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PLANT MICROBE INTERACTIONS

Plant Growth Promotion at Low Temperature by Phosphate-Solubilizing *Pseudomonas* Spp. Isolated from High-Altitude Himalayan Soil

Priyanka Adhikari[1] · Rahul Jain[2] · Avinash Sharma[3] · Anita Pandey[1,4]

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Abstract

Scarcity of arable land, limited soil nutrient availability, and low-temperature conditions in the Himalayan regions need to be smartly managed using sustainable approaches for better crop yields. Microorganisms, able to efficiently solubilize phosphate at low temperatures, provide an opportunity to promote plant growth in an ecofriendly way. In this study, we have investigated the ability of psychrotolerant *Pseudomonas* spp., isolated from high altitudes of Indian Himalaya to solubilize P at low temperature. Quantitative estimation of phosphate solubilization and production of relevant enzymes at two different temperatures (15 and 25 °C) was performed for 4 out of 11 selected isolates, namely, GBPI_506 (*Pseudomonas* sp.), GBPI_508 (*Pseudomonas palleroniana*), GBPI_Hb61 (*Pseudomonas proteolytica*), and GBPI_CDB143 (*Pseudomonas azotoformans*). Among all, isolate GBPI_CDB143 showed highest efficiency to solubilize tri-calcium phosphate (110.50 ± 3.44 μg/mL) at 25 °C after 6 days while the culture supernatants of isolate GBPI_506 displayed the highest phytase activity (15.91 ± 0.35 U/mL) at 15 °C and alkaline phosphatase (3.09 ± 0.07 U/mL) at 25 °C in 6 and 9 days, respectively. Out of five different organic acids quantified, oxalic acid and malic acid were produced in maximum quantity by all four isolates. With the exception of GBPI_508, inoculation of bacteria promoted overall growth (rosette diameter, leaf area, and biomass) of *Arabidopsis thaliana* plants as compared to uninoculated control plants in growth chamber conditions. The plant growth promotion by each bacterial isolate was further validated by monitoring root colonization in the inoculated plants. These bacterial isolates with low-temperature phosphate solubilization potential along with phosphatases and phytase activity at low temperature could be harnessed for sustainable crop production in P-deficient agricultural soils under mountain ecosystems.

Keywords Low temperature · Plant growth promotion · *Arabidopsis thaliana* · Fluorescence microscopy · Indian Himalayan region

Introduction

Phosphorus (P) is an important nutrient which plays a vital role in plant's growth and development. Phosphorus is required in majority of the plant-based metabolic processes like photosynthesis, energy transfer, signal transduction, and macromolecular biosynthesis [1–3]. Although, it is available in abundant form in soil in both inorganic and organic forms but these are not available forms for the plants. Soil microbes are the key players for the efficient uptake and mobilization of P, making it available to the plant roots [4–6]. Soil microorganisms can easily transform the unavailable form of P to orthophosphate (Pi), the only form of P that plant root can directly assimilate from the soil. These microorganisms are

Priyanka Adhikari and Rahul Jain contributed equally to this work.

✉ Anita Pandey
anita.pandey@geu.ac.in; anitapandey333@gmail.com

[1] Center for Environmental Assessment and Climate Change, G.B. Pant National Institute of Himalayan Environment, Almora, Uttarakhand 263643, India

[2] Biotechnology Division, CSIR – Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh 176061, India

[3] National Centre for Cell Science, Pune, Maharashtra 411007, India

[4] Department of Biotechnology, Graphic Era (Deemed to be University), Bell Road, Clement Town, Dehradun, Uttarakhand 248002, India

referred as phosphate-solubilizing microorganisms (PSMs) and are considered effective components of the agricultural soils that contribute towards improving global food production [4, 7].

Inorganic forms of P found in soils, in general, are a mixture of crystalline unstructured calcium, aluminum, and iron phosphate. Solubilization of inorganic insoluble phosphate salts by different microorganisms depends on their ability to produce and release organic acids in the surrounding environment. These organic acids decrease the pH of the soil thereby causing release of the metal ion of insoluble phosphates in soil, resulting in the solubilization of phosphate salts. Therefore, PSMs can help in minimizing application of synthetic P fertilizers and enhancing fertility of soils [8, 9]. Contrary to inorganic P, up to 80% of total P in soil account for organic P in the form of phytic acid (phytate) which remain strongly bound to soil reducing its bioavailability to plants [10]. The enzyme phytase along with acid/alkaline phosphatases produced by soil microorganisms mineralize organic form of P in soil to release the plant-available orthophosphate [11].

The microbial diversity of cold environments, such as the Indian Himalayan region (IHR) and the Tropical Andes, is receiving increased attention with reference to their prospective biotechnological applications including the promotion of agriculture [12–17]. With continuous concern over food security and global increase in population, a burden on agriculture sector is increasing day by day to fulfill the forthcoming food demand in a sustainable way. This burden can be partially reduced by increasing agriculture productivity in mountainous environment where a low crop yield is observed due to less availability of nutrients, minimum land availability, and low temperatures, by applying native microbial strains as a potential source of biofertilizers [18]. In our earlier reports, we have identified several psychrotolerant *Pseudomonas* isolates possessing various properties of biotechnological and agricultural relevance [14, 18–20]. Therefore, the current study aims at characterizing the phosphate-solubilizing/mineralizing potential of psychrotolerant *Pseudomonas* spp. isolated from Himalayan soil. Through this study, the long-term goals will be to develop effective cold applicable biofertilizers for mountain ecosystems.

Material and Methods

Bacterial Isolates

A total of 11 *Pseudomonas* spp. isolates, isolated from soils at high-altitude sites in Indian Himalaya, were selected for the current study. The strains were preserved and maintained at the in-house microbial culture collection established in the microbiology lab of GB Pant National Institute of Himalayan Environment, Uttarakhand, India. All strains were reactivated from the glycerol stocks and routinely subcultured on tryptone yeast extract (TYE) agar by incubation at 25 °C for 24 h. Identification of all isolates based on 16S rRNA gene sequencing has been reported earlier (Table S1) [20].

Qualitative and Quantitative Estimation of Phosphate Solubilization

Qualitative estimation on phosphate solubilization by bacteria was performed on Pikovskaya's agar at four temperatures (5, 15, 25, and 35 °C). Formation of clear zones of phosphate solubilization around the bacterial colonies after 48 h incubation was indicative of the bacterial ability to solubilize inorganic phosphorus.

Quantitative estimation of phosphate solubilization was performed in Pikovskaya's broth at 15 and 25 °C. The cultures were inoculated, separately, in 50 mL Pikovskaya's broth containing tri-calcium phosphate (TCP) in 250 mL Erlenmeyer flasks and incubated. The estimation of solubilized phosphorus was performed every 3rd day of incubation up to 21 days by the method of Murphyand Riley [21] using a UV-Vis spectrophotometer (Amersham Biosciences Ultrospec 3100 Pro, Sweden). Reduction in the pH of cell-free supernatant obtained after centrifugation (10,000×*g*, 5 min) was also measured simultaneously using a pH meter. All the experiments were performed in triplicates.

Production of Acid and Alkaline Phosphatases

Quantitative estimation of alkaline and acidic phosphatase activity was performed at 15 and 25 °C using the cell-free culture supernatants obtained from Pikovskaya's broth as explained above. For assaying alkaline phosphatase, 0.4 mL culture supernatant was mixed with 1 mL of glycine-sodium hydroxide buffer (0.1 M, pH 9.0). This was followed by addition of 0.5 mL p-nitrophenyl phosphate (1 mg/mL) and 0.1 mL of $MgCl_2$ to the reaction mixture. Same procedure was followed for acid phosphatase assay, except citrate buffer (0.1 M, pH 5.0) which was used instead of glycine-sodium hydroxide buffer. The reaction mixtures were incubated at 25 °C for 30 min. After incubation, 100 μL of NaOH (5 M) was added and the release of p-nitrophenol (pNP) was measured at 400 nm using a UV-Vis spectrophotometer. Activities of both the enzymes were measured every 3rd day up to 21 days in triplicates.

Production of Phytase

The cultures were screened for their ability to release inorganic phosphate from sodium and calcium phytate at four different temperatures (5, 15, 25, and 35 °C) using phytase

screening medium (PSM) (10.0 g/L D-glucose, 2.0 g/L $CaCl_2$, 5.0 g/L NH_4NO_3, 0.5 g/L KCl, 0.5 g/L $MgSO_4.7H_2O$, 0.01 g/L $FeSO_4.7H_2O$, 0.01 g/L $MnSO_4.H_2O$, 4.0 g/L sodium phytate/calcium phytate substrate, 15.0 g/L agar, pH 7.0). Positive isolates for the production of phytase enzyme formed a clear hollow zone around the colony due to the hydrolysis of calcium and/or sodium phytate after 48 h of incubation.

The phytase production was quantitatively estimated in 50 mL PSM broth in 250 mL Erlenmeyer flasks at 15 and 25 °C. A total of 150 μL of cell-free supernatant containing crude enzymes was added to 600 μL substrate solution (2 mM sodium phytate in 0.2 M Tris-Cl buffer, pH 6.5) and incubated for 30 min. This was followed by addition of 750 μL of 5% trichloroacetic acid to stop the reaction. The estimation of released inorganic phosphate was performed as described previously.

Organic Acid Estimation by HPLC

Production of organic acids (oxalic acid, malic acid, succinic acid, citric acid, and lactic acid) was estimated using reverse-phase high-performance liquid chromatography (RP-HPLC) (Shimadzu LC-2030 Plus solution, Japan) equipped with prominence photo diode array (PDA) detector (SPD M20A) and the data were analyzed using the Shimadzu LC solution software. Two milliliters of culture supernatants from Pikovskaya's broth previously inoculated with the different test cultures was collected, centrifuged, and filtered through 0.2 μm syringe filters. The organic acids in the filtrate were determined by HPLC (C18 column, 5 μm, 250 × 4.6 mm), using 1 mM H_2SO_4 in 1 mM Na_2SO_4 (pH 2.8) as eluent at a flow rate of 1 mL/min. Organic acid detection was performed every 3rd day and up to 21 days. Deionized water was used as blank.

PCR Amplification of gcd Gene

Genomic DNA was isolated following the method of Chen and Kuo [22]. Primer pair gcdF 5′-GACCTGTGGGACATGGACGT-3′ and gcdR 5′-GTCCTTGCCGGTGTAGSTCATC-3′ [23] was used for the amplification of glucose dehydrogenase encoding gene (*gcd*). Polymerase chain reaction (PCR) was performed on a SimpliAmp thermal cycler (Applied Biosystems). The following conditions were used for PCR: 1 cycle of 94 °C for 1 min; 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min and 1 cycle of 72 °C for 5 min. After PCR, the amplified product was run on 1.2% agarose gel and photo documentation was performed using a gel doc system. Similarly, we also amplified an internal sequence of genes involved in cofactor pyrroloquinoline quinone biosynthesis (*pqqE* and *D*), and *Pseudomonas*-specific phytases. Details of the primers and their sequences used in this study are provided in Table S2.

Plant-Based Bioassays

Model plant *Arabidopsis thaliana* (ecotype Col-0) was used for plant bioassays to determine potential of bacterial isolates in promoting plant growth under growth chamber conditions. Seeds were surface sterilized and germinated in sterile coco-peat mix after stratification at 4 °C for 48 h. Germinated seeds were allowed to grow for 2 weeks in a plant growth chamber (23 ± 2 °C, 12 h photoperiod, and 70-80% relative humidity). For inoculum preparation, each isolate was grown in 50 mL TY broth at 25 °C for 24 h under shaking (150 rpm). Bacterial cells were then centrifuged (6000×*g*, 5 min) and washed with sterile distilled water, resuspended in 10 mL of sterile saline solution, and finally used for priming and inoculation (cell concentration = 10^8 cfu/mL). Individual plants after 2 weeks of growth were root primed by dipping in to bacterial suspension for 1 h and air-dried. After root priming, each plant was transferred into a fresh pot (5 × 7 cm) filled with coco-peat mix and 1 mL of bacterial suspension was inoculated around the roots. One plant per pot and 10 plants per treatment were maintained. Control plants were root primed and inoculated with equal volume of sterile saline solution. The inoculated plants were kept in a plant growth chamber at same conditions as stated above and harvested 3 weeks post inoculation. After harvesting, leaf area (mm^2) for individual leaf in all treatments was calculated using the ImageJ v1.53a (NIH, USA) software. Rosette diameter (cm), fresh weight (mg), and dry weight (mg) of shoots and roots were also measured. The plants were photographed at regular intervals and on the day of harvesting.

Fluorescence Imaging for Root Colonization

Fresh roots of 2-3 plants were collected post harvest and washed with sterile distilled water to remove any adhered particles. Roots were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) for 2 h at 4 °C, washed in PBS, and stained with 1:1000 diluted solution of Syto 9 and Baclight red (Invitrogen, USA), mixed in equimolar concentrations. After staining for 10 min, roots were washed with PBS and fluorescent images were obtained using EVOS® FL Auto Cell Imaging System (Invitrogen, USA). Images were captured at both GFP and RFP channel and merged. Images were processed using Image J for an optimized view of root colonization by bacteria. At least 20 root pieces were observed and a representative image of each treatment is shown.

Statistical Analysis

Analysis of variance (ANOVA) following Duncan's multiple range test (DMRT) was performed by SPSS v20 software package for measuring statistical difference ($p < 0.05$). Box plot analysis was performed for finding variability in the

production of organic acids secreted during phosphate solubilization at 15 and 25 °C.

Results

Phosphate Solubilization

Qualitative estimations for phosphate solubilization and phytate mineralization were done on the basis of zone of clearance around the bacterial colonies (Fig. S1). Out of 11 isolates, only four *Pseudomonas* species, namely GBPI_506, GBPI_508, GBPI_Hb61, and GBPI_CDB143, were considered for the quantitative estimation of phosphate solubilization, phytase, and phosphatases enzyme production at 15 and 25 °C based on high activity recorded in qualitative assays (Fig. S2).

In quantitative estimations at 15 °C, isolate CDB_143 (109.47 μg/mL; 12th day) solubilized maximum phosphorus followed by GBPI_Hb61 (104.61 μg/mL; 3rd day). Isolates GBPI_508 (80.40 μg/mL; 9th day) and GBPI_506 (91.84 μg/mL; 6th day) solubilized comparatively lower amount of phosphorus at 15 °C. When monitored at 25 °C, the solubilization efficiency of isolates CDB_143 and GBPI_Hb61 was similar to that of 15 °C i.e. 110.89 μg/mL (6th day) and 103.55 μg/mL (3rd day), respectively. However, the isolates GBPI_506 (50.13 μg/mL; 15th day) and GBPI_508 (55.13 μg/mL; 18th day) solubilized lesser amount of phosphorus at 25 °C as compared to 15 °C ($p < 0.05$) (Fig. 1).

The pH of the culture broth was also monitored during the course of phosphate solubilization by the bacteria. A higher amount of solubilized phosphorus was accompanied with low pH of the culture broth. The lowest pH of the medium recorded during the phosphate solubilization process was pH 3.05 as compared to the initial pH of 7.0 (Fig. 1).

Estimation of Phytase and Phosphatase Production

Maximum phytase production by all four bacterial isolates was recorded at 15 °C except isolate GBPI_CDB143, where maximum activity (14.31 U/mL) was recorded at 25 °C. Isolate GBPI_506 showed maximum phytase activity of 15.91 U/mL on the 6th day at 15 °C. No significant difference ($p < 0.05$) in the maximum phytases activity of isolate GBPI_Hb61 was recorded at 25 °C (12.16 U/mL) and 15 °C (12.09 U/mL); however, this difference in the enzyme activity was significant ($p < 0.05$) in case of isolate GBPI_508 (Table S3).

Phosphatase production varied with respect to the days of incubation. Acid phosphatase activity in the culture supernatants was not detected after prolonged incubations in case of all the bacterial isolates (Table S2). In contrast, alkaline phosphatase activity was detected in culture supernatant till 21st day of incubation. Isolate GBPI_508 produced maximum acid as well as alkaline phosphatase enzymes at both the temperatures i.e. 15 and 25 °C; however, no significant difference ($p < 0.05$) was observed in the maximum activity of both the enzymes produced at 15 and 25 °C.

Production of Organic Acids

The organic acids produced by four isolates in the Pikovskaya's broth were analyzed, at both the temperatures. HPLC analysis of five standard organic acids including oxalic acid (retention time, $t_R = 2.727$), malic acid ($t_R = 3.963$), lactic acid ($t_R = 5.131$), citric acid ($t_R = 6.350$), and succinic acid ($t_R = 9.402$) was performed based on their retention time. Out of five different organic acids quantified by HPLC, all four bacterial isolates were more efficient in producing oxalic and malic acids in comparison to other organic acids (Fig. 2). At 25 °C, maximum amount of oxalic acid (0.439 μg/mL) was produced by GBPI_506; malic acid (0.376 μg/mL), lactic acid (0.041 μg/mL), and citric acid (0.086 μg/mL) by GBPI_CDB143, and succinic acid (0.059 μg/mL) by GBPI_Hb61. Similarly, at 14 °C, maximum amount of oxalic acid (0.216 μg/mL) and malic acid (0.289 μg/mL) were produced by GBPI_508; maximum lactic acid (0.093 μg/mL) and citric acid (0.087 μg/mL) by GBPI_506; and succinic acid (0.089 μg/mL) by GBPI_Hb61. The box plot analysis showed a high variability in the organic acid particularly oxalic acid and malic acid produced at 15 and 25 °C (Fig. 2).

PCR Amplification

The gene coding for glucose dehydrogenase was successfully amplified for all 11 *Pseudomonas* isolates under study using PCR. The approximate molecular size of the amplified product was 875 bp (Fig. 3). Amplification of genes involved in pyrroloquinoline quinone biosynthesis, a cofactor for glucose dehydrogenase was also intended, without success. However, when amplification of phytase encoding genes was performed using a set of designed primers, multiple DNA bands were observed (data not shown).

Effect of Bacterial Inoculation on Plant Biomass

Visual analysis of plants inoculated with bacteria clearly showed an increased overall plant growth in comparison to control except in the case of isolate GBPI_508, where a reduced and stunted growth was observed (Fig. 4a). Total rosette diameter was maximal in plants inoculated with GBPI_Hb61 (1.9-fold) followed by GBPI_CDB143 (1.59-fold) and GBPI_506 (1.54-fold) with a significant difference ($p < 0.05$) from untreated plants (2.08 cm) (Fig. 4b). Moreover, similar order for leaf area of inoculated plants was observed with maximum leaf area for GBPI_Hb61 (4.25-fold) followed by GBPI_CDB143 (2.52-fold) and

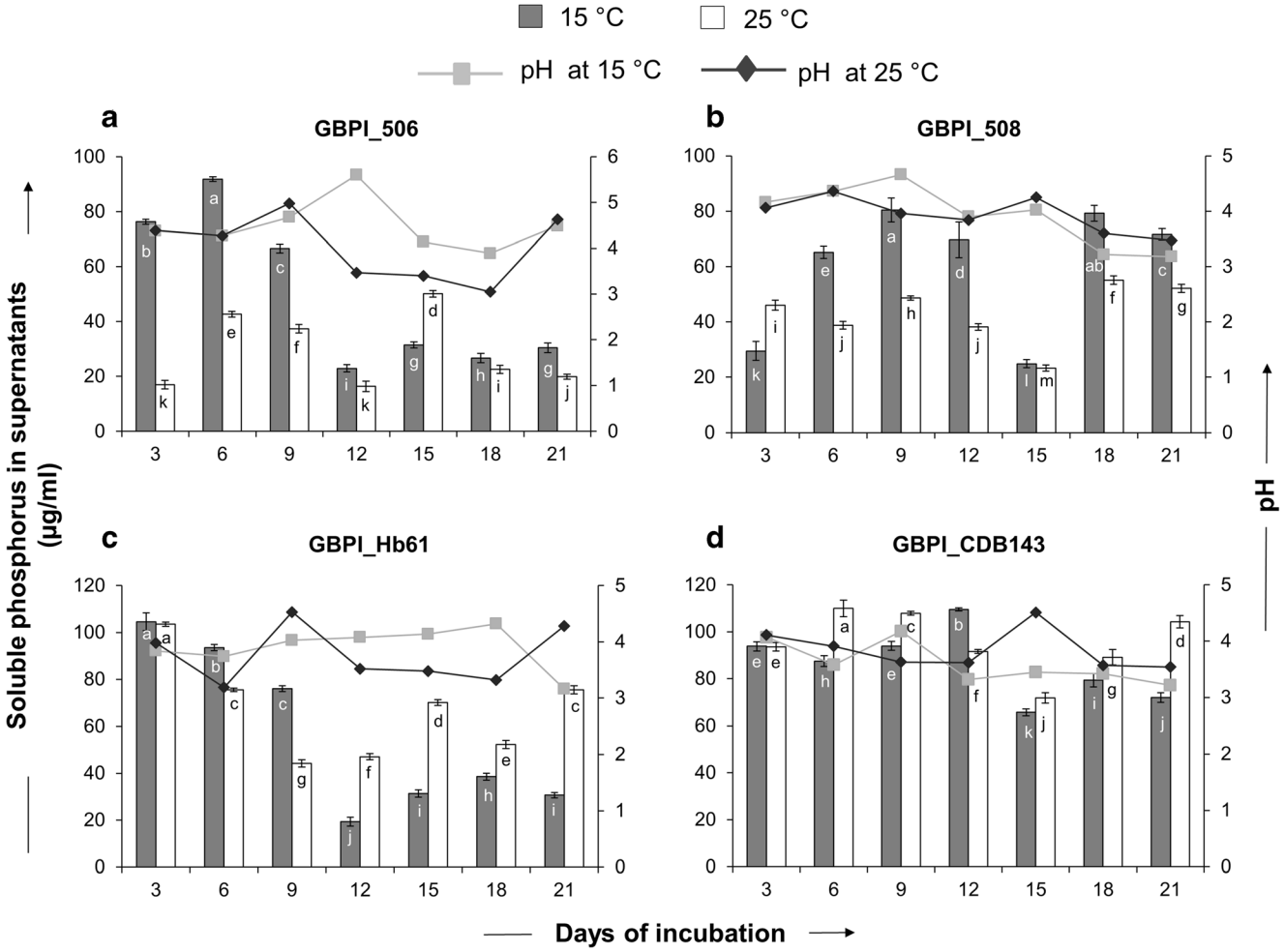

Fig. 1 Kinetics of phosphate solubilization by *Pseudomonas* spp. isolates and pH of supernatants at 15 and 25 °C. Different letters above the bars indicate significant difference ($p < 0.05$). Column bars indicate mean value ± standard error ($n = 3$)

GBPI_506 (2.19-fold) in comparison to untreated plants (12.11 mm^2) (Fig. 4c). Inoculation with GBPI_508 reduced the overall plant growth as indicated by minimum rosette diameter and leaf area.

In case of plant biomass, fresh weight was observed maximum for GBPI_Hb61 (3.01 and 3.98-fold for shoot and roots, respectively) followed by GBPI_506 (1.53 and 1.79-fold for shoot and roots, respectively). This increase in fresh biomass was significant ($p < 0.05$) in comparison to control plants. However, no significant difference in plant biomass was recorded for plants inoculated with the isolate GBPI_CDB143. In line with visual observations, shoot biomass of plants inoculated with isolate GBPI_508 was recorded minimum. Conversely to biomass on fresh weight basis, no significant improvement in biomass on dry weight basis was observed in all treatments, except GBPI_Hb61. GBPI_Hb61 recorded a 2.26 and 2.04-fold increase in shoot and root biomass, respectively, as compared to dry biomass of control plants (Fig. 4d).

Root Colonization by Inoculated Bacteria

Evidences from fluorescence microscopy supported the plant bioassay-based results (Fig. 5). In case of control plants as well as plants inoculated with GBPI_508, only rare root colonization was observed. However, the roots of plants inoculated with remaining three pseudomonads including GBPI_Hb61, GBPI_CDB143, and GBPI_506 showed an abundant colonization throughout the roots. The internalization of bacteria was observed through the root hairs as bacterial threads were clearly seen occupying spaces in root hairs (Fig. 5c).

Discussion

Decreased agriculture productivity in low-temperature mountain environments is one of the major concerns for the self-sustainability of local farmers. Moreover, low nutrient availability in cold soil due to reduced cellular processes further

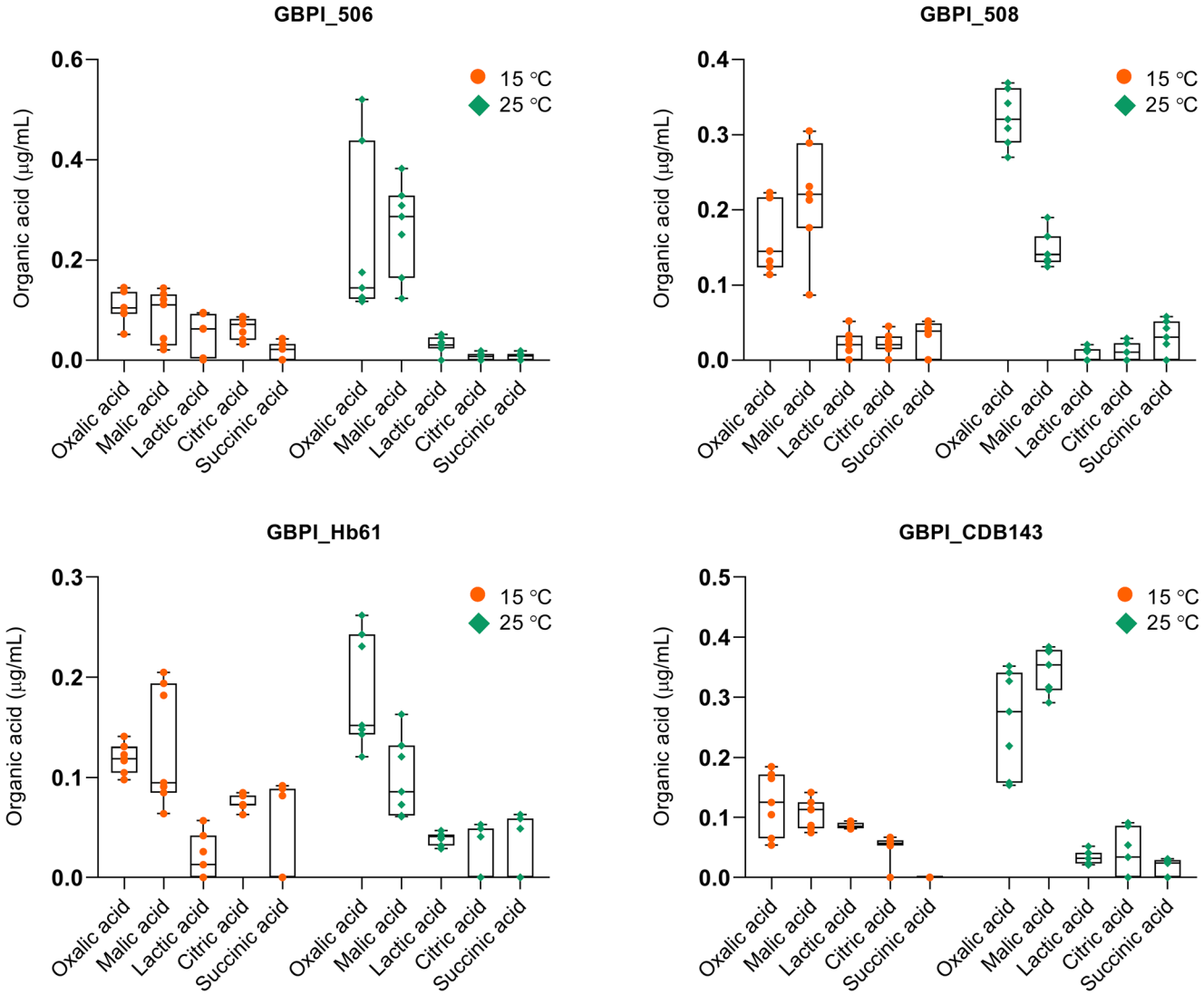

Fig. 2 Variability in organic acids production by four pseudomonads in Pikovskaya's broth during phosphate solubilization at 15 and 25 °C detected using HPLC

decreases the plant growth. The efficacy of the inorganic P fertilizer is very low due to their fixation in acidic as well as alkaline soils; both soil types predominate in mountain regions. Further, due to high cost and human health issues of inorganic fertilizers [24], use of microbes as bioinoculants has emerged as an alternate strategy for supplying nutrients

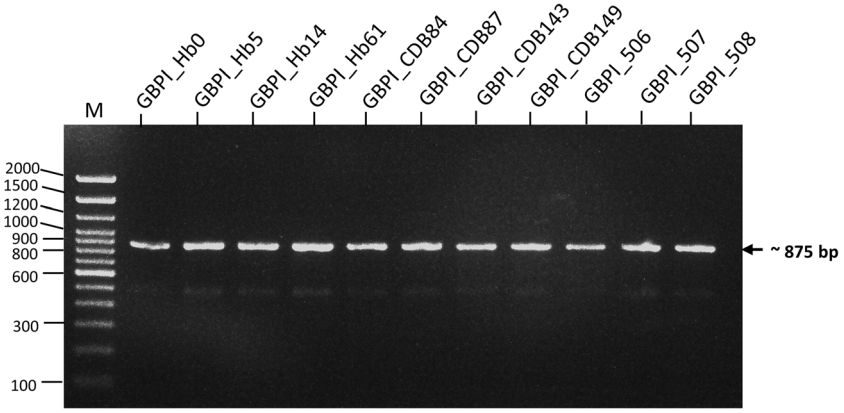

Fig. 3 PCR amplification of the gene encoding glucose dehydrogenase (*gcd*) in *Pseudomonas* spp. under study. Amplicon of ~875 bp was detected in 1.2% agarose gel

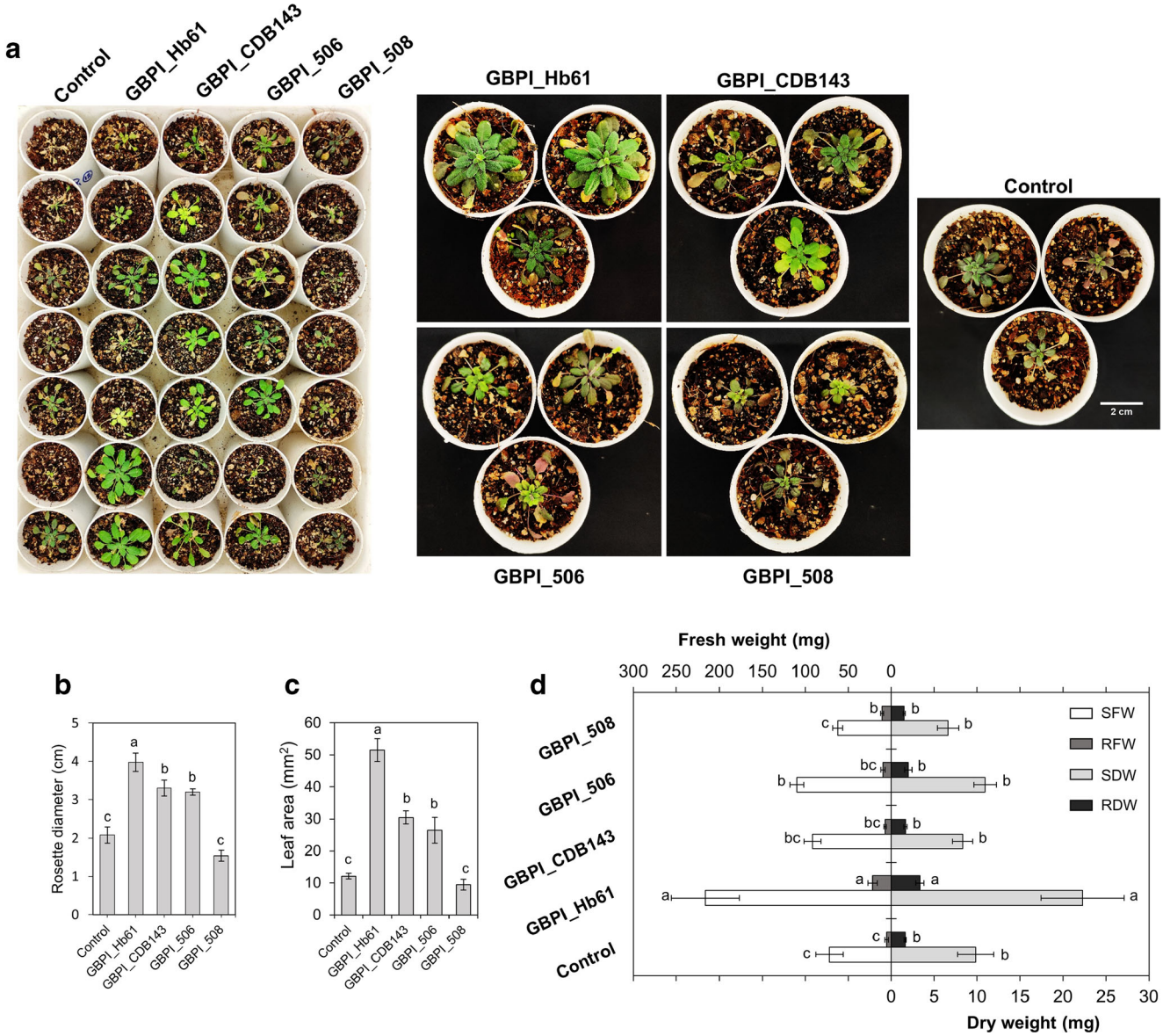

Fig. 4 Effect of bacterial inoculation on *Arabidopsis thaliana* plant growth in controlled conditions. **a** Plant bioassay performed on *A. thaliana* after individually inoculating 4 *Pseudomonas* isolates. Control plants were inoculated with equal volume of sterile saline solution. Rosette diameter (**b**) and leaf area (**c**) of plants in each treatment. Fresh and dry biomass of plant root and shoot following bacterial inoculation (**d**). Error bars indicate standard errors (n = 3-6). Different letters represent significant difference ($p < 0.05$) among treatments as calculated by DMRT. SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight and RDW, root dry weight

including soluble phosphorus to the plants at lower costs and to enhance soil fertility [25]. Therefore, application of native PSMs and other useful microbial inoculants tolerant to low temperatures in soils can be an important strategy to restore soil for sustainable agriculture [8, 13]. *Pseudomonas* is one of the most diverse bacterial genera in agricultural soils and plays an important role in maintaining soil fertility [26]. Their potential as plant growth-promoting bacteria is well known and these organisms have been reported for their applications as biofertilizers and biocontrol agents [14, 27, 28].

The current study reveals the ability of *Pseudomonas* spp. to solubilize phosphate at wide range of temperature from as low as 5 °C up to 35 °C. This potential of phosphate solubilization was higher at 15 °C in case of isolates GBPI_506, GBPI_508, and GBPI_Hb61 while isolate GBPI_CDB143 solubilized more phosphorus at 25 °C, with overall solubilizing efficiency in a range from 16.97 to 110.78 μg/mL. Since the average temperature throughout the year remains low in Himalayan mountains, it is very important to screen microorganisms for use in agricultural lands with higher efficiency of phosphate solubilization at low temperature. Low temperature-tolerant bacteria with plant growth-promoting attributes supplied in the form of biofertilizers may perform efficiently under such conditions to mobilize nutrients (P)

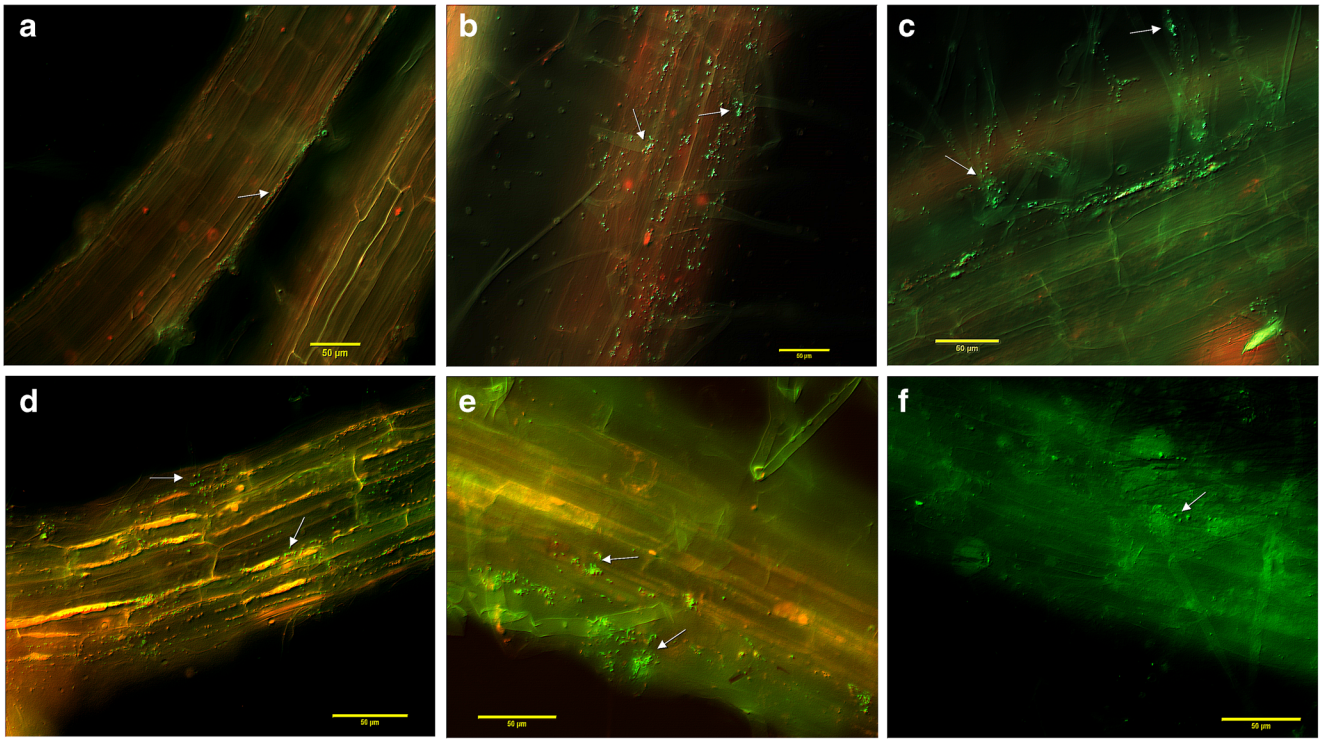

Fig. 5 Fluorescence microscopy of *Arabidopsis thaliana* roots inoculated with GBPI_Hb61 (**b**-**c**), GBPI_143 (**d**), GBPI_506 (**e**), and GBPI_508 (**f**) under study. Arrows show the bacterial cells colonizing root cells/surface. Scale bar = 50 μm. Root hairs were seen predominantly colonized by bacteria forming a bacterial thread leading to internalization (**c**). Note the rare microbial colonization pattern in case of control (**a**) and GBPI_508 (**f**)

[14]. For instance, maize yield increased by inoculation of cold-tolerant *Pseudomonas corrugata* as shown by pot and field experiments under rain-fed conditions in the Himalayan region [29]. Psychrotolerant *Pseudomonas* spp. (RT5RP2 and RT6RP) isolated from the rhizoplane of high-altitude wild grass can solubilize rock phosphate at temperatures ranging from 4 to 30 °C [30]. Similarly, *Pseudomonas palleroniana* N-26 and *Pseudomonas jessenii* MP-1 isolated from the Western Indian Himalayan soils were reported as efficient phosphate solubilizers by Tomer et al. [31]. Plant growth promotion abilities including solubilization of tri-calcium phosphate and enhanced plant growth of wheat by *Pseudomonas* spp. isolated from Antarctic soils have been reported [32]. Several *Pseudomonas* isolated from the Himalayan region are reported for their ability to solubilize insoluble form of P in the soils [12, 33].

Phosphate solubilization by the microbes was concomitant to a decrease in the pH of the growth media. This generally occurs due to the release of several low molecular organic acids that facilitate phosphate solubilization through their hydroxyl and carboxyl groups by chelating cation bound to phosphate thus converting it to soluble form [23, 34]. The HPLC analysis permitted to detect the release of oxalic acid and malic acid as the most prevalent organic acids while lactic acid, citric acid, and succinic acid as less accumulated organic acids in the culture supernatants at 15 as well as 25 °C. Our results are in agreement with the previous findings, where a decrease of up to 3-4 units in pH has been reported along with the release of organic acids during the course of phosphate solubilization [35, 36]. For instance, *Pseudomonas* sp. MS16 produces gluconic acid, acetic acid, and oxalic acid in the growth media during phosphate solubilization [37]. Similarly, de Amaral Leite et al. [38] reported release of oxalic acid, quinic acid, and succinic acid by phosphate-solubilizing *Pseudomonas* sp. UFPI B5-8A. Moreover, the efficacy of oxalic acid in solubilizing a high amount of insoluble phosphorus has been proven [39–41] and similar findings are also observed in the current study. Presence of a membrane-bound key enzyme, glucose dehydrogenase (gcd) involved in gluconic acid biosynthesis [37, 42] might play a role in phosphate solubilization by these isolates.

Besides solubilizing mineral phosphate, *Pseudomonas* spp. were able to mineralize phosphorous from phytate. Isolates GBPI_506 and GBPI_143 produced maximum phytase at 15 °C and 25 °C, respectively. Phytate is an abundant natural organic form of phosphorus in many soils and can be a source for microorganisms to release plant-available orthophosphate. Shulse et al. [43] engineered root-associated bacteria including *Pseudomonas simiae* WCS417r, *Ralstonia* sp. strain UNC404CL21Col, and *Pseudomonas putida* KT2440 to release plant-available phosphorus from phytate. All these three strains can also solubilize tri-calcium phosphate. In another

study, heterologous expression of bacterial phytase (BPP and HAP family) in *Arabidopsis thaliana* has shown to stimulate plant growth without any side effects on plant physiology and seed germination [44]. The ability to release phosphorus was further complemented by the presence of acid and alkaline phosphatase activities in the bacterial isolates. Several evidences report acid and alkaline activities in phosphate-solubilizing bacteria such as *Serratia* sp. and *Pantoea* sp. [36, 45].

In plant-based bioassays, it was clearly observed that three out of the four tested isolates promoted the growth of *A. thaliana*. The rosette diameter, leaf area, and plant biomass were significantly improved in inoculated plants as compared with control. Several lines of evidence suggest that P availability by the beneficial microbes in the rhizosphere as well as endosphere or expression of genes involved in phosphorus uptake has beneficial impact on the plant growth [37, 44, 46, 47]. Colonization by microorganisms in root environment determines their efficacy for showing beneficial impacts on plants [48]. In our results, maximum effect of bacterial inoculation on *Arabidopsis* growth in case of GBPI_Hb61, GBPI_CDB143, and GBPI_506 was correlated to the abundant bacterial colonization in and around the roots.

Presence of multifaceted phosphorus-mobilizing features in all the *Pseudomonas* isolates through utilization of different substrates provide an opportunity for formulating biofertilizers which not only can release bioavailable P in soil but also are efficient in low temperatures. As the efficiency of *Pseudomonas* to transform P into bioavailable forms through mineralization or solubilization varies depending on the species and substrates [49, 50], a consortium of multiple and compatible *Pseudomonas* species will be an efficient approach for P-biofertilizer formulation.

Conclusion

Himalayan soil represents a vast reservoir of microbial diversity that deserves to be thoroughly explored for the potential applications and conservation. In this study, diverse low temperature-tolerant *Pseudomonas* spp. isolated from such environment have been evaluated for important traits of plant growth promotion i.e. inorganic phosphate solubilization through organic acid secretion and organic P mineralization through phytase and phosphatase production. Bacterial efficacy to promote plant growth at low temperature by phosphate solubilization/mineralization provides an insight on how the potentials of these organisms can be utilized for making agricultural system resilient to climate change. Application of plant-beneficial microorganisms in agricultural lands not just increases the crop productivity but also provides an ecofriendly strategy for increasing the economy of local farmers.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-021-01702-1.

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Declarations

Conflict of Interest The authors declare no competing interests.

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