg⁻¹). Difference-method estimates, not isotope-based recoveries.

Apparent recovery of applied P was low in all treatments (Table 07). Uninoculated plants recovered 11.0% and 12.9% of the P added at 15 and 30 mg P kg⁻¹; inoculated plants recovered 12.5–15.1%. Measured against the uninoculated plants without P, which counts both the bacterium and the fertilizer, recovery rose to 15.3–20.5%. These figures bracket the global means of 12.4% for cereal crops [20] and 12.6% for inorganic P added to terrestrial ecosystems [1], and they confirm that most of the applied P stayed in the soil, as expected for calcareous soils [3]. They also show what the inoculants did not do. Within each inoculation, recovery differed from that of the control by only –0.1 to +4.1 percentage points, while plants given no fertilizer took up 3.6–7.3 mg more P per plant after inoculation. Most of the benefit therefore came from soil P that was already there, rather than from better use of the fertilizer.

3.7. Proposed mechanism and limitations

Figure 7 brings these results together. Acidification and organic anions released near the root dissolve Ca–P, part of the released phosphate is taken up and part is lost again to CaCO₃ surfaces, while CaCO₃ itself consumes protons and limits how far the pH can fall. Mineralization of organic P adds a further source [7,9]. The larger roots of inoculated plants (+12.2%) suggest that a growth-promotion effect, such as auxin

production, also enlarged the soil volume explored; total uptake was closely related to root dry mass ($r = 0.84$). This study did not measure auxins or organic acids in soil, so the relative weight of each route remains open.

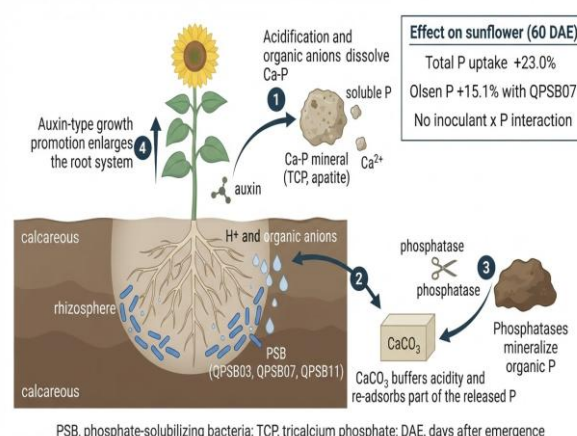


Figure 07: Proposed routes by which the selected PSB increased P supply to sunflower in calcareous soil: acidification and organic-anion release dissolve Ca–P, CaCO₃ buffers part of the acidity and re-adsorbs part of the released P, phosphatases mineralize organic P and auxin-type growth promotion enlarges the root system.

Several limits should be kept in mind. The pot trial used one soil, one season and a 60-day vegetative harvest, so seed yield and oil were not assessed. Survival and colonization of the inoculants were not tracked, although this is often where inoculants fail [11]. Screening relied on TCP, which overstates solubilization of native Ca-P [23], and the identities are still presumptive. The next steps are 16S rRNA sequencing, re-isolation or qPCR tracking of the strains, tests on rock phosphate, mixtures of the *Bacillus*-like and *Pseudomonas*-like isolates and multi-site field trials with sunflower in Al-Qadisiyah, with formulations suited to dryland conditions [32].

4. CONCLUSION

Native PSB were easy to recover from the calcareous soils of Al-Qadisiyah. Their ability to dissolve TCP varied more than five-fold, from 59.7 to 332.0 mg L⁻¹, and followed the fall in broth pH closely. Halo size on NBRIP agar was only a moderate guide and would have placed the best broth solubilizer, QPSB07, sixth. Broth assays should therefore be used to rank candidates. The active isolates were *Bacillus*-like and *Pseudomonas*-like rods. In an unsterilized calcareous soil low in Olsen P, the three selected isolates raised sunflower P uptake by about 23.0% across P rates, with the largest relative gains where little or no P was applied. QPSB07 also raised shoot P and residual Olsen P, and its plants at 15 mg P kg⁻¹ took up as much P as uninoculated plants given about 24.7 mg P kg⁻¹. Recovery of fertilizer P stayed low (11.0–15.1%), which leaves room for biological help. Molecular identification, tracking of the strains in soil and multi-site field trials are needed before any fertilizer saving can be recommended.

5. FUNDING

This research received no external funding.

6. CONFLICTS OF 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.

7. DATA AVAILABILITY

The raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

8. ACKNOWLEDGEMENTS

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