



Multifarious plant growth promoting ability of *Pseudomonas extremorientalis* RPB22 enhances chickpea (*Cicer arietinum*) growth

SAMADHAN YUVRAJ BAGUL^{1,2*}, RITU RAJ PATEL³, RITU VISHWAKARMA², HILLOL CHAKDAR², PANDIYAN K^{1,4}, KUMAR M², MAGESHWARAN VELLAICHAMY², RAGHUNANDAN B L⁵ and ALOKK KUMAR SRIVASTAVA²

ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh 275 103, India

Received: 29 July 2024; Accepted: 06 January 2026

ABSTRACT

The present study was carried out during 2020–2021 at ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh to assess the plant growth-promoting potential of a bacterial isolate obtained from the hot water spring at Tattapani, Chhattisgarh. The hot spring exhibited a temperature of 80°C and a pH of 7.5. Molecular characterization identified the isolate as *Pseudomonas extremorientalis* strain RPB22. This strain showed tolerance to salinity up to 3% NaCl and temperature of 50°C. Strain RPB22 efficiently solubilized inorganic tricalcium phosphate (333 ± 9 µg/mL) and potassium (10.5 mg/L). High-Performance Liquid Chromatography (HPLC) analysis revealed the production of several organic acids, predominantly malic acid, followed by oxalic acid. Seed treatment of chickpea with strain RPB22 led to a 2.75-fold and 2.17-fold increase in fresh root and shoot dry weight, respectively, while root volume increased 1.8-fold compared to the untreated control. These findings highlight the potential of *P. extremorientalis* RPB22 as a thermo-tolerant, multifaceted plant growth-promoting bacterium suitable for biofertilizer development in arid and semi-arid environments exposed to high temperatures.

Keywords: Hot spring, HPLC, Organic acid, Phosphate, Plant growth promotion, Thermo-tolerance

Over the past few decades, microbial communities adapted to extreme environmental conditions have attracted considerable attention due to their unique physiological and biochemical traits. Extremophilic microorganisms exhibit remarkable resilience across a spectrum of harsh conditions, including extremes of temperature, salinity, pH levels, and radiation exposure (Mirete *et al.* 2016). Among these environments, thermal springs have captured significant attention from researchers globally. The microbial communities inhabiting these locales serve as invaluable reservoirs for diverse biotechnological applications, yielding antibiotics, novel compounds, and even new species (Sayeh *et al.* 2010, Zhang *et al.* 2010). Typically, the water temperature in hot springs varies between 40–60°C, constituting ideal niches for microbial diversity (Badhai

et al. 2015, Saxena *et al.* 2017). The composition of microorganisms is profoundly influenced by environmental factors such as temperature, pH, dissolved hydrogen sulfide concentrations, and the physical and chemical properties of water and sediments. Generally, the pH in most hot springs falls within the range of 7–7.5 (Purcell *et al.* 2007, Soy *et al.* 2019). These hot springs represent biodiversity hotspots for microbes, offering promising avenues for the discovery of novel genes, molecules, and hydrolytic enzymes essential for industrial, medical, and agricultural applications (Saxena *et al.* 2017). Despite the abundance of thermal springs in India, there has been limited exploration of hot springs of India. The Tattapani hot spring in Balrampur, Chhattisgarh, India, remains relatively unexplored.

Certain bacteria isolated from extreme environments possess plant growth-promoting (PGP) traits, including ammonia production, nitrogen fixation, phosphate solubilization, organic acid production, iron chelation through siderophore production, and indole-3-acetic acid (IAA) production for root development (Ahemed *et al.* 2014). While plants have inherent mechanisms to cope with extreme conditions, PGP bacteria from hot springs can enhance plant growth under unfavourable environmental conditions. However, only limited studies have reported plant growth-promoting (PGP) bacteria isolated from hot springs

¹ICAR-Directorate of Medicinal Aromatic Plant Research, Boriavi, Anand, Gujarat; ²ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh; ³Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh; ⁴ICAR-Central Institute for Research on Cotton Technology, Mumbai, Maharashtra; ⁵College of Natural Farming, Natural Farming Science University, Halol, Panchmahal, Gujarat.
*Corresponding author email: samadhan.bagul@icar.org.in

exhibiting multiple beneficial traits such as phosphate, zinc, and potassium solubilization, along with IAA and siderophore production (Verma *et al.* 2018, Mukherjee *et al.* 2020). The exploration of such thermotolerant PGP bacteria is particularly relevant, as chickpea is highly sensitive to elevated temperatures, which adversely affect flowering, pod set, and overall yield (Jha *et al.* 2025). Therefore, isolation and characterisation of heat-tolerant *Pseudomonas* strains with multifaceted PGP attributes could provide an effective biological strategy to mitigate heat-induced yield losses in chickpea cultivation.

MATERIALS AND METHODS

The present study was carried out during 2020–2021 at ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh. Soil sample was collected in a sterile thermoflask from hot spring of Tattapani (23° 40'48.69" N, 83° 39'11.06" E), Balrampur, Chhattisgarh, in February 2020 (Fig. 1). Physicochemical parameters like pH and electrical conductivity were recorded following standard procedure (Jackson 1958) at ICAR-National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh. The sample collected from hot spring was processed on nutrient agar media for the isolation of bacterial isolate using standard spread plate technique. One gram of soil was serially diluted in 9 mL of 0.85% NaCl, and 0.1 mL of the 10⁻⁶ dilution was spread onto nutrient agar plates. The plates were then incubated for 24–48 h at 30°C and at 60°C in Biological Oxygen Demand (BOD) incubator. After incubation, growth was observed on petri-plates. Pure colonies of the bacteria were picked and streaked again on the respective media. The pure cultures of bacteria were then transferred to 20% sterile glycerol stock and preserved at -20°C for further use. Colony morphology of bacterial cultures was observed. Isolates with distinct colony morphology based on shape, colour, texture, pigmentation and Gram stain reaction were recorded. Strain RPB 22 was selected on the basis of multifarious plant growth promoting attributes for further

study. Genomic DNA was extracted using the Nucleo-pore gDNA Mini Kit (Genetix Biotech). The 16S rRNA gene was amplified with universal primers 27F and 1492R in a 25 µL PCR reaction (Taq buffer, dNTPs, Taq polymerase, primers, template). PCR products (~1,500 bp) were resolved on 1.2% agarose gel, purified using the QIAquick kit (Qiagen), and sequenced at ICAR-NBAIM (ABI 3130xl Genetic Analyzer). The sequences were quality-checked and compared with NCBI and EzTaxon databases (Yoon *et al.* 2017), identifying species with ≥98.6% similarity. All 16S rRNA gene sequences were deposited in NCBI. Bacterial isolate obtained were screened for growth at different temperature (40°C, 50°C, 70°C and 80°C) in water bath, pH (4–11) and salt concentrations (0–10% NaCl w/v), respectively. Qualitative phosphate solubilization was assessed on Pikovskaya's agar containing 0.5% tri-calcium phosphate (Pikovskaya 1948). Potassium solubilisation was evaluated on Aleksandrov agar (pH 7.2 ± 0.2) supplemented with 0.2% potassium aluminium silicate (Rajawat *et al.* 2016). Zinc solubilisation was tested on mineral salt medium enriched with 0.1% each of zinc oxide and zinc phosphate (Gontia-Mishra *et al.* 2017). All the plates were incubated at 30°C for 4–5 days and observed for halo zone formation and solubilisation index was calculated using formula as follows:

$$\text{Solubilization index} = (\text{Zone around the colony} + \text{Colony diameter}) / (\text{Colony diameter})$$

Overnight grown culture of bacterial isolate was centrifuged at 10000 rpm for 10 min at 4°C, supernatant was collected and filter sterilized with 0.45 µm (Axiva) filter. 2 mL supernatant was mixed with 4 mL Salkowski's reagent [2 mL 0.5M FeCl₃, 49 mL water and 70% Perchloric acid (HClO₄)]. The mixture was incubated in dark at room temperature for 30 min and the intensity of pink colour was measured at 530 nm with UV/Visible Spectrophotometer (Scan Drop Analytek Jena), using standard curve (Brick *et al.* 1991). Overnight grown culture of the bacterial isolate was inoculated onto CAS agar plate. Siderophore production

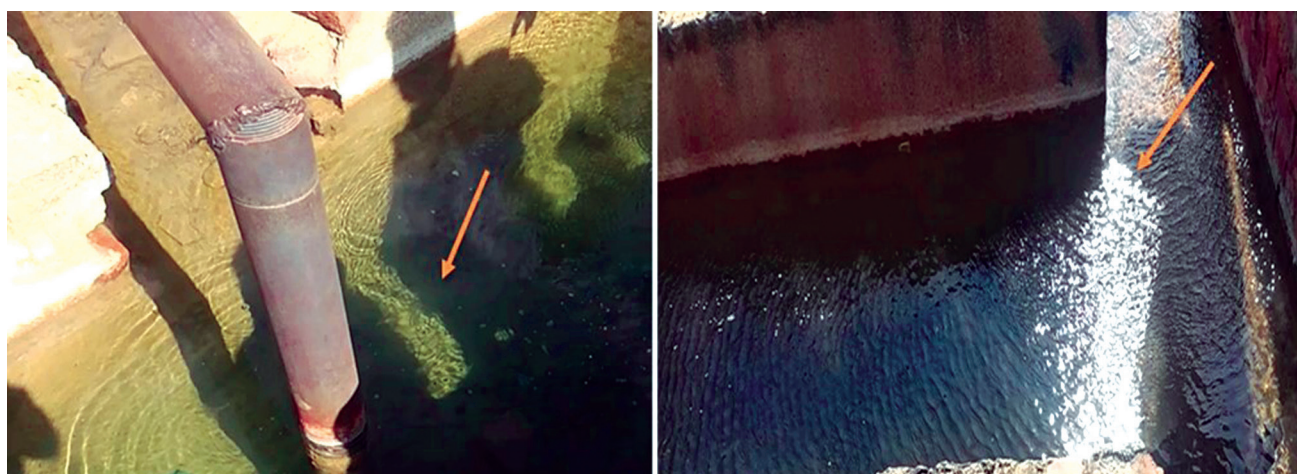


Fig. 1 Tattapani hot spring with a bore hole from geothermal area and hot water flowing stream. Arrow indicates sample collected site.

was indicated by yellow halos around the colonies after the incubation at $30 \pm 2^\circ\text{C}$ for 2–3 days (Schwyn and Neilands 1987). Different inorganic source of phosphorous such as tri-calcium phosphate, Mussoorie rock phosphate and aluminium phosphate was amended in the National Botanical Research Institute Phosphate (NBRIP) broth at a concentration of 0.5% each separately and incubated for 5 days at $30 \pm 2^\circ\text{C}$. Phosphorus in the culture supernatant was measured by method described by Fiske and Subbarow (1925). Quantitative estimation of K solubilisation provided with 0.5% potassium aluminium silicate was done with protocol given by Rajawat *et al.* (2016).

Culture supernatant of RPB22 was analysed by High Performance Liquid Chromatography (HPLC) for organic acid produced during different sources of inorganic P solubilisation. Waters C18 column was used with a mobile phase consisting of methanol (20%) and Millipore water (80%). Flow rate was adjusted to 0.8 mL/min, column temperature of 40°C , injection volume 20 μL and UV detector 210 nm as the conditions reported by (Oteino *et al.* 2015). Supernatant were filtered through 0.22 μm filter (Axiva) before analysis. Standard organic acid, viz. oxalic acid, gluconic acid, mallic acid, citric acid and succinic acid was procured from Sigma Aldrich and all the standards were prepared in a mobile phase with a final concentration of 1 mg/mL. To evaluate plant growth assay chickpea cultivar (Pusa 362) seeds were obtained from ICAR-Indian Institute of Seed Research, Mau, Uttar Pradesh to assess the growth promoting ability of RPB22. Seeds were surface sterilised with 2% sodium hypochlorite for 5 min and washed five times with sterilised water. Thirty surface sterilised seeds were soaked with and without RPB22 culture in NB for 40 min (10^8 CFU/mL) broth water (Gopalakrishnan *et al.* 2016). The chickpea seeds (Five each) were inoculated in the pot of size 30 cm \times 30 cm filled with 2 kg sterile field soil in triplicates in a nethouse. After 30 days, measurement of root parameters was taken by Hewlett Packard scanner and analyzed using WIN RHIZO programme V. 2002 C software (Regent Instruments Inc. Ltd. Quebec, Canada). Experiment was conducted in completely randomized design with three replications each. Data were analysed by Microsoft Office Excel to calculate standard deviation and standard error of mean.

RESULTS AND DISCUSSION

Analysis of sample: Sample collected from hot spring soil had slightly alkaline pH (7.5). Temperature of the thermal spring was recorded as 85°C . Electrical conductivity of the sample was recorded as 1.45 mS/cm. The conductivity of soil depends on temperature and the concentration of dissolved ions or total chemical components in the water. Thermal springs, distributed worldwide, harbour extreme environments that maintain their unique properties consistently over time. Previous studies have documented the surface water temperature of the Manikaran hot spring as 95°C (Sharma *et al.* 2016), while the Tattapani hot spring registered a temperature of

90°C and a pH of 7.8 (Kumar *et al.* 2014). In our study, we noted slight variations in temperature and pH, likely attributable to seasonal fluctuations and increased human activities surrounding the hot spring.

Isolation, identification and characterization of bacterial isolate: The diverse colony morphology observed underscores the rich microbial diversity within the hot spring. Researchers have previously examined colony morphology in thermophilic bacterial isolates, noting variations in size, colour, and shape (Nshimiyimana *et al.* 2018). Munoz-Torres *et al.* (2023) recorded *Pseudomonas*, *Bacillus*, *Pantoea* from Jurasi hot springs of Atacama Desert, Chile. Similarly, Banerjee *et al.* (2024) isolated exopolysaccharide producing *Staphylococcus* spp. BSP3 from Chilean hot spring. Ghati *et al.* (2013) documented the successful isolation of three thermophilic bacteria from the Bakreshwar hot spring in West Bengal, India, identifying them as *Acinetobacter lwoffii* strain 412, *Bacillus cereus* AGP-03, and *Geobacillus stearothermophilus* strain G1017-C12. Saxena *et al.* (2017) expanded on this research, reporting the presence of several genera in the Manikaran hot spring of India, including *Bacillus*, *Geobacillus*, *Anoxybacillus*, *Brevundimonas*, and *Pseudomonas*. Notably, our study marks the first report of cultivable diversity in the Tattapani hot spring of Chhattisgarh, where temperatures exceed 80°C . In the present study, RPB22 isolate has been sequenced and BLAST search results of 16S rRNA gene showed 99.86% similarity with *Pseudomonas extremorientalis* from NCBI and EZtaxon. The 16s rDNA sequence of the isolate deposited to NCBI database and accession number was obtained (MT560341). The identified strain has been deposited under the accession number (NAIMCC-B-02402) in the culture collection of National Agriculturally Important Microbial culture collection (NAIMCC), Mau, Uttar Pradesh.

Screening of bacterial isolates for temperature and salinity tolerance: Bacterial isolate was screened for temperature, pH and salinity tolerance. RPB22 could tolerate up to 50°C temperature and tolerance to 3% NaCl concentration. In a separate study, Mukherjee *et al.* (2020) identified *Klebsiella* spp. strain PMnew from the Paniphala hot spring in West Bengal, which displayed tolerance to temperatures ranging from 18– 65°C and NaCl concentrations up to 20%. Similarly, Pandey *et al.* (2015) conducted an investigation into the Soldhar and Ringigad hot springs in Uttarakhand, revealing that the isolates exhibited robust growth across temperatures ranging from 20– 80°C and displayed resilience to NaCl concentrations of up to 12%. Thermophilic *Pseudomonas aeruginosa* Ch39 was isolated from Chignahuapan hot springs in Mexico was reported to grow at 45°C temperature (Castelan-Sanchez *et al.* 2025). Several *Pseudomonas* spp. have been reported to thrive at temperature optima of 40– 60°C in the Indian Himalayan regions (Verma *et al.* 2022). These findings align closely with earlier reports, indicating the consistent adaptability of these extremophilic bacteria to harsh environmental conditions.

Indole acetic acid (IAA) and siderophore production: The diverse properties exhibited by bacteria thriving in

extreme environments hold significance not only for plant health but also for various biotechnological applications. For instance, they can be directly utilized for biological control of plant pathogens and for promoting plant growth. Plant growth promoting bacteria are known to predominantly produce auxins, particularly IAA, which play a pivotal role in enhancing the development of host plant root systems and thereby fostering overall plant growth (Tanimoto 2005). Strain RPB22 produced 13 µg/mL of IAA without tryptophan, which increased to 16.4 µg/mL when supplemented with 300 µg/mL tryptophan. Munoz-Torres *et al.* (2023) reported bacteria exhibiting various plant growth promoting traits isolated from hot spring. Many of the isolates exhibited indole acetic acid (IAA) production both with and without tryptophan supplementation. However, RPB26 demonstrated a more pronounced increase in IAA production with higher concentrations of tryptophan. Similarly, Mukherjee *et al.* (2020) isolated *Klebsiella* spp. strain PMnew from the Paniphala hot spring in West Bengal, which produced 125.01 ± 0.01 µg/mL of indole acetic acid when treated with 5 mM tryptophan. *B. licheniformis* BHUJP-H3, isolated from a hot spring in the Leh and Ladakh region of India, exhibited IAA production of 34.76 ± 1.12 µg/mL at 300 µg/mL after 72 h (Verma *et al.* 2018). Various studies have investigated siderophore production by different bacterial species, including *Pseudomonas putida* IPL/IH/R-6 and *Pseudomonas plecoglossicida* IPL/IH/D-1, *Bacillus subtilis*, *Bacillus* spp., *Bacillus amyloliquefaciens* (4RH), *Kliseibella* spp., and strains BAN 87 and BAN 86 (Amaresan *et al.* 2014, Verma *et al.* 2018, Mukherjee *et al.* 2020, Sood *et al.* 2024). Ahmad *et al.* (2005) reported that *Azotobacter* and *Pseudomonas* exhibited increased IAA production with higher concentrations of tryptophan, as the amino acid serves as a key precursor in IAA biosynthesis under broth culture conditions. However, it's important to recognize that IAA concentration may vary among different strains and can be influenced by factors such as growth conditions, media composition, and nutrient availability (Sridevi *et al.* 2007). Siderophores serve as essential iron chelators crucial for promoting plant growth (Verma *et al.* 2018). Although significant siderophore production was observed in this study, it was notably absent at high temperatures.

Qualitative estimation of P, K and Zn from bacterial isolates: Bacterial isolate has been screened for phosphate, potassium and zinc solubilisation and showed phosphate, potassium and zinc solubilising activity by forming a clear halo zone. RPB22 exhibited the capability to solubilize all three inorganic sources of phosphate, potassium, and zinc in broth culture. However, it was unable to solubilise phosphorus in media containing MRP (Mussoorie Rock Phosphate) and AP (Aluminum Phosphate) amendments. This observation aligns with previous findings indicating that isolates proficient in solubilizing phosphorus in broth culture may not necessarily produce clear halo zones on agar-containing media (Perez *et al.* 2007). In this study Phosphate Solubilising Index (PSI) of 2.4 and Potash Solubilising Index (KSI) of 2.0 was recorded for *P. extremorientalis* RPB22.

Sood *et al.* (2024) reported that *Pseudomonas putida* IPL/IH/R-6 and *Pseudomonas plecoglossicida* IPL/IH/D-1 exhibited maximum potassium solubilization with a KSI value of 2.0, while strain IPL/IH/R-6 also demonstrated phosphate solubilization with a PSI value of 3.0. Amaresan *et al.* (2014) reported that 34 isolates from Andaman Barren Island demonstrated the ability to solubilize inorganic phosphate, while Verma *et al.* (2018) noted that the thermophilic *B. licheniformis* BHUJP-H3 displayed significantly enhanced phosphate solubilization (33.01 µg/mL) compared to other *Bacillus* species studied from hot springs in the Leh and Ladakh region. In their investigation, Mukherjee *et al.* (2020) explored the phosphate solubilization activity of *Klebsiella* spp. strain PMnew and revealed its capacity to produce organic acids (550.16 ± 0.04 µg/mL) for phosphate solubilisation. *Pseudomonas koreensis* SH-29 exhibited phosphorous solubilisation (140.8 µg/L) at 45°C (Ahmad *et al.* 2023). Alonazi *et al.* (2025) reported that a native isolate, *Pseudomonas thivervalensis* K26, obtained from the rhizosphere of *Acacia* spp., exhibited heat tolerance up to 50°C and demonstrated multiple plant growth-promoting traits. Such thermotolerant *Pseudomonas* strains can serve as valuable bioinoculant candidates for chickpea cultivation in heat-prone regions, where they may enhance nutrient uptake, root development, and overall plant resilience under high-temperature stress conditions.

Quantitative estimation of P and K and organic acid production: On the basis of multifarious plant growth promoting traits, RPB22 was selected for quantitative estimation of P and K. Isolate RPB22 could solubilise 333 µg/mL of TCP, 164 µg/mL MRP and 123 µg/mL AP. Potassium solubilisation was recorded as 7.5 mg/L. It has also recorded that apart from TCP, RPB22 failed to produce halo zone in PVK agar media when supplemented with MRP and AP however, in liquid culture solubilisation was recorded. The HPLC profile of isolate RPB22 (Fig. 2) unveiled the presence of multiple organic acids, with a total concentration of 25 g/L, predominantly malic acid followed by succinic acid. The highest production was observed when the growth media were amended with AP, malic acid was recorded as highest (10.81 g/L) when culture broth was amended with aluminium phosphate followed by 7.19 g/L when TCP (Tri-Calcium Phosphate) was supplied in the growth media. Interestingly, succinic acid was only secreted in the presence of TCP and AP while, oxalic acid was secreted in the MRP and AP amended media but absent in the TCP amended media (Table 1). However, gluconic acid and citric acid was not detected. Apart from these organic acids in the culture broth, peaks of unknown. In a study conducted by Yadav *et al.* (2015), thermotolerant strains were shown to produce various organic acids, including oxalic, malic, succinic, and formic acid, with strain BISR-HY66 being the sole producer of succinic acid. The quantity and profile of organic acids produced by *Pseudomonas* strains vary depending on both the type of substrate provided and the inherent metabolic characteristics of the strain. This variability highlights the importance of

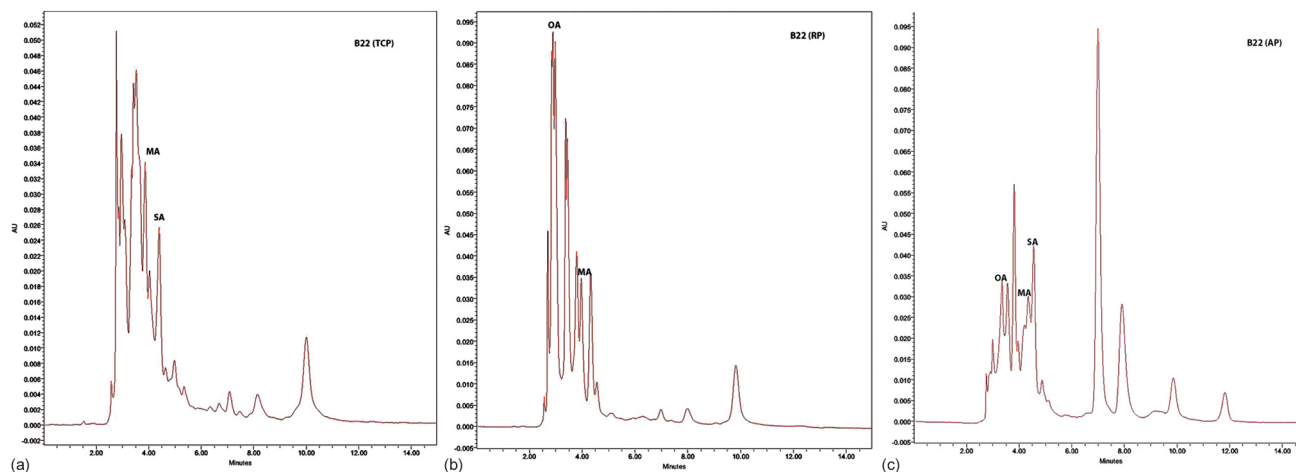


Fig. 2 HPLC profile of phosphate solubilizing *Pseudomonas extremorientalis* RPB22 with (a) Tri-Calcium Phosphate (TCP); (b) Rock Phosphate (RP); (c) Aluminium phosphate (AP). MA, Malic acid; SA, Succinic acid; OA, Oxalic acid.

selecting appropriate bacterial isolates and substrates to optimize nutrient solubilization and plant growth-promoting efficiency (Vyas and Gulati 2009). The growth of plants is significantly influenced by soil nutrients and the microbiota present in the rhizosphere. In the present study, *Pseudomonas extremorientalis* RPB22 exhibited variation in organic acid production, consistent with observations from previous reports. The strain also demonstrated diverse plant growth-promoting traits, including the solubilization of inorganic phosphate, potassium, and zinc, as well as the production of siderophores and indole-3-acetic acid (IAA), highlighting its multifaceted potential to enhance nutrient availability and promote plant growth.

Plant growth assay: On the basis of multifarious PGP traits *Pseudomonas extremorientalis* RPB22 was used for seed treatment in chickpea cultivar (Pusa 362). The treatment showed enhanced root and shoot parameters of chickpea plant (Fig. 3). Increased root length (29.5 cm) and fresh weight (3 g) was observed in RPB22 treated chickpea plant as compared to uninoculated plant. Root volume was increased by 1.8-fold in RPB22 treated plant as compared to control plant. Root parameters like surface area, projected area of root, diameter and volume of root was also increased. Number of links in treated plant was recorded as (1364 ± 112) which was higher than the control (1049 ± 472) plant (Table 2). Treatment with RPB22 resulted in increased values of shoot

and root parameters in chickpea plants. These findings aligned with previous studies on bioinoculants isolated from hot springs, as reported by Patel *et al.* (2017) and Verma *et al.* (2018). Thakur and Yadav (2023) reported that *Streptomyces* spp. AB-11, isolated from the rhizosphere of *Ajuga parviflora*, exhibited tolerance up to 45°C. Inoculation

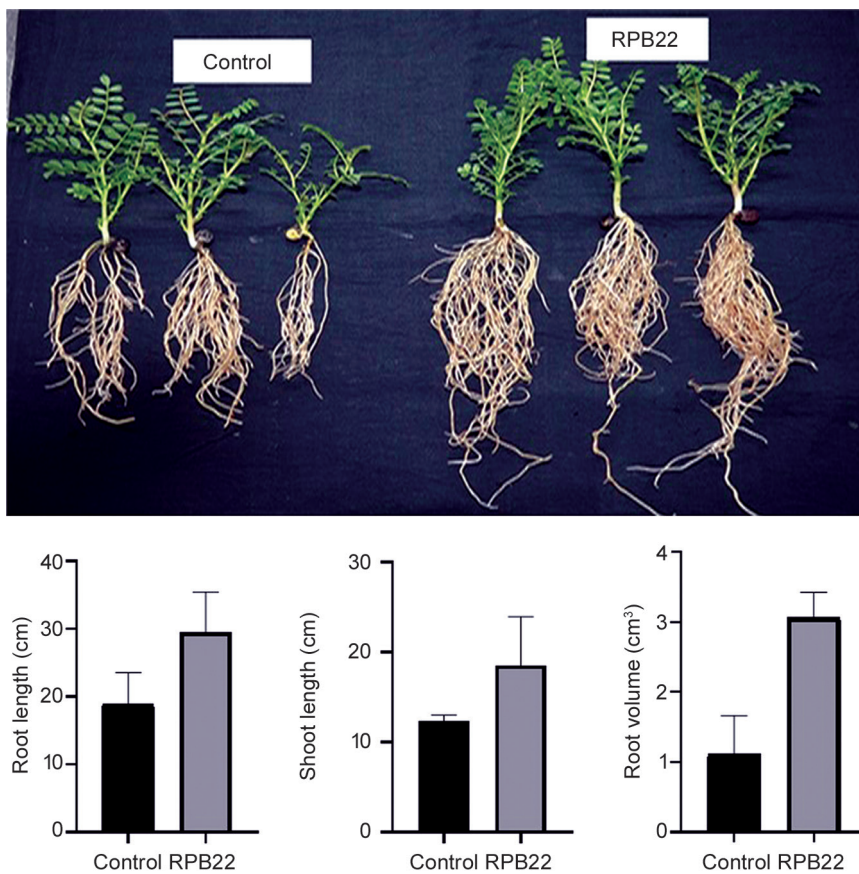


Fig. 3 Plant growth promotion assay of chickpea after 30 days of growth and effect of *Pseudomonas extremorientalis* RPB22 inoculation on plant growth parameters of chickpea (Pusa 362) after 30 days of growth.

Bars represent mean \pm SD (n = 3).

Table 1 Organic acid profile for phosphate solubilising strains RPB22

*Isolate/source of P	P solubilisation ($\mu\text{g/mL}$)	Organic acids (g/L)					Total known acids (g/L)
		Gluconic	Oxalic	Mallic	Citric	Succinic	
* <i>Pseudomonas extremorientalis</i> RPB22							
TCP	333 \pm 0.9	ND	ND	7.19	ND	1.53	8.72
MRP	164 \pm 1.2	ND	0.33	2.39	ND	ND	2.72
AP	124 \pm 1.2	ND	0.22	10.81	ND	3.21	14.24
Total							25.68

Values presented are mean of three replicates \pm SD (Standard deviation). TCP, Tri-calcium phosphate; MRP, Mussoorie rock phosphate; AP, Aluminum phosphate; ND, Not detected.

Table 2 Plant growth parameters of chickpea (Pusa 362) after 30 days of growth

Treatment	Root length (cm)	Shoot length (cm)	Root fresh wt. (g)	Shoot fresh wt. (g)	Root dry wt. (g)	Shoot dry wt. (g)	Projected area (cm^2)	Surface area (cm^3)	Average diameter (cm)	Root volume (cm^3)	links
Control	18.9 \pm 4.64	12.3 \pm 1.0	0.8 \pm 1.1	0.41 \pm 0.5	0.05 \pm 0.0	0.1 \pm 0.02	16.3 \pm 6.1	51.1 \pm 19	0.8 \pm 0.15	1.1 \pm 0.5	1049 \pm 472
<i>Pseudomonas extremorientalis</i> RPB22	29.5 \pm 5.9	18.5 \pm 5.7	3 \pm 0.9	1.3 \pm 0.2	0.2 \pm 0.1	0.3 \pm 0.1	34 \pm 5.3	106 \pm 17	1.2 \pm 0.04	3.1 \pm 0.4	1364 \pm 112

Values are mean of three replicates \pm SD (Standard deviation).

of chickpea with this strain resulted in a 25% increase in root length and a 37.1% enhancement in fresh weight compared to the uninoculated control, indicating its potential role in promoting plant growth under high-temperature conditions. The observed enhancement in plant growth is likely influenced by IAA production, which promotes root elongation and branching. However, overall growth reflects a coordinated interactions of multiple plant responses activated by the microbial inoculant, including improved nutrient acquisition through phosphate, potassium, and zinc solubilization, siderophore-mediated iron availability, and enhanced stress tolerance (Thakur and Yadav 2023). This multifaceted interaction between the plant and *Pseudomonas extremorientalis* RPB22 underscores the importance of thermotolerant, multifunctional PGPR in supporting plant growth under challenging environmental conditions such as high temperature and nutrient-limited soils.

Based on its multifaceted plant growth-promoting (PGP) attributes, *Pseudomonas extremorientalis* RPB22 was quantitatively evaluated for its influence on phosphorus (P) and potassium (K) uptake, revealing significant improvements in root and shoot growth parameters by coordinated interplay among multifaceted PGP organisms. These results highlight the potential of isolate RPB22 as a promising bioinoculant candidate, particularly for use in arid and semi-arid regions, due to its thermotolerance and broad-spectrum PGP activities. Moreover, the application of such thermotolerant microbial inoculants in chickpea enhances nutrient mobilization through organic acid production, especially when supplemented with insoluble phosphate sources such as tricalcium phosphate (TCP), aluminium phosphate (AP), and rock phosphate (RP). This

approach not only improves phosphorus availability and plant growth under heat stress but also reduces reliance on chemical fertilizers, thereby contributing to sustainable crop production and soil health maintenance under climate change scenarios.

REFERENCES

- Ahmad F, Ahmad I and Khan M S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turkish Journal of Biology* 29(1): 29–34.
- Ahmad M, Imtiaz M, Nawaz M S, Mubin F, Sarwar Y, Hayat M, Asif M, Naqvi R Z, Ahmad M and Imran A. 2023. Thermotolerant PGPR consortium B3P modulates physio-biochemical and molecular machinery for enhanced heat tolerance in maize during early vegetative growth. *Annals of Microbiology* 73: 34. <https://doi.org/10.1186/s13213-023-01736-5>
- Ahemed M and Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University-Science* 26: 1–20. <https://doi.org/10.1016/j.jksus.2013.05.001>
- Alonazi M A, Alwathnani H A, AL-Barakah F N I and Alotaibi F. 2025. Native plant growth-promoting rhizobacteria containing ACC Deaminase promote plant growth and alleviate salinity and heat stress in maize (*Zea mays* L.) plants in Saudi Arabia. *Plants* 14(7): 1107. <https://doi.org/10.3390/plants14071107>
- Amaresan N, Kumar K, Sureshbabu K and Madhuri K. 2014. Plant growth-promoting potential of bacteria isolated from active volcano sites of Barren Island, India. *Letters in Applied Microbiology* 58(2): 130–37. Doi: 10.1111/lam.12165
- Badhai J, Ghosh T S and Das S K. 2015. Taxonomic and functional characteristics of microbial communities and their correlation with physicochemical properties of four geothermal springs in Odisha, India. *Frontiers in Microbiology* 6: 1166. <https://doi.org/10.3389/fmicb.2015.00116>

- org/10.3389/fmicb.2015.01166
- Banerjee S, Barjas C B, Tapia J, Fabi J P, Banerjee D S, Barjas G C, Tapia J, Fabi J P, Delattre C and Banerjee A. 2024. Characterization of Chilean hot spring-origin *Staphylococcus* spp. BSP3 produced exopolysaccharide as biological additive. *Natural Products and Biology* **14**: 15. <https://doi.org/10.1007/s13659-024-00436-0>
- Brick J M, Bostock R M and Silverstone S E. 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied Environmental Microbiology* **57**: 535–38. [10.1128/aem.57.2.535-538.1991](https://doi.org/10.1128/aem.57.2.535-538.1991)
- Castelan-Sanchez H G, Fernandez Doderio J V, Rojas-Vargas J, Martínez-Ocampo F, Hurtado-Ramirez J M, Ríos-Vazquez D I, Sánchez-Alonso P, Vazquez-Cruz C and Rojas-Ruiz N E. 2025. Thermophilic *Pseudomonas aeruginosa* strain Ch39 isolated from Chignahuapan hot springs in Puebla, Mexico. *Letters in Applied Microbiology* **78**: 4. <https://doi.org/10.1093/lambio/ovaf059>
- Fiske C H and Subbarow Y. 1925. The colourimetric determination of phosphorus. *Journal of Biology and Chemistry* **66**: 375–400.
- Ghati A, Sarkar K and Paul G. 2013. Isolation, characterization and molecular identification of esterolytic thermophilic bacteria from an Indian hot spring. *Current Research in Microbiology and Biotechnology* **1**(4): 196–202.
- Gontia-Mishra I, Sapre S and Tiwari S. 2017. Zinc solubilising bacteria from the rhizosphere of rice as prospective modulator of zinc biofortification in rice. *Rhizosphere* **3**: 185–90. <https://doi.org/10.1016/j.rhisph.2017.04.013>
- Gopalakrishnan S, Vadlamudi S, Samineni S and Kumar S S. 2016. Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. *Springer Plus* **5**: 1882. [Doi 10.1186/s40064-016-3590-6](https://doi.org/10.1186/s40064-016-3590-6)
- Jackson M L. 1958. *Soil Chemical Analysis*. pp. 498. Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Jha U C, Nath C P, Paul P J, Nayyar H, Kumar N, Dixit G P, Sen S, Kumar Y and Prasad P V V. 2025. Decoding the heat stress resilience in Chickpea (*Cicer arietinum* L.): Multi-trait analysis for genotypic adaptation. *Scientific Report* **15**(1): 25055. [Doi: 10.1038/s41598-025-07573-7](https://doi.org/10.1038/s41598-025-07573-7).
- Kumar M, Yadav A N, Tiwari R, Prasanna R and Saxena A K. 2014. Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. *Annals of Microbiology* **64**: 741–51. doi.org/10.1007/s13213-013-0709-7
- Mirete S, Morgante V and Gonzalez-Pastor J E. 2016. Functional metagenomics of extreme environments. *Current Opinion in Biotechnology* **38**: 143–49. [Doi: 10.1016/j.copbio.2016.01.017](https://doi.org/10.1016/j.copbio.2016.01.017)
- Mongra A C. 2012. Distribution pattern of cyanobacteria in hot water springs of Tattapani, Himachal Pradesh, India. *Journal of Academia and Industrial Research* **1**: 363–70.
- Mukherjee T, Banik A and Mukhopadhyay S K. 2020. Plant growth promoting traits of a thermophilic strain of the *Klebsiella* group with its effect on rice plant growth. *Current Microbiology* **77**(10): 2613–22. [Doi: 10.1007/s00284-020-02032-0](https://doi.org/10.1007/s00284-020-02032-0)
- Munoz-Torres P, Marquez S L, Sepulveda-Chavera G, Cardenas-Ninasivincha S, Arismendi-Macuer M, Huanca-Mamani W, Aguilar Y, Quezada A and Bugueno F. 2023. Isolation and identification of bacteria from three geothermal sites of the Atacama Desert and their plant-beneficial characteristics. *Microorganisms* **11**: 2635.
- Nshimiyimana J B, Khadka S, Mwizerwa M E, Akimana N, Adhikari S and Nsabimana A. 2018. Thermophiles: Isolation, characterization and screening for enzymatic activity. *Bioscience Discovery* **9**(3): 430–37.
- Oteino N, Lally R D, Kiwanuka S, Lloyd A, Ryan D, Germaine K J and Dowling D N. 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology* **6**: 745.
- Pandey A, Dhakar K, Sharma A, Priti P, Sati P and Kumar B. 2015. Thermophilic bacteria that tolerate a wide temperature and pH range colonize the Soldhar (95°C) and Ringigad (80°C) hot springs of Uttarakhand, India. *Annals of Microbiology* **65**(2): 809–16. <https://doi.org/10.1007/s13213-014-0921-0>
- Patel J K and Archana G. 2017. Diverse culturable diazotrophic endophytic bacteria from poaceae plants show cross-colonization and plant growth promotion in wheat. *Plant and Soil* **417**(1–2): 99–116.
- Perez E, Sulbaran M, Ball M M and Yarzabal L A. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry* **39**(11): 2905–14. <https://doi.org/10.1016/j.soilbio.2007.06.013>
- Pikovskaya R I. 1948. Mobilisation of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiologia* **17**: 362–70.
- Purcell U, Sompong L C, Yim T G, Barraclough Y and Peerapornpisal S B. 2007. Pointing the effects of temperature, pH and sulphide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. *FEMS Microbiology and Ecology* **60**: 456–66. [Doi: 10.1111/j.1574-6941.2007.00302.x](https://doi.org/10.1111/j.1574-6941.2007.00302.x)
- Rajawat M V, Singh S, Saxena A and Prasanna A. 2016. A modified plate assay for rapid screening of potassium-solubilising bacteria. *Pedosphere* **26**(5): 768–73. [https://doi.org/10.1016/S1002-0160\(15\)60080](https://doi.org/10.1016/S1002-0160(15)60080)
- Sayeh R, Birrien J L, Alain K, Barbier G, Hamdi M and Prieur D. 2010. Microbial diversity in Tunisian geothermal springs as detected by molecular and culture-based approaches. *Extremophiles* **14**(6): 501–14. [Doi: 10.1007/s00792-010-0327-2](https://doi.org/10.1007/s00792-010-0327-2)
- Saxena R, Dhakan D B, Mittal P, Waiker P, Chowdhury A, Ghatak A and Sharma V K. 2017. Metagenomic analysis of hot springs in central India reveals hydrocarbon degrading thermophiles and pathways essential for survival in extreme environments. *Frontiers in Microbiology* **5**(7): 2123. <https://doi.org/10.3389/fmicb.2016.02123>
- Schwyn B and Neilands J B. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* **160**(1): 47–56. [Doi: 10.1016/0003-2697\(87\)90612-9](https://doi.org/10.1016/0003-2697(87)90612-9)
- Sharma A, Kohli P, Singh Y, Schumann P and Lal R. 2016. *Fictibacillus halophilus* sp. nov., from a microbial mat of a hot spring atop the Himalayan Range. *International Journal of Systematic and Evolutionary Microbiology* **66**(6): 2409–16. [Doi: 10.1099/ijsem.0.001051](https://doi.org/10.1099/ijsem.0.001051)
- Sood G, Prakash V and Arora S. 2024. Diversity of multi trait plant growth-promoting culturable bacteria from natural geothermal springs. *Geomicrobiology Journal* **41**(5): 543–51. [Doi: 10.1080/01490451.2024.2335941](https://doi.org/10.1080/01490451.2024.2335941)
- Soy S, Nigam V N and Sharma S R. 2019. Cellulolytic, amylolytic and xylanolytic potential of thermophilic isolates of Surajkund hot spring. *Journal of Bioscience* **44**: 124. [Doi: 10.1007/s12038-019-9938-7](https://doi.org/10.1007/s12038-019-9938-7)
- Sridevi M and Mallaiah K V. 2007. Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green

- manure crop, *Sesbania sesban* (L.) Merr. *Iranian Journal of Biotechnology* **5**(3): 178–82.
- Thakur R and Yadav S. 2023. Thermotolerant and halotolerant *Streptomyces* spp. isolated from *Ajuga parviflora* having biocontrol activity against *Pseudomonas syringae* and *Xanthomonas campestris* acts as a sustainable bioadditive in growth promotion of *Cicer arietinum*. *Physiological and Molecular Plant Pathology* **127**: 2023. <https://doi.org/10.1016/j.pmpp.2023.102059>
- Tanimoto E. 2005. Regulation of root growth by plant hormones—Roles for auxin and gibberellin. *Critical Review in Plant Science* **24**: 249–65. Doi: 10.1080/07352680500196108
- Verma J P, Jaiswal D K, Krishna R, Prakash R, Yadav J and Singh V. 2018. Characterization and screening of thermophilic bacillus strains for developing plant growth promoting consortium from hot spring of Leh and Ladakh region of India. *Frontiers in Microbiology* **9**: 1293. <https://doi.org/10.3389/fmicb.2018.01293>
- Verma J, Sourirajan A and Dev K. 2022. Bacterial diversity in 110 thermal hot springs of Indian Himalayan Region (IHR). *3 Biotech* **12**(9): 238. Doi: 10.1007/s13205-022-03270-8
- Yadav A N, Verma P, Kumar M, Pal K K, Dey R, Gupta A, Padaria J C, Gujar G T, Kumar S, Suman A, Prasanna R and Saxena A K. 2015. Diversity and phylogenetic profiling of niche-specific *Bacilli* from extreme environments of India. *Annals of Microbiology* **65**: 611–29. <https://doi.org/10.1007/s13213-014-0897-9>
- Yoon S H, Ha S M, Kwon S, Lim J, Kim Y, Seo H and Chun J. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematics and Evolutionary Microbiology* **67**(5): 1613. <https://doi.org/10.1099/ijsem.0.001755>
- Vyas P and Gulati A. 2009. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology* **9**: 174. <https://doi.org/10.1186/1471-2180-9-174>
- Zhang J, Tang S K, Zhang Y Q, Yu L Y, Klenk H P and Li W J. 2010. *Laceyella tengchongensis* sp. nov., a thermophile isolated from soil of a volcano. *International Journal of Systematics and Evolutionary Microbiology* **60**: 2226–30. Doi: 10.1099/ijse.0.011767-0