



Characterization of Phosphate-Solubilizing *Pseudomonas lurida* OR400772, and Potential Application in Tricalcium Phosphate Contaminated Soil to Alter *Vicia faba* Growth, and Gene Expression Patterns

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Abstract

Phosphorus (P) fertilizers augment agricultural production despite their adverse environmental impacts, as they deplete plants of P through frequent soil P fixation. Therefore, this study investigated and characterized the potential alternative, phosphate-solubilizing *Pseudomonas lurida* (PI) "OR400772" from the alfalfa rhizosphere. The in vitro characterization of P solubilizing activity on Pikovskaya's (PVK) media, in addition to other plant growth-promoting activities of PI strain, was investigated. The in vivo validation of PI strain in a pot experiment for *Vicia faba* L. growth in tricalcium phosphate (TCP)-containing soil was evaluated. The HPLC analysis demonstrated that the synthesis of citric acid and gluconic acid promotes the solubilization of phosphorus by PI, consequently lowering the media's pH to 3.2. Furthermore, PI strain was able to produce ammonia, indole acetic acid (IAA), and hydrogen cyanide (HCN). PI-inoculated *V. faba* plants growing in TCP-containing soil exhibited a significant increase in plant biomass, with particularly notable increase in root development i.e. root dry weight was 10.3 times larger, and exhibited higher rhizosphere colonization index. The total soluble protein rose significantly by 83.86 % compared to the control, with reduced free amino acids and enhanced photosynthetic pigments. The gene expression profiles of ribulose-bisphosphate carboxylase oxygenase (rbcL), plasma membrane H⁺-ATPase (PM-H⁺-ATPase), and calcium-dependent protein kinase (CDPK5); key genes of metabolic pathways related to photosynthesis, nutrient uptake, and signaling mechanisms, suggesting molecular mechanisms underlying the observed growth promotion. This study showed that *P. lurida* may be a better biofertilizer than synthetic compounds in agricultural systems. This may help preserve soil fertility and the environment.

Graphical Abstract

A concise summary of the experimental methodology for the P solubilization of *P. lurida* "OR400772" (PI) and its activities to promote plant growth. The experimental design entails an in vitro assay to demonstrate the P-solubilizing activity on Pikovskaya's media and the plant growth-promoting features, including ammonia production, indole acetic acid (IAA), hydrogen cyanide (HCN), and some enzymatic activities such as catalase, protease, and cellulase, as well as Zn solubilization. The second component of the experiment configuration consisted of an in vivo pot experiment that was designed to demonstrate the plant growth-promoting affinities of the PI strain on *V. faba* L. plant growth in soil that was mixed with tricalcium

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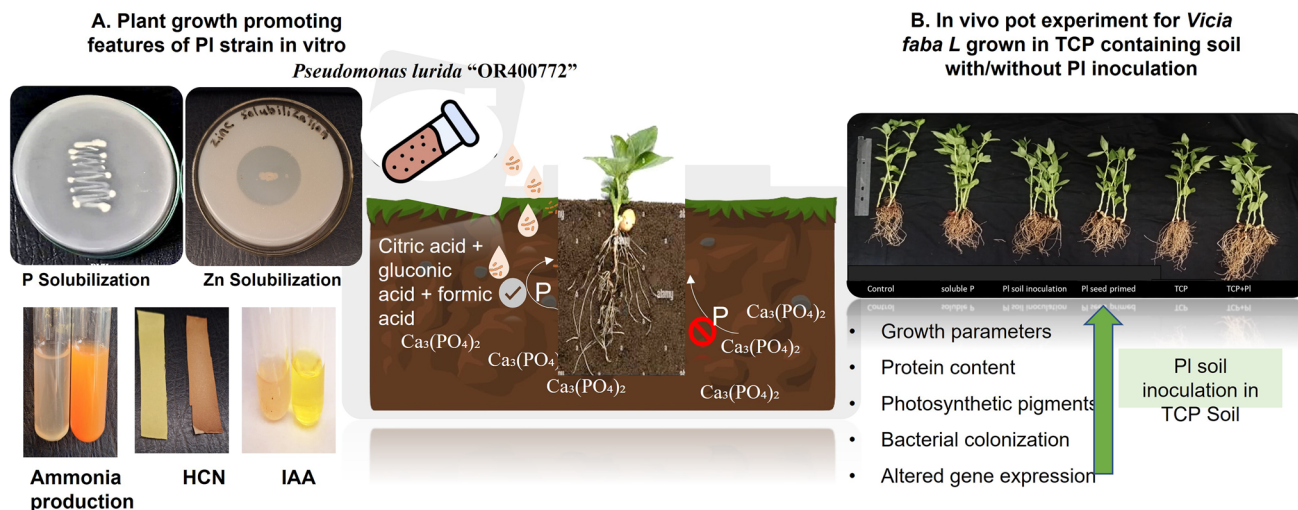
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phosphate (TCP); an insoluble form of P. The impact on plant growth, primary metabolites, genome, and gene expression analysis of three genes (Ribulose-bisphosphate carboxylase oxygenase, P-type plasma membrane (PM) H^+ -ATPase, and Calcium-dependent protein kinase) that are involved in stress and development signaling pathways is delineated.



Keywords Calcium-dependent protein kinase · ISSR fingerprinting · Lytic enzymes · Metabolites · Plant growth promoting rhizobacteria · Plasma membrane (PM) H^+ -ATPase · Rhizosphere colonization · Photosynthesis

Abbreviations

CDPK	Calcium-dependent protein kinase
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
Pi	Inorganic P
ISSR	Inter Simple Sequence Repeats
PM- H^+ -ATPase	Plasma membrane H^+ -ATPase
PVK	Pikovskaya's media
TCP	Tricalcium phosphate
PSB	Phosphate-solubilizing bacteria
P	Phosphorus
PSM	Phosphorus-solubilizing microorganisms
PI	<i>P. lurida</i>
RbcL	Ribulose-bisphosphate carboxylase oxygenase

1 Introduction

Phosphorus (P) is a necessary macronutrient for plants since it enters a variety of metabolic processes, notably the synthesis of nucleic acids and energy generation (Bechtaoui et al. 2021). P plays a function in strong root system creation, photosynthesis, plant development, and, eventually, crop production (Khan et al. 2023). Therefore, is it difficult for plants to thrive under P starvation.

Organic phosphorus accounts for more than 20 % of total phosphorus in soil, originating from biological metabolic processes such as orthophosphate absorption and microbial, plant, or animal wastes (Zhu et al. 2024). Nevertheless, P can be present in an insoluble form, adsorbed onto the edges of clay minerals, and bonded to organic matter through metallic cations (Ca, Mg, Fe, or Al). The unavailability of phosphorus in top-soil is a result of its precipitation with cations, immobilization, adsorption, and interconversion to insoluble organic forms, resulting in only 0.1 % of it being available for plant assimilation (Rawat et al. 2021). Consequently, phosphate chemical fertilizers are frequently administered to the soil to enhance the availability of phosphate to plants, thereby supporting their growth and development (Bechtaoui et al. 2021). According to U.S. Geological Survey Mineral Commodity Summaries 2024, the global consumption of phosphate fertilizers and industrial applications raised from 47 million tons in 2020 to 49 million tons in 2024. Unfortunately, plants only absorb a small portion, whereas the highest proportion of inorganic P (Pi) becomes less available, forming a complex precipitated form of $Ca_3(PO_4)_2$ or even in apatite (Ibrahim et al. 2022). Another concern is that P fertilizers can negatively influence soil pH, organic carbon (C) and nitrogen (N) contents, soil cation, granulometric composition, and other soil characteristics, which can affect soil biota (bacteria, fungus, protozoa, nematodes, earthworms, plant roots, arthropods, etc.) and therefore P bioavailability (Basílio et al. 2022). On

top of that, excess agricultural fertilizer can then be transferred into surrounding ecosystems via runoff or transport through freshwater bodies, damaging freshwater and marine systems through eutrophication (Ceulemans et al. 2014). Accumulation of heavy metals derived from mineral P fertilizers has major negative effects on the environment and/or human health due to their poisonous properties (Basílio et al. 2022). Thus, many studies shed light on the need for alternative sources to increase P availability in soil, and reducing P fertilizer is a target for new forms of sustainable agriculture. The use of microbial inoculants with P-solubilizing activity in soils is regarded as an environmentally conscious alternative to delimit the further applications of chemical-based P fertilizers (Silva et al. 2023). Phosphorus solubilizing microorganisms (PSM) are a key component of the soil biogeochemical cycle of P, being particularly involved in a range of biochemical events such as solubilization, mineralization, and immobilization of P (Bargaz et al. 2021). The most thoroughly studied phosphorus (P) solubilizing microorganisms are the phosphate-solubilizing bacteria (PSB), with the greatest number of genera and species belonging to the *Proteobacteria* phylum, including the genera *Bacillus*, *Pseudomonas*, and *Acinetobacter* (Chang et al. 2024). Research has shown that numerous PSB strains possess growth-promoting properties, such as the ability to synthesize hormones, antibiotics, and enzymes that enhance plant development and safeguard against pathogen assaults (Alotaibi et al. 2022; Biswas et al. 2024). Insoluble P is converted to soluble forms by the emission of organic acids (e.g., oxalic, citric, gluconic, succinic, α -ketoglutaric, and fumaric acids) by PSBs. Indeed, these compounds chelate cations (Al, Fe, and Ca) that are attached to P through their hydroxyl and carboxyl groups, thereby providing a form of P ($\text{H}_2\text{PO}_4^{4-}$ and HPO_4^{2-}) that is readily absorbed by plants (Cheng et al. 2023; Ducouso-Détrez et al. 2024; Zhu et al. 2024). By catalyzing the hydrolysis of P-ester bonds, these microorganisms can facilitate organic P mineralization by producing P-hydrolyzing enzymes, primarily phytases and phosphatases (Chang et al. 2024). As a result, the immobilized reservoir of P can be released into the soil through the application of PSM, which can be mineralized and released as accessible P over (Rawat et al. 2021). Tomato seed inoculation with these isolates cultured in rock phosphate soil improves seed germination, shoot and root growth, nutritional content, total chlorophyll, and root colonization, causing systemic resistance to tomato bacterial canker disease (Bakki et al. 2024). The inoculation of *Acinetobacter calcoaceticus* into paddy soil led to an increase in the average concentrations of organic acids, including acetic acid, gluconic acid, oxalic acid, citric acid, and alkaline phosphatase. This increase in the average content of soil labile P by 9.2 %, activated insoluble inorganic P and organic P. This is resulting in a significant increase in

shoot dry weight. Therefore, can increase the availability of phosphorus for subsequent rice crops (Chang et al. 2024).

In this investigation, it was hypothesized that the moderate phosphate solubilization capacity of the rhizosphere bacterial isolate *P. lurida* (PI) OR400772 could facilitate the growth and development of *V. faba*. Therefore, the current objectives were as follows: (1) quantify the organic acid production to characterize the P-solubilization mechanisms of PI; (2) evaluate the plant growth-promoting characteristics of the PI strain, including its capacity to produce IAA, HCN, ammonia, lytic enzyme, and Zn-solubilization; (3) evaluate PI as a bio-inoculant by evaluating morphological, physiological, and genomics parameters in *V. faba*; and (4) investigate the molecular basis of the plant response to PI inoculation by analyzing the expression of key genes involved in nutrient uptake (plasma membrane H^+ -ATPase), signaling (calcium-dependent protein kinase), and photosynthesis (ribulose 1,5-bisphosphate carboxylase). This research examines a knowledge gap in the regulation of stress-responsive genes in plants by phosphate-solubilizing bacteria in TCP- containing soil.

2 Materials and Methods

2.1 Bacterial Isolation and Purification

The bacterial isolate was obtained from the rhizosphere of alfalfa (*Medicago sativa*) cultivated in an open field at EL-Warraq, Giza governorate, Egypt. Following the methodology outlined by Al-Zereini (2014), bacterial isolation was performed by applying a 100 μL of soil suspension (1:10 dilution) onto the tryptic soy agar (TSA) plates. After 24 h of incubation at 28 °C, the plates containing the preponderant bacterial colonies, surrounded by a clear zone, were selected, purified, and utilized in the present study under the label PI.

2.2 Molecular Identification by 16S rDNA Sequencing

DNA extraction was performed from overnight broth grown culture according to Prabha et al. (2013). The DNA quality and quantity was assessed in 1 % agarose gel that electrophoresed in 1 $\times$ TAE buffer. Polymerase chain reaction was performed using Cosmo DNA polymerase red master mix (Willowfort:WF-1020201) according to manufacture instructions in 50 μL reaction volume. Amplification, purification and sequencing of DNA fragment corresponding to the 16S-rRNA encoding gene was performed as described by Hamada and Soliman (2023). Both 27F and 1492 R's 16S rDNA primers spanning the full length of the gene were used (Lagier et al. 2015).

Sequence data were compared with available sequences of bacteria in the National Centre for Biotechnology

Information (<https://www.ncbi.nlm.nih.gov/>) using BLAST search. The phylogenetic tree analysis was conducted using MEGA version 11 with 100 bootstrapping (Tamura et al. 2021).

2.3 Qualitative Screening for Phosphate (P) Solubilization Ability

Isolate PI was screened for its P solubilizing ability according to Prasad et al. (2021) as follows: The test was carried out on Pikovskaya's (PVK) agar medium using TCP as a source of P. A loopful of an overnight-grown bacterial culture was used to inoculate Petri plates with PVK agar medium and incubated at 28 ± 2 °C. After at least 7 days of static incubation, the appearance of a clear zone around the bacterial growth was detected as a positive result. The solubilization zone and colony diameters were measured, and the results were expressed as the solubilization index (SI) according to Blanco-Vargas et al. (2020) as follows: $SI = A/B$, where A is the colony growth diameter in mm plus solubilization halo diameter in mm, and B is colony diameter (mm). Results corresponded to the average of three independent replicas $\pm$ SE.

2.4 Quantitative Determination of Phosphate Solubilization Ability

According to Desai and Amaresan (2022), with a slight modification, the quantitative estimation of P solubilization was performed as follows: 50 mL of PVK broth medium (pH 7) was prepared with different TCP concentrations (0.5, 0.25, and 0.125 g 100 mL⁻¹). After media sterilization, 100 μ L of 24 h-old PI culture with 2×10^6 CFU mL⁻¹ was inoculated and then incubated at 28 ± 2 °C for 7 and 14 days in a shaking incubator (Vision, Korea) at 150 rpm. Using the vanadomolybdophosphoric acid colorimetric method, the available phosphate was estimated. The absorbance was determined at 400 nm using a Jenway 6405 UV/Vis spectrophotometer (UK). The amount of available phosphate was determined in reference to a KH₂PO₄ standard curve. Furthermore, the final pH of the supernatant was measured; the results were taken after 7 and 14 days from the beginning of the experiment, and the mean of the triple experimental replica was tabulated as $\pm$ SE.

2.5 Evaluation of Plant Growth Promoting Characteristics of PI Strain

2.5.1 Ammonia Production

Ammonia production assay was carried out according to Geetha et al. (2014) as follows: A 24-h-old culture was

transferred into test tubes with 10 mL of peptone water medium (Biolife, Sarasota, Florida) and kept at 28 °C in a standing incubator for 7 days. Nessler's solution (0.5 mL) was added to each tube to detect the results. The appearance of a bright yellow color was proof that ammonia was being produced.

2.5.2 HCN and IAA Production

Both HCN and IAA production assays were determined according to Lukkani and Reddy (2014); Ortiz-Ojeda et al. (2017), with minor modifications, respectively. To evaluate the production of HCN, A loopful of overnight culture was distributed onto nutrient agar plates containing 4.4 g L⁻¹ glycine. After inoculation, the plates were turned upside down, and a sterile strip of Whatman filter paper no. 1 impregnated with picric acid solution (yellow) (0.5 % picric acid in 2 % sodium carbonate) was put on the lid. Then, the plates were wrapped with parafilm and incubated statically at 28 °C for 4 days. The development of orange, red, or brown color on the filter paper strip after the incubation period indicates a positive result of HCN production. For IAA evaluation, a 24-h aged culture was spread onto LB agar plates. After incubation for 10 days at 28 °C, bacterial growth was covered with freshly prepared Salkowski's reagent (used to detect IAA production) and incubated in the dark for 1 h at room temperature. The appearance of a pink-to-rose color indicates the production of IAA.

2.5.3 Screening for Lytic Enzymes Production

For detecting amylase activity, overnight culture of PI strain was spotted in the center of nutrient agar (NA) plates supplemented with 0.2 % soluble starch; the plates were incubated for 4–7 days at 28 °C, and starch hydrolysis was detected by flooding plates with iodine solution (Shanmugasundaram 2015). Catalase test was performed according to Reiner (2013) by the tube method. For screening cellulase activity, Bushnell Haas medium (BHM) agar plates containing carboxymethyl-cellulose (CMC) as a sole carbon source were used, and in the middle of the BHMCMC agar plates, a loopful of PI overnight culture was inoculated. Then, the plates were incubated at 28 °C for 7 days (Singh et al. 2013). After the incubation period, the cellulase activity was determined using the plate staining method according to Teather and Wood (1982). A clear zone surrounding the bacterial growth indicates a positive cellulase finding. Protease enzyme was qualitatively screened on gelatin agar medium according to Vishnu Priya et al. (2016). The proteolytic action was noticeable as a clear area around the bacterial growth.

2.5.4 Zn- Solubilization Qualitative Assay

The efficacy of PI strain to solubilize zinc oxide was determined by a plate assay on mineral salts medium (MSM), as described by Othman et al. (2022). The outcome was detected as a clear zone surrounding the bacterial colony after 4 days of incubation at 28 °C.

2.6 HPLC Analysis of Organic Acids

In an effort to comprehend the process through which phosphate is solubilized by the PI strain. HPLC was utilized to identify the organic acids produced by this strain, which could account for its solubilization capability. In order to prepare the sample, the supernatant of a PI culture that had been previously grown in PVK broth medium at 28 °C for 14 days in an incubator agitated at 150 rpm was obtained. The supernatant was filtered and sterilized through a Milipore cellulose nitrate membrane filter with a pore size of 0.22 µL (CHM, Spain) after being centrifuged for 10 min at 4 °C and 10,000 rpm. Then the sterilized supernatant was analyzed by High Performance Liquid Chromatography (HPLC) at the Food Safety and Quality Control laboratory, Dairy Science and Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. The Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) was utilized with a flow rate of 0.6 mL min⁻¹, an ultraviolet (UV) detector set at 214 nm, a column oven temperature of 65 °C, and a RezexTM column for the organic acids with mobile phase 0.005 M H₂SO₄. To analyze the HPLC profile of the culture filtrate, the ClarityChrom chromatography data system software was utilized to compare the peak regions of the culture filtrate with the elution profiles of purified standard organic acids, including citric acid, gluconic acid, formic acid, and malic acid.

2.7 In Vivo Pot Experiment and Assessing Morphological Attributes

V. faba L. seeds (Giza 843 cultivar) were obtained from the Agricultural Research Center (ARC), Giza, Egypt. In vivo experiment was done according to Hamada and Soliman (2023) with slight modifications, as follows: plastic pots, each 30 cm in diameter and 25 cm in height, containing- 1.5 kg of mixed autoclaved soil containing 3:1 sand: Peatmoss, were used for seedlings growth. The seeds were surface-sterilized in 70 % ethyl alcohol for 10 min, then rinsed three times in dist. water. Before planting, the seeds were soaked in water for 8 h. The experiment was designed with six treatments in 15 replicas per treatment in a Randomized Complete Block (RCB) design as follows:

- 1 Control (C): Seeds were sown directly in the plastic pot without any soil amendments.
- 2 Tricalcium Phosphate (TCP): The soil was mixed with TCP at 2.5 g kg⁻¹, and seeds were sown in the amended soil.
- 3 P-Soil inoculation: soil was mixed with PI bacterial suspension containing 2 × 10⁶ CFU mL⁻¹ (10 mL kg⁻¹ soil), and seeds were sown in the amended soil.
- 4 Tricalcium Phosphate plus bacteria (TCP + P): soil was mixed with TCP (2.5 g kg⁻¹) and the PI bacterial suspension containing 2 × 10⁶ CFU mL⁻¹ (10 mL kg⁻¹ soil), and seeds were sown in the amended soil.
- 5 P-seed primed: Seeds were soaked for 1 h in PI bacterial suspension containing 2 × 10⁶ CFU mL⁻¹, air-dried, and then sown in the plastic pot.
- 6 Soluble Phosphate (SPH): Soil was mixed with potassium dihydrogen phosphate (KH₂PO₄) at 2.2 g kg⁻¹ as a source of soluble phosphate, and seeds were sown in the amended soil. note: The amount of P used in this treatment is equivalent to the amount used in TCP treatment.

We conducted a comparison between P-soil inoculation and P-seed priming to assess the efficacy of bacterial inoculation strategies. To identify the optimal biofertilizer administration strategy for *P. lurida*. Soil inoculation and seed priming are frequently implemented in agricultural settings. Every technique has the potential to enhance the availability of plant-solubilized phosphate, bacterial colonization, and field implementation. By comparing different strategies, we learned how bacterial administration affects plant growth and gene expression. This impacts the development of sustainable agricultural application protocols. The soil was mixed with TCP or KH₂PO₄ before the pots were filled with soil. Bacterial suspension preparation involved the collection of cells from an overnight PI culture through centrifugation at 4 °C and 5000 rpm for 10 min. The cells were subsequently washed twice with sterile distilled water and suspended in sterile distilled water to achieve a final concentration of 2 × 10⁶ CFU mL⁻¹. The bacterial inoculation of soil was accomplished by pouring the prepared bacterial suspension into each pot twice on the first and fourteenth days following the seed sowing, using a 10 mL of PI suspension per kg of soil. All pots were watered as required to maintain soil moisture, and the experiment was conducted for 30 days. The plant samples were collected from the soil and washed to get rid of any soil clumps. The shoots were dissected from roots, and their lengths, fresh, and dry weights were measured.

2.7.1 Colonization Index of the *V. faba*'s Rhizosphere

At the end of the pot experiment, the bacterial load that colonized the rhizosphere zone of the different treatments was

determined according to Bakki et al. (2024), the method with minor modifications as follows: the seedlings were uprooted, and the trapped soil around their roots was removed and air dried. From each treatment, 5 g of rhizosphere soil was weighted out and stirred in 45 mL of sterile water for 5 min. Following this, serial decimal dilutions were done in sterile water. Finally, 0.1 mL of each dilution was swabbed on nutrient agar plates and incubated overnight at 28 °C. The bacterial growth was noted by counting colonies, and rhizosphere colonization was expressed as colony-forming units CFU g⁻¹ soil. Each experiment was performed in triplicas.

2.7.2 Physiological Attributes

The effect of different treatments on the primary metabolites of the plants was assessed, such as proteins, amino acids, soluble sugars, and photosynthetic pigments.

Protein concentration was performed using the Folin–Ciocalteu reagent according to Lowry et al. (1951). Protein content was calculated as mg g⁻¹ FW (fresh weight) in reference to a bovine serum albumin (BSA) standard curve. Free amino acids were quantified using the ninhydrin solution as described by Lee and Takahashi (1966). Amino acid content was calculated as mg g⁻¹ FW by referring to a standard curve prepared from glycine. Total soluble sugars were quantified using anthrone reagent as described by Plummer (1878) and calculated as mg g⁻¹ FW. Photosynthetic pigments were quantified according to the method described by Metzner et al. (1965) amended by Lichtenthaler (1987) the concentration of chlorophyll a, b, and carotenoids was calculated using the following equations: Chlorophyll a = 10.3 A₆₆₄ – 0.918 A₆₄₅, Chlorophyll b = 19.7 A₆₄₅ – 3.87 A₆₆₄, and Carotenoids = 4.3 A₄₅₂(0.0265 Chl.a + 0.426 Chl.b). After that, the results were calculated as mg g⁻¹ FW.

2.7.3 DNA Extraction and Genome Profiling

The ISSR analysis was applied to investigate the effect of different treatments on the genetic profiling of the

30-day-old *V. faba* plants. The total genomic DNA was extracted from pooled randomly liquid nitrogen-frozen leaves of each treatment, using the cetyltrimethylammonium bromide (CTAB) method (Dellaporta et al. 1983). To determine the "intactness" of the extracted DNA, it was resolved on 1 % agarose gel prepared in 1 × TAE buffer containing 0.5 µg mL⁻¹ ethidium bromide. For genetic profiling, the PCR was carried out as described in Soliman et al. (2021) using the 5 ISSR primers. ISSR markers and the size of the bands were scored using Gel Analyzer 19.1 (www.gelanalyzer.com) with reference to 100 bp DNA ladder (GeneDireX- Cat.No. DM001-R500).

2.7.4 Gene Expression Analysis

RNA Extraction, DNase Treatment, and cDNA Preparation All the steps were carried out on ice; the plant tissue (pooled leaves of six replicas) was ground in liquid nitrogen and mixed well with Zymo Direct-Zol RNA extraction buffer (Miniprep kit—Zymo Research, R2050) according to the manufacturer's instructions. The DNA contaminant was removed from isolated RNA samples using the DNase Kit (ThermoScientific cat # EN0521). Successful DNase treatment was confirmed by the absence of any PCR product after 39 cycles using ribosomal gene primers and DNase-treated RNA as a template. 2 µg of DNase-treated RNA was used for cDNA synthesis with Maxima reverse transcriptase (ThermoScientific) and oligo (dt)₁₈ primers (cat.# 18064–014, Life Technologies-Invitrogen), following the manufacturer's instructions.

Quantitative Real-Time PCR (qPCR) HERA PLUS SYBR green qPCR master mix (cat# 600,882, Agilent Technologies-Stratagene) and PCR was performed using a QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) with cycling parameters: 95 °C (2 min) then 39 cycles of 95 °C (15 s), and 60 °C (20 s). Each reaction was performed in duplicate using the corresponding primer pairs for each specific gene as listed in Table 1. Primers were synthesized

Table 1 List of the primers used for gene expression analysis

Description	F-primer sequence (5' → 3')	R-primer sequence (5' → 3')	References
Ribulose-bisphosphate carboxylase oxygenase large partial (Rubisco) (rbcl) gene	ATTCGCAAATCTTCCAGACG	GAGCCTGTTCTTGGAGAAGA	(Ammar et al. 2017)
P-type plasma membrane (PM) H ⁺ -ATPase	ACTGGCCATAGCAAAGGAGA	GAAATACCCCAGCAAATCCA	
Calcium-dependent protein kinase sk5-like (CDPK5)	CTCTGCACACACAACCCAAC	GTCCCATGGATCCTGACAAC	
rRNA encoding gene	CTTGCAGTCAAGCTCCCTTC	CCTTGTCCCAAGACAGACCA	

by Eurogentec service (<http://www.eurogentec.com/eu-home.html>). The expression differences were assessed by the comparative Ct method. The cycle threshold (Ct) values were automatically calculated and exported to MS Excel for further analysis. The fold change in gene expression was calculated based on $2^{-\Delta\Delta C_t}$ according to Livak and Schmittgen (2001).

2.8 Statistical Analysis

All data are represented as an average of six independent replicas $\pm$ SE, otherwise stated in the corresponding section. The statistical significance was analyzed at ($p < 0.05$) using the GraphPad InStat GraphPad Software (version 3.05), San Diego, California USA, www.graphpad.com. Significant differences between treatments were analyzed using One-way ANOVA coupled with the Tukey–Kramer Multiple Comparisons Test. All data were passed a normality test using the Kolmogorov–Smirnov method integrated in the software.

3 Results

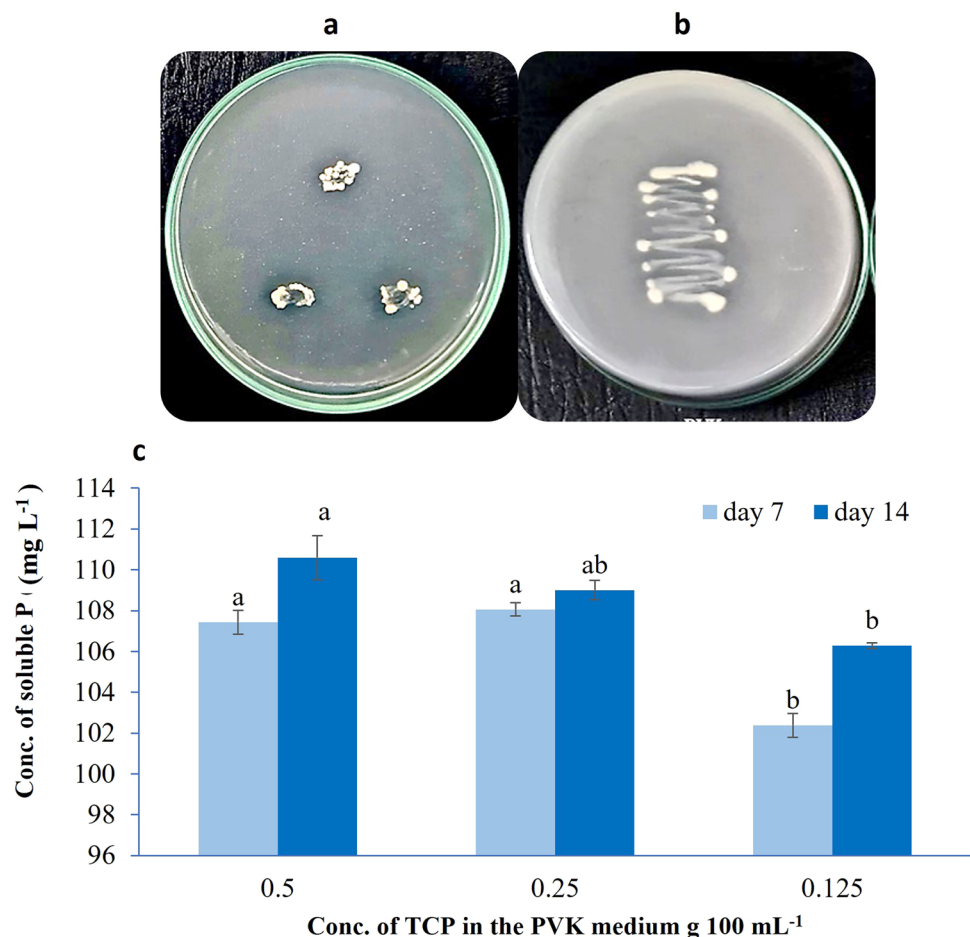
3.1 Sample Isolation and Molecular Identification of the Soil Isolate

The results of the BLAST search of the 16S rDNA sequence amplified from the PI strain suggesting it to be *P. lurida* as the sample has 100 % percent of identity and query coverage to this strain on NCBI data collection samples (Supplementary Fig. 1). The 16S rDNA sequence of the PI strain was deposited in the gene-bank with accession number OR400772.

3.2 Solubilization Ability of P Using *P. lurida* (PI) Strain

The qualitative test results indicated that the bacterial strain PI was capable of producing visible zones on PVK agar medium after seven days of incubation, indicating phosphate solubilization (Fig. 1a and b). The value

Fig. 1 Qualitative and quantitative detection of P solubilization on Pikovskaya's (PVK) medium: **a** and **b**) the clear zone formation around the *P. lurida* “OR400772” (PI) bacterial growth (spotted colony; **a** and spiral; **b**) indicating P solubilization. **c**) Quantitative estimation of P solubilization shows the concentration of soluble phosphate detected after 7 and 14 days of incubation of PI strain in the PVK broth medium. Error bars indicate the standard error of the mean of three independent replicas. Different small letters indicate significance at $P < 0.05$ according to the Tukey–Kramer Multiple Comparisons Test



of the P solubilization index (SI) was determined to be 1.6 ± 0.06 mm. To quantify the amount of solubilized P, a quantitative detection was determined using vanadomolybdate method in PVK broth medium. After a 7-days incubation period, significant results ($P=0.0012$) revealed that the soluble phosphate concentrations were 107.46, 108.06, and 102.39 mg L⁻¹ at TCP concentrations of 5, 2.5, and 1.2 g L⁻¹, respectively. However, after 14 days of incubation, the soluble phosphate concentrations were as follows ($P=0.012$): 110.60, 109.03, and 106.27 mg L⁻¹, respectively. The data indicated that the amount of soluble phosphate generated using PI strain did not differ significantly ($P<0.09$) between the observations made on day 7 and day 14 of incubation (Fig. 1c). As the concentration of TCP increased in the media the corresponding amount of soluble P produced increased, in which the media containing 5, and 2.5 g L⁻¹ TCP produced the highest concentration of soluble P detected. Consequently, in the in vivo experiment, a concentration of 2.5 g L⁻¹ TCP was applied.

Phosphate solubilization via PI strain was accompanied by a reduction in the pH of the medium. At TCP concentrations of 5, 2.5, and 1.2 g L⁻¹, the pH dropped from an initial value of 7.2 to 3.2, suggesting acid production.

3.3 Characteristic Features of *P. lurida* “OR400772” (PI) Strain

To determine whether the PI strain possessed any additional characteristics that would make it an effective plant growth-promoting bacterial strain, such as the capacity to synthesize ammonia, HCN, IAA, and lytic enzymes such as amylase, catalase, cellulase, and protease, a series of tests was completed. The results indicated that the OR400772 strain was capable of producing ammonia, HCN, IAA, catalase, cellulase, and protease, as illustrated in Fig. 2. Additionally, PI strain was employed to solubilize zinc oxide (ZnO), and the results were successful in that a clear zone encircled the bacteria's cell growth.

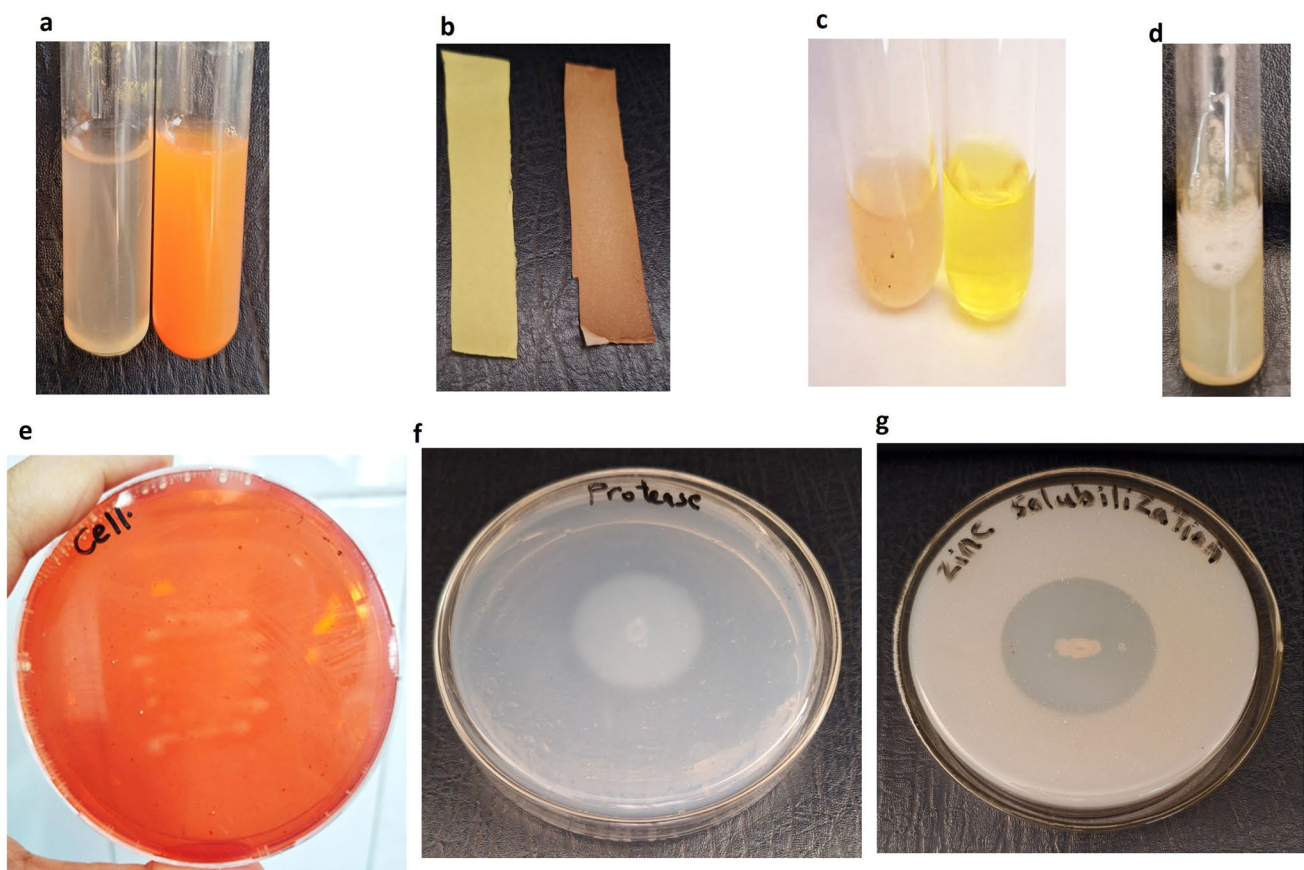


Fig. 2 Plant growth-promoting characteristics of *P. lurida* OR400772: **a**, **b**, and **c** represent results of ammonia (golden yellow), hydrogen cyanide (HCN) (brown), and indole acetic acid (IAA)

production (pink), respectively, and **d**, **e**, **f**, and **g** represent results of catalase, cellulase, protease, and zinc solubilization activities, respectively

Table 2 Names, retention times, and quantity of organic acids produced by *P. lurida* OR400772 during P solubilization on PVK broth medium using HPLC analysis

Name of organic acid	Retention time (min)	Concentration of organic acid PI culture filtrate (mg L ⁻¹)
Citric acid	8.342	6965.6 ± 120.6a
Gluconic acid	9.9	6772.8 ± 117.3a
Formic acid	13.886	301.8 ± 5.2 b
Malic acid	7.8	ND

(ND) refers to not detected

Data indicate means of 3 independent replicas ± standard error (SE). Different small letters indicate significance at $P < 0.05$ according to the Tukey–Kramer Multiple Comparisons Test

3.4 Production of Organic Acids by *P. lurida* “OR400772” (PI) Strain

As phosphate solubilization was accompanied by an abrupt decrease in pH, indicating acid production, the most prevalent acid produced by PI strain was

HPLC-analyzed to validate the concept. The HPLC analysis identified three distinct peaks with retention times of 8.342, 9.9, and 13.886 min, which were indicative of the presence of citric, gluconic, and formic acids, respectively. The sample contained the following acid concentrations: 6965.6, 6772.8, and 301.8 mg L⁻¹, respectively, which may account for the decrease in pH in the medium (Table 2).

3.5 In Vivo Application of PI Strain in Agricultural Purposes: “*V. faba* Pot Experiment”

To assess the capacity of *P. Lurida* to solubilize insoluble phosphate in soil to increase its availability to plants, thereby facilitating their growth and development. As detailed in the materials and methods section, an in vivo pot experiment was conducted on *V. faba* plants in which the PI strain was applied to soil containing TCP in comparison to other treatments. A number of molecular, physiological, and growth characteristics of the cultivated plants were evaluated.

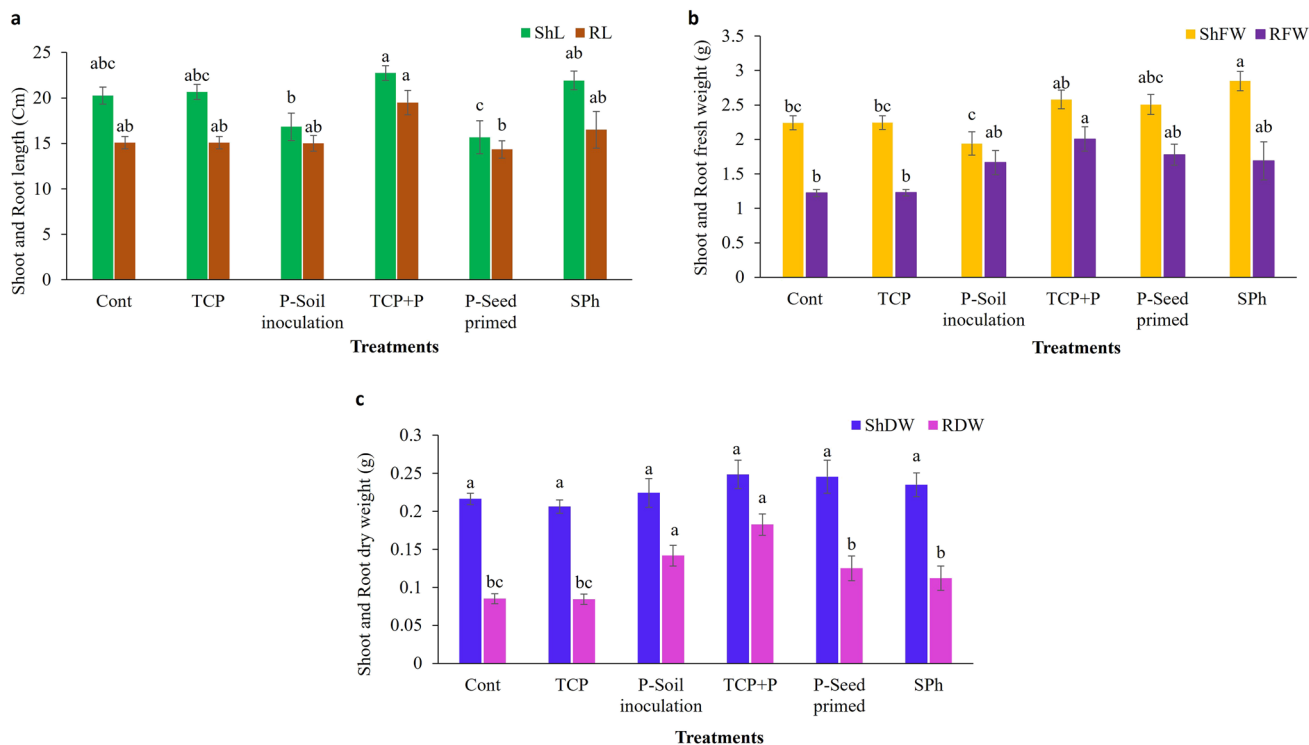


Fig. 3 Quantitative evaluation of the vegetative growth attributes of *V. faba* L. plants grown in soil amended with tricalcium phosphate (TCP), *P. lurida* OR400772 (PI) strain, and/or soluble phosphate (SPh). The effect of different treatments on **a**) shoot and root lengths, **b**) fresh weight of shoot (ShFW) and root (RFW), and **c**) dry weight

of shoot (ShDW) and root (RDW). Error bars indicate the standard error of the mean of six independent replicas. Different small letters indicate significance at $P < 0.05$ according to the Tukey–Kramer Multiple Comparisons Test

3.5.1 Growth Attributes

The growth of plants is not significantly influenced by the presence of TCP in the soil, as there are no significant differences between the control and TCP plants. However, a slight enhancement was observed when the PI strain was inoculated into the TCP amended soil (supplementary Fig. 2). In comparison to the TCP and control groups, the fresh weight of the shoots and roots was significantly increased, reaching 13 % and 39 % increase, respectively. Similar positive effects were observed in the fresh weights of both shoots and roots for plants that were grown in soil amended with soluble phosphate, P-soil inoculated soil, and P-seed primed plants (Fig. 3a and b). The addition of PI strain to soil, regardless of whether TCP is present or not, significantly increased root dry weight, reaching 10.3 and six-fold more than controls, respectively (Fig. 3c). The data indicated that the production of organic acids by the PI strain resulted in an increase in the soluble P content in the soil as a result of TCP solubilization, which directly influences root growth.

3.5.2 Colonization Index of *V. faba*'s Rhizosphere

Significant variation ($P \leq 0.02$) in the bacterial count was observed among the various regimens in the rhizosphere colonization results. The TCP + P treatment exhibited the maximum bacterial colonization value ($372.0 \times 10^4 \pm 23.44$ CFU g⁻¹ soil), followed by PI inoculated soil without TCP and PI seed primed ($148.0 \times 10^4 \pm 1.15$ CFU g⁻¹ soil). On the other hand, the control, TCP, and soluble phosphate groups exhibit low concentrations of rhizosphere bacteria (Fig. 4a and b). This data indicated that the high colonization index of the bacteria

surrounding the rhizosphere of the plant could be the cause of the promotive effect noted on *V. faba* growth.

3.5.3 Essential Metabolites Attributes

The plants that were grown in soil inoculated with the PI strain exhibited a substantial increase ($P < 0.001$) in the total soluble protein concentration, which reached 91.9 ± 4.5 mg g⁻¹ FW. In the presence of TCP, the concentration was 110.32 ± 2.1 mg g⁻¹ FW, compared to 65.9 ± 1.2 mg g⁻¹ FW for plants that were grown in soil containing TCP without bacterial inoculation. As anticipated, the protein concentration was also substantially increased by the presence of SPh, reaching 81.15 ± 6.12 ; however, this increase was less pronounced than that of the TCP + P treatment (Fig. 5a). In contrast, the levels of free amino acids were substantially reduced in the plants that were grown in soil that had been inoculated with PI and soil that has been amended with soluble phosphate (Fig. 5b). With the exception of *P. lurida* inoculated soil, the soluble sugar contents did not exhibit any substantial changes within the various treatments (Fig. 5c). Interestingly, the TCP + P grown plants exhibited a highly significant increase in chl a, b, carotenoids, and total pigment contents, with values of 96, 57.9, 91, and 82 % higher than those of the control plants, respectively (see Table 3).

3.5.4 Genomic Attributes

The ISSR markers were used to screen the potential impact of different treatments on the *V. faba* plant's genome. Here in five ISSR primers were used; four of them were able to produce stable, reproducible markers (Table 4). A total of 21 ISSR markers, ranging in size from 1387 to 107 bp, with an average of 5.25 markers per primer, were produced. The current findings

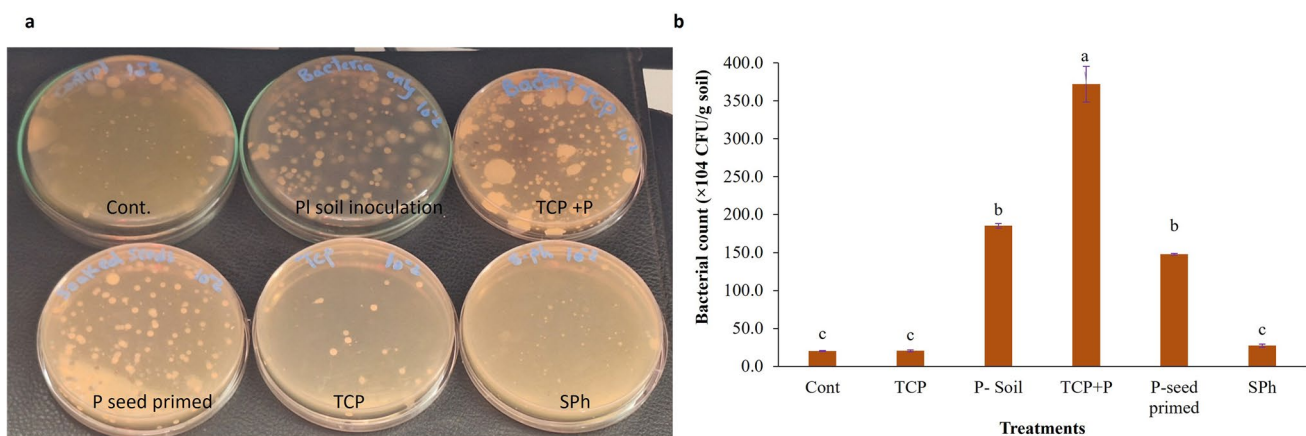


Fig. 4 Bacterial colonization in the rhizosphere of *V. faba* L. plants of different treatments. **a)** bacterial colonies on nutrient agar petri plates and **b)** corresponding histogram of bacterial counts. The count was expressed as colony-forming units per gram of soil (CFU g⁻¹

soil). Error bars indicate the standard error of the mean of three independent experiments. Different small letters indicate significance at $P < 0.05$ according to the Tukey–Kramer Multiple Comparisons Test

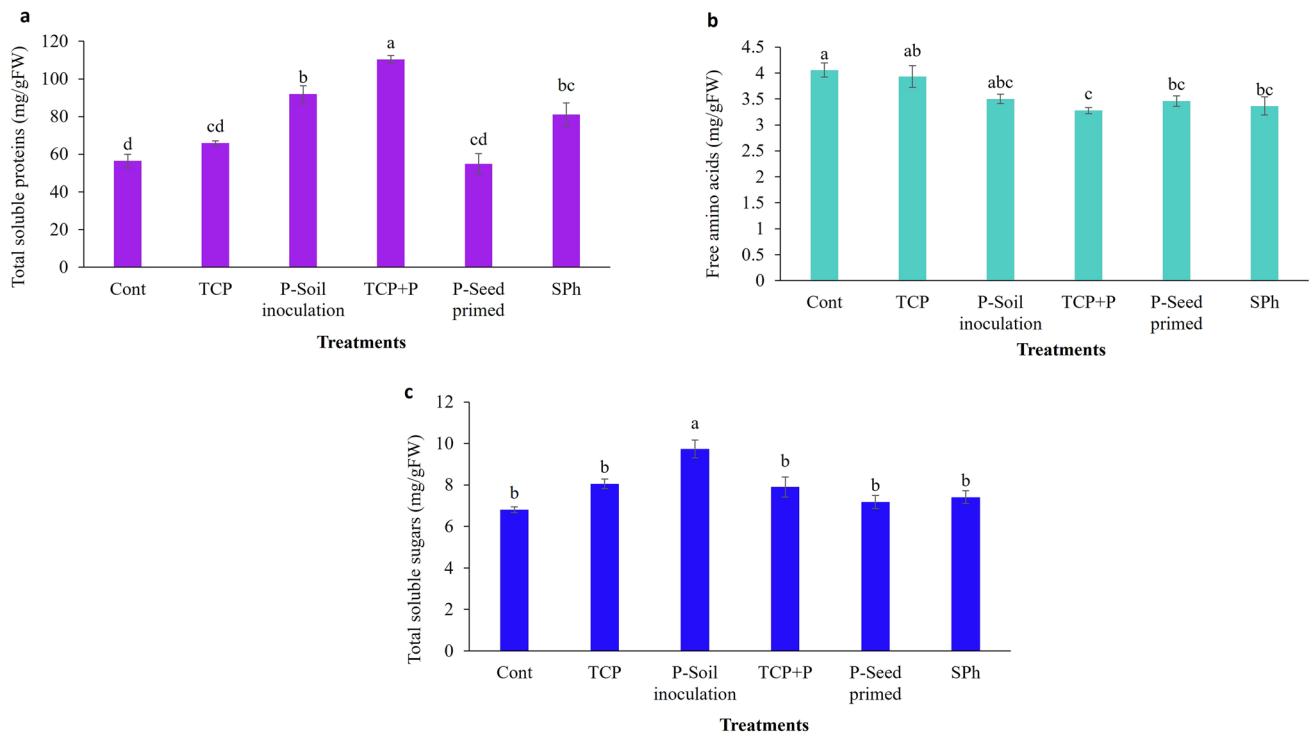


Fig. 5 Quantitative evaluation of the essential metabolites attributes of *V. faba* L. plants grown in soil amended with tricalcium phosphate (TCP), *P. lurida* OR400772 (PI) strain, and/or soluble phosphate (SPh). The effect of different treatments on **a**) total soluble protein,

b) free amino acids, and **c**) total soluble sugars. Error bars indicate the standard error of the mean of six independent replicas. Different small letters indicate significance at $P < 0.05$ according to the Tukey–Kramer Multiple Comparisons Test

suggest that the ISSR profile detects no changes in the genomic profiling as a consequence of various treatments (Fig. 6).

3.5.5 Gene Expression Profiling

The real-time PCR analysis revealed differential gene expression patterns of the three selected genes, Ribulose-bisphosphate carboxylase oxygenase (Rubisco) large subunit (*rbcl*), Calcium-dependent protein kinase sk5-like (CDPK), and P-type plasma membrane (PM) H^+ -ATPase in different treatments. The results indicated that the expression level of *rbcl* was significantly upregulated in the plants grown in TCP + P containing soil compared to those of P- soil inoculated and P-seed primed. Significant down-regulation was observed in the P-seed primed plants compared to other treatments (Fig. 7a).

The expression of CDPK5 and PM- H^+ -ATPase encoding genes showed highly significant upregulation in the plants that were grown in soil amended with soluble phosphate compared to all other treatments, reaching 32- and 284-fold increases compared to the control. Worth noting, however nonsignificant, the plants of TCP + P treatments show 1.8 and 2.4-fold expression compared to the control for CDPK5 and PM- H^+ -ATPase genes, respectively (Fig. 7b and c). The gene expression analysis has suggested molecular

mechanisms that underlie the observed growth promotion of *V. faba* plants by the PI strain in TCP-containing soil.

4 Discussion

The ability of a few soil microorganisms to convert insoluble forms of P into an accessible form is a significant property in plant growth-promoting bacteria for enhancing plant yields. The introduction of phosphate-solubilizing bacteria (PSB) as inoculants promotes plant P absorption (Bakki et al. 2024). PSB comprise approximately 20–40 % of the soil microbiota and are distinguished by their metabolic plasticity, capacity to colonize roots, and varied enzyme and metabolite synthesis. They have the ability to solubilize phosphate minerals such as dicalcium and tricalcium phosphate, hydroxyapatite, and phosphate rock (Cheng et al. 2023). This study involved characterization and a potential employment of a rhizosphere-isolated bacterial strain in agriculture purpose. The blast alignment of the 16S-rDNA sequences of the current isolate “PI” identified it as *P. lurida* with accession number OR400772 (Supplementary Fig. 1). *Pseudomonas*, is an example of phosphate-solubilizing bacteria, inhabits a variety of environments and has a wide metabolic diversity (Bouizgarne et al. 2023; Bakki et al. 2024). *P. lurida*, is a

Table 3 Quantitative evaluation of the total pigments (chl a, chl b and carotenoids) of *V. faba* plants grown in soil amended with tricalcium phosphate (TCP), *P. lurida* (PI) strain, and/or soluble phosphate (SPh)

	Chl a ± SE	Chl b ± SE	Carotenoids ± SE	Total pigments ± SE
Cont	2.6 ± 0.00d	1.9 ± 0.01c	1.8 ± 0.03c	6.3 ± 0.03c
TCP	3.4 ± 0.01c	2.5 ± 0.20b	2.5 ± 0.11b	8.4 ± 0.29b
P-Soil inoculation	3.2 ± 0.05c	1.8 ± 0.07c	1.5 ± 0.06c	6.4 ± 0.05c
TCP + P	5.1 ± 0.04a	3.0 ± 0.05a	3.4 ± 0.04a	11.5 ± 0.13a
P-Seed primed	3.9 ± 0.29b	2.0 ± 0.20c	1.7 ± 0.01c	7.6 ± 0.50b
SPh	2.7 ± 0.05d	1.8 ± 0.03c	1.7 ± 0.04c	6.1 ± 0.13c

Different small letters indicate significance at ($P < 0.05$) according to Tukey–Kramer Multiple Comparisons Test

The data represent the mean of 6 independent replicas ± SE

Table 4 List of ISSR primers (codes and sequences) used to profile *V. faba* plant's genome that was grown in soil amended with tricalcium phosphate (TCP), *P. lurida* (PI) strain, and/or soluble phosphate (SPh)

Primer code	Sequence (5' → 3')	Total	Size range (bp)
I-842	(GA) ⁸ CTG	2	490–354
I-844	(CT) ⁸ GC	6	730–107
I-885	(CGT)(ACT)(CGT)(GA) ⁵	9	1387–272
I-889	(AGT)(CGT)(AGT)(AC) ⁵	-	-
ISSR-5	(ACG) ⁴ GAC	4	1194–380
Total number of markers		21	

The total markers amplified for each primer and the size range of the markers produced are included in the table

Gram-negative bacterium, which was first described from the rhizosphere of grasses (Behrendt et al. 2007). The isolated strain “OR400772” showed a potential ability to solubilize the insoluble phosphate of TCP compound to soluble P. The qualitative assay on the PVK media showed a clear halo around the bacterial growth with SI 1.6 ± 0.06 mm indicating P-solubilization activity. In liquid culture, the maximum solubilizing efficiency of P reached 108.06 mg L^{-1} soluble P after 7 days of incubation at 28°C . Results were nearly in agreement with a previous study by Adhikari et al. (2021), who observed that *P. azotoformans* GBPI_CDB143 strain solubilizing tri-calcium phosphate at 25°C after 6 days with a value of $110.50 \pm 3.44 \text{ mg L}^{-1}$.

Inorganic phosphate complexes like Fe–P, Al–P, and Ca–P in the soil is solubilized by different mechanisms like organic acid production, inorganic acid and H_2S production, proton release from NH_4^+ , indirect mechanisms, direct oxidation pathways, and exopolysaccharide production and siderophore production (Rawat et al. 2021). The decrease of the initial pH (7.2) of the liquid PVK media to acidic pH (3.2) post PI inoculation and incubation up to 14 days, indicating production of acids. During phosphate solubilization, bacteria make low-molecular-weight organic acids like acetic acid, glycolic acid, oxalic acid, malonic acid, succinic acid, and 2-ketogluconic acid, which make the periplasmic space more acidic. In fluorescent *Pseudomonas* spp., gluconic acid is the most commonly released organic acid. It converts insoluble forms of phosphate into soluble forms that plants may easily intake. As a result, organic acid emission causes the bacterial cells' external environment to become acidic, lowering their pH (Bakki et al. 2024). This explained the high production of gluconic acid and citric acid (6772.8 and 6965.6 mg L^{-1} , respectively) by PI strain. Organic acids secreted by PSB can directly solubilize mineral phosphate as a result of their hydroxyl and carboxyl groups by chelating cations (Fe, Al, and Ca) bound to phosphate, converting it to a soluble form (Adhikari et al. 2021). The primary

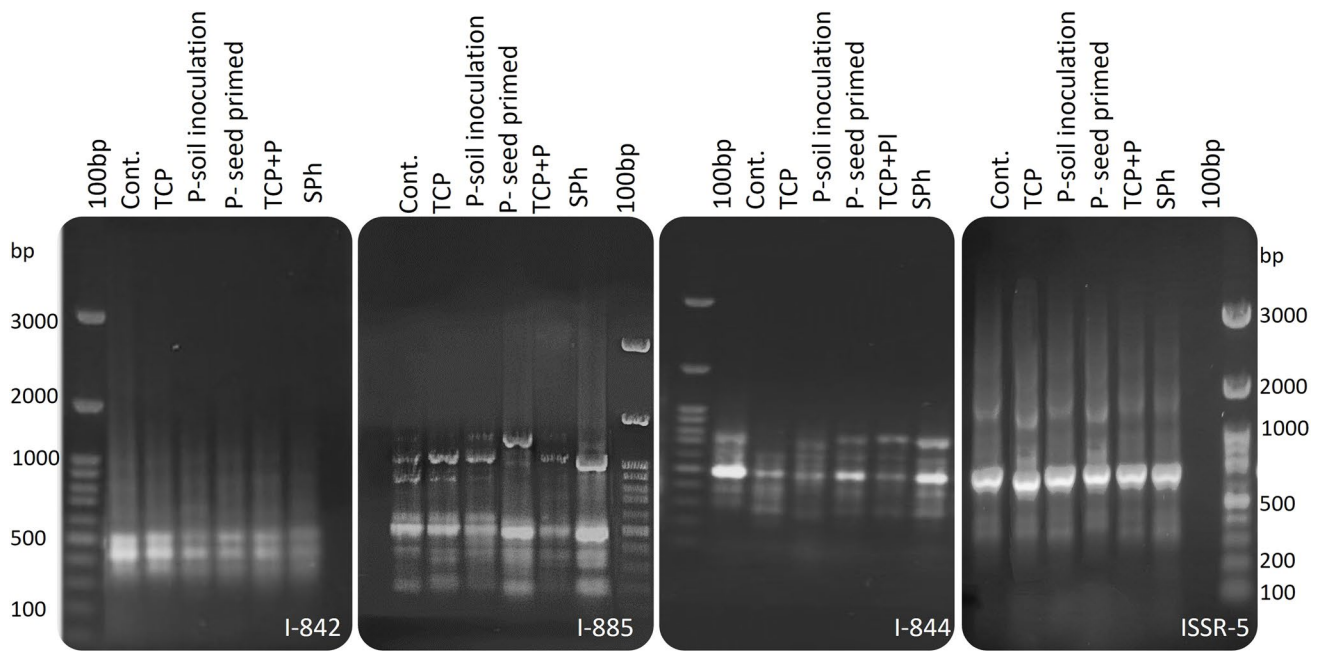


Fig. 6 Genome profiling using ISSR markers of *V. faba* L. plants grown in soil amended with tricalcium phosphate (TCP), *P. lurida* OR400772 (Pl) strain and/or soluble phosphate (SPh). The primer's ID is indicated at the end of each gel image. 100 bp refers to the DNA ladder

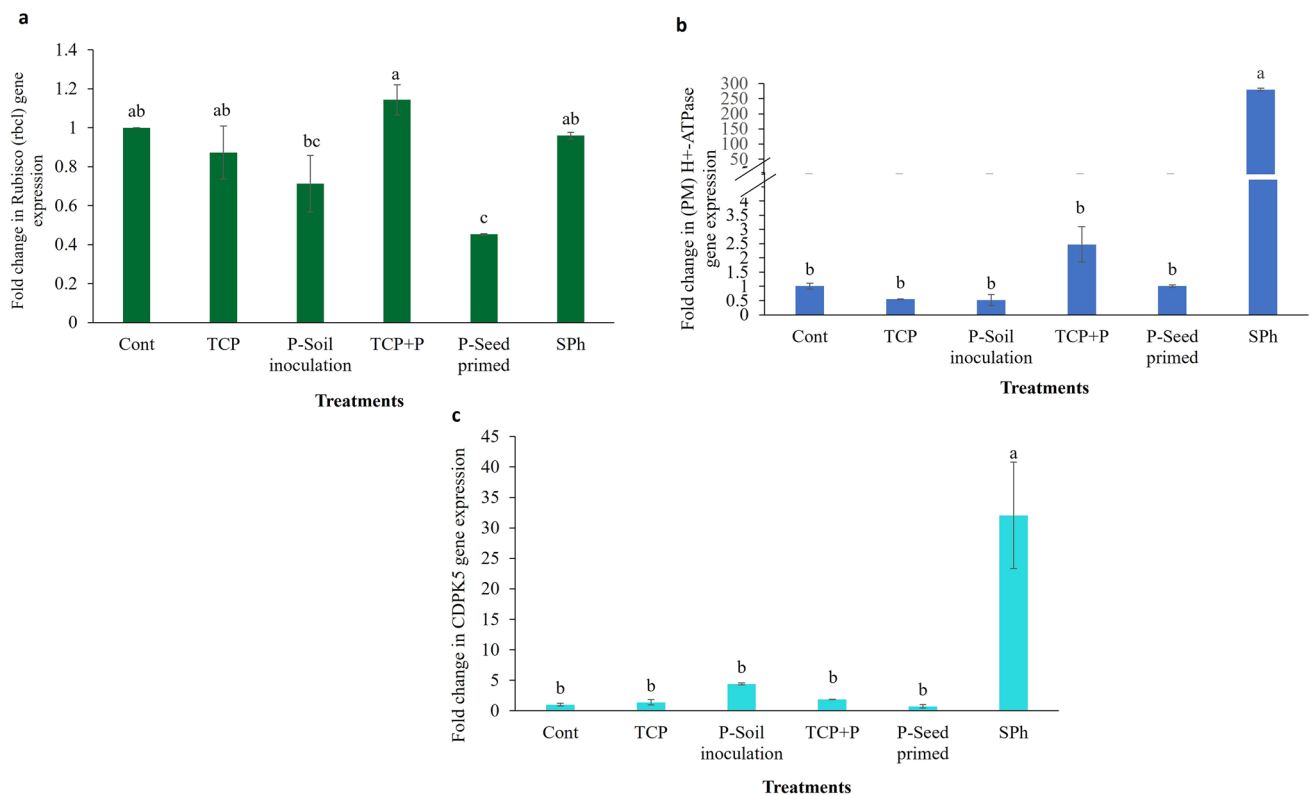


Fig. 7 Quantitative gene expression analysis of *V. faba* L. plants grown in soil amended with tricalcium phosphate (TCP), *P. lurida* OR400772 (Pl) strain, and/or soluble phosphate (SPh). The effect of different treatments on the expression level of **a**) Ribulose-bis-phosphate carboxylase oxygenase (Rubisco/rbcl), **b**) P-type plasma

membrane (PM) H⁺-ATPase, **c**) Calcium-dependent protein kinase sk5-like (CDPK5). Error bars indicate the standard error of the mean of three independent replicates. Different small letters indicate significance at ($P < 0.05$) according to the Tukey–Kramer Multiple Comparisons Test

mechanism that initiates the P-solubilization ability of the PI strain is the organic acid production, as evidenced by the positive correlation observed between the P-solubilization potential, acids produced, and pH decrease. *Pseudomonas* sp. strain AZ5 and *Bacillus* sp. strain AZ17 both showed evidence of plant stimulation through P and Zn solubilization, as well as the generation of organic acids and IAA (Zaheer et al. 2019). The current strain was intended to be in vivo utilized to evaluate its potential to enhance plant growth in soil containing TCP. As a result, additional experiments were conducted to evaluate its capacity to activate other plant growth promoting rhizobacteria PGPR characteristics. PI strain exhibited the capacity to solubilize zinc in vitro, produce IAA phytohormone, HCN gas, and ammonia, in addition to phosphate solubilization. It also possessed numerous enzymatic activities (catalase, cellulase, and protease) that were directed at its substrates (Fig. 2). These results were consistent with the findings of Kumar et al. (2021), which found that *P. lurida* strain EOO26 exhibited various PGP properties, including IAA generation, phosphate solubilization, siderophore synthesis, ACC deaminase activity, nitrogen fixation, production of ammonia, and hydrogen cyanide. These properties are capable of alleviating metal and other abiotic stress and promoting plant growth. In accordance with this, (Astriani et al. 2020) reported that the soybean rhizosphere isolate *P. plecoglossicida* has the capacity to solubilize phosphate at a concentration of 75.39 mg L⁻¹ and to produce indole acetic acid as one of the plant growth hormones. The rhizospheres' strains *P. aeruginosa*, *P. fluorescens*, and *P. syringae* were found to be capable of producing protease, lipase, and α -amylase enzymes, which served as a biocontrol tool against the tomato bacterial wilt-causing organism *Ralstonia solanacearum* (Mohammed et al. 2020). The production of siderophores, phosphorus solubilization, and the secretion of various antagonistic compounds against various plant pathogens are among the numerous beneficial traits that *Pseudomonas* species possess, which are responsible for controlling plant growth (Sah et al. 2021). Plant growth promoting rhizobacteria (PGPR) colonize the roots and improve plant growth by several PGP mechanisms such as solubilization of essential minerals as, phosphorus, potassium, and zinc, biological nitrogen fixation, synthesis of phytohormones, ACC deaminase, siderophores, ammonia, hydrogen cyanide, and secondary metabolites (Kour et al. 2020; Hamada and Soliman 2023; Biswas et al. 2024).

The PI strain's attributes render it a potent biocontrol agent and biofertilizer for the plant. The application of PI to soil in the in vivo pot experiment increased the growth and development of *V. faba*. Growth parameter assessments showed that PI inoculation to soil significantly promoted shoot and root fresh weight and root dry weight, equivalent to plants cultivated in soluble phosphate fertilized soil. Substantial increases of 92, 65, and 200 % in *Zea mays* L. shoot

length, fresh weight, and number of leaves were recorded with bacterial isolate *Stutzerimonas stutzeri* (HH2) at low phosphate levels (10-, and 20-mM TCP), whereas, at 30 mM TCP, an increase of 165 % was observed in root length of plants treated with bacterial isolate *Bacillus bingmayongensis* (KH3) compared to the control (Tariq and Ahmed 2024). The growth patterns of wheat plants that were inoculated with four P-solubilizing bacterial strains independently, namely *Bacillus subtilis* ZE15 and ZR3 and *Bacillus megaterium* ZE32 and ZR19, have shown a promotive effect on the growth. The most noticed significant effect was recorded for the root parameter; including root length, diameter, and volume, similar to the current observation (Iqbal et al. 2020). This suggested that the production of organic acids by PSB strains leads to increased soluble P content in the soil, directly influencing root growth (Oteino et al. 2015; Setiawati et al. 2022). In line with this, Mumtaz et al. (2017) stated that *Bacillus* sp. ZM20, *Bacillus aryabhattai* ZM31, and S10, and *Bacillus subtilis* ZM63 can produce organic acids, which in turn are capable of P and Zn solubilization and possess other characteristics that allow them to promote the growth of maize crops. Rhizosphere microbiota is directly influenced by soil properties, root exudates, and available nutrients (Ling et al. 2022). The bacterial colony counts have shown more colonies in the rhizosphere of TCP + P's faba bean plants. High abundance of bacterial colonies of endophytic strains *Bacillus subtilis* ZE15 and *Bacillus megaterium* ZE32 was recorded for the rhizosphere of wheat plants, which may be attributed to their superior colonization ability compared to other rhizobacterial strains (Iqbal et al. 2020). Otherwise, the microbial activity of these strains releases plant nutrients around the root system: amino acids, carbohydrates, nucleic acid derivatives, growth factors, enzymes, and other factors, triggering the abundant existence of bacterial colonies in the rhizosphere area (Ling et al. 2022). In turn, total microorganisms of the biosphere soil play a key role in the availability of nutrients to plants and thus plant health (Trivedi et al. 2020). Seed priming with PI strain enabled bacteria to adhere to the seed surface, which may subsequently colonize the soil. Nevertheless, their numbers would be restricted in comparison to the intended soil colonization, which would be evident in the plant growth compared to the soil-inoculated treatments (Hamada and Soliman 2023).

As a plant grows, it continues to build up proteins that would be used to construct new cellular components, stabilize the membrane, and be employed in the biosynthesis of enzymes and metabolism. This increased protein synthesis would be concomitant with continuous use of free amino acids, the building blocks for protein (Gupta et al. 2022). This scenario is well established in the current finding, as the promotive effect of the plants that were cultivated in P-inoculated soil may be attributed to the significant increase in the

total soluble protein contents, which reached $91.9 \pm 4.5 \text{ mg g}^{-1}$ FW, while it was $110.32 \pm 2.1 \text{ mg g}^{-1}$ FW for TCP+P' plants, compared to $65.9 \pm 1.2 \text{ mg g}^{-1}$ FW for plants that were grown in soil containing TCP without bacterial inoculation. Similar promotion was observed for the SPh' plant in which the protein concentration was 81.15 ± 6.12 . In contrast, a substantial reduction in free amino acids was observed in those treatments. Similarly, significant increases in tomato protein, sugar contents, chlorophyll a, b, and total chlorophyll were observed in tomato seedlings grown in TCP-containing soil and inoculated independently with PSB isolates named *Pseudomonas* isolates (PsT-04c, PsT-94 s, PsT-116, PsT-124, and PsT-130) and *Bacillus* isolate (BaT-68 s) (Bakki et al. 2024). In accordance with, the TCP+P grown plants exhibited a highly significant increase in chl a, b, carotenoids, and total pigment contents, with values of 96, 57.9, 91, and 82 % higher than those of the control plants, respectively. This enhancement in photosynthetic pigments may explain the congruent enhancement in total protein. As total pigments increase, the plants would do more photosynthesis, thus increasing metabolic processes by which plants grow (Mumtaz et al. 2017; Iqbal et al. 2020; Bakki et al. 2024).

Surprisingly, no significant differences were found in DNA profiling of all treatments, suggesting that PI inoculation and SPh supplementation enhance growth without altering the underlined DNA profiling. In contrast, the DNA profiling using ISSR detected a 25 % total variation in the genetic content of *Malva parviflora* plants bio-inoculated with a plant growth-promoting *Beauveria bassiana* and exposed to drought stress (Abdelhameed et al. 2024). This may result from the generation of ROS that interact with the DNA causing its damage however, in the current study no stress was applied. On the other hand, the gene expression profiling of three selected genes involved in signaling response displayed significant variation that was treatment-dependent. Changes in gene expression were linked to an extensive array of physiological and biological processes (Srivastava et al. 2023). During photosynthesis, *rbcl* is the principal regulatory enzyme accountable for CO_2 fixation. Its activity is subject to the influence of numerous environmental factors, one of which is the availability of phosphate. The significant upregulation of *rbcl* expression in TCP+P' plants indicated that these bacteria possess plant growth-promoting traits like IAA and ammonia production, which would account for the observed increase in photosynthetic pigments and thus ultimately enhance photosynthetic capacity, improving plant growth and development. P availability in the soil enables plants to allocate a greater quantity of resources to the synthesis of RuBisCO, thereby resulting in an upregulation of the enzyme's expression and activity (Pieters et al. 2001). By restricting photophosphorylation in the chloroplasts, P deprivation reduces the ATP/ADP

ratio, consequently diminishing the overall efficiency of the Calvin cycle and impairing RuBisCO activation (Malhotra et al. 2018; Bechtaoui et al. 2021). The application of 200 mg kg^{-1} of P enhanced the photochemical efficacy of PSII (FV/FM) in Brazil nut trees, resulting in a 50 % increase in photosynthesis, light and nutrient use efficiency, and dry biomass (Corrêa et al. 2022).

The P-type H^+ -ATPase gene encodes adenosine triphosphate (ATP)-driven proton (H^+) pumps that couple electrochemical proton gradients across membranes with ATP hydrolysis. Proton-pumping enzymes, specifically plant plasma membrane H^+ -ATPases, are essential for nearly all physiological processes. These include nutrient uptake, pH regulation, stomatal opening, cell proliferation, and stress adaptation (Młodzinska-Michta 2022). As this gene was significantly upregulated in the SPh treatment and also, however, to a non-significant level in the TCP+P' plants (Fig. 7b), its differential expression may indicate that it plays a significant role in the P signaling and transportation. Prior research has established that fluctuations in PM H^+ -ATPase gene expression is influenced by environmental stimuli and signals, including but not limited to low pH (Zeng et al. 2012), salt stress (Cao et al. 2020; Yao et al. 2020), heavy metal stress (Soliman et al. 2025), iron deficiency, and phosphorus deficiency (Yuan et al. 2017). Consequently, the quantity of PM H^+ -ATPases could be modulated to regulate hydrolysis and pump operations in response to varying conditions. The uptake rate of N, P, and potassium (K) was enhanced and the flux rates of cations (H^+ , NH_4^+ , and K^+) were increased in rice roots when *Oryza sativa* PM H^+ -ATPase 1 (*OSA1*) was overexpressed, as compared to gene-knockout *osa1* mutant lines (Ding et al. 2022). Similarly, N and P uptakes in tomato roots were enhanced by overexpression of the *SIHA8* gene, which encodes a PM H^+ -ATPase (Liu et al. 2020).

The extensive distribution of CDPKs throughout the plant indicates that these kinases possess considerable capacity to modulate a multitude of signal transduction pathways and exert a substantial impact on the growth and development of plants (Valmonte et al. 2014; Simeunovic et al. 2016; Kiselev and Dubrovina 2025). The expression of CDPK5 gene exhibited a slight increase in P-soil inoculated and TCP+P plants. However, a substantial upregulation in expression was observed in SPh plants, which may indicate that CDPK5 is involved in P signaling (Fig. 7c). CDPK5 is normally recognized for its upregulation in phosphate starvation conditions. Nevertheless, the upregulation of CDPK5 that was observed in the presence of soluble phosphate implies that it might also play a role in regulating the uptake and utilization of phosphate when it is easily accessible. Plants may at first interpret the existence of soluble phosphate as a possible constraint, which initiates the activation of the signaling pathway mediated

by CDPK5 to fine-tune phosphate homeostasis. Plants may be required to precisely modulate the uptake, distribution, and utilization of phosphate even when soluble phosphate is present to avoid misallocated or over-accumulated phosphate resources. The potential involvement of CDPK5 upregulation in a broader signaling network that regulates phosphate availability in conjunction with nutrient uptake, root development, and stress responses is worth considering. The potential function of phosphate-accumulating bacteria (*P. putida*) in mitigating phosphate deficiency in plants is proposed to be through the mediation of P availability to plants via multiple pathways, according to comparative transcriptome analysis (Srivastava et al. 2023). A comprehensive understanding of the direct and indirect regulation of gene expression by PSB in relation to plant development and growth necessitates the implementation of transcriptome analysis on plants inoculated with PSB.

5 Conclusion

P. lurida (PI)"OR400772" that was isolated from the rhizosphere of an alfalfa plant was able to solubilize insoluble phosphate (P) of tricalcium phosphate (TCP) into soluble form in vitro by producing organic acids, including citric and gluconic acids, which decreased the pH of the media. In addition to its capacity to solubilize P and Zn, it possesses other plant growth-promoting characteristics, including the production of ammonia, indole acetic acid, and hydrogen cyanide, as well as a variety of enzymatic activities, including catalase, protease, and cellulase. As a result, it has the potential to be employed as a bioinoculant in soils that are deficient in P. The use of PI inoculant in a soil containing TCP exhibited a promoter effect on the growth and primary metabolites of the plants that was comparable to or occasionally superior to that of the plants grown in soluble phosphorus fertilized soil. This promotional response may be initiated by modifying the gene expression response of ribulose-bisphosphate carboxylase oxygenase, which directly affects photosynthesis and P-type plasma membrane (PM) H⁺-ATPase, as well as the Calcium-dependent protein kinase gene that may be directly or indirectly involved in P signaling in plants. These findings corroborate our hypothesis that *P. lurida* can be an effective bioinoculant for sustainable agriculture in phosphorus-limited environments. It offers a viable alternative to chemical fertilizers while maintaining plant growth results that are equivalent or superior. A substantial advancement in the understanding of plant–microbe interactions in the context of photosynthesis, nutrient acquisition, and signaling mechanisms is provided by the new mechanistic knowledge regarding the impact of this bacteria on plant molecular pathways.

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Data Availability The datasets generated and/or analyzed during the current study are available in the NCBI GenBank repository- <https://www.ncbi.nlm.nih.gov/nuccore/OR400772.1/>

Declarations

Ethics Approval and Consent to Participate The plant related work was undergoing following the institutional, national, and international guidelines and legislation.

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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